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Adalimumab Clearance, rather than Trough Level, May Have Greatest Relevance to Crohn's Disease Therapeutic Outcomes Assessed Clinically and Endoscopically

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ABSTRACT

Objective: We postulated that adalimumab (ADA) drug clearance (CL) may be a more critical determinant of therapeutic outcome than ADA concentration. This was tested in Crohn's disease (CD) patients undergoing ADA maintenance treatment.

Methods: CD patients from 4 cohorts received ADA induction and started maintenance therapy. Therapeutic outcomes consisted of endoscopic remission (ER), sustained -reactive protein (CRP) based clinical remission (defined as CRP levels below 3 mg/L in the absence of symptoms) and fecal calprotectin (FC) level below 100µg/g. Serum albumin, ADA concentration and anti-drug antibody status were determined using immunochemistry and homogenous mobility shift assay, respectively. CL was determined using a nonlinear mixed effect model with Bayesian priors. Statistical analysis consisted of Mann-Whitney test, and logistic regression with calculation of odds ratio. Repeated event analysis was conducted using a nonlinear mixed effect model.

Results: In 237 enrolled patients (median age 40 years, 45% females), median CL was lower in patients achieving ER as compared to those with persistent active endoscopic disease (median 0.247 L/day vs 0.326 L/day, respectively) ($p<0.01$). There was no significant difference in ADA concentration between patients in endoscopic remission compared to those with recurrence (median 9.3 µg/mL vs 11.7 µg/mL, respectively). Sustained CRP-based clinical remission and FC levels below 100µg/g were generally associated with lower CL and higher ADA concentration. Repeated event analysis confirmed those findings with better performances of CL than concentration in associating with ER and other outcomes.

Conclusion: Lower ADA Clearance is associated with an improved clinical outcome for patients with Crohn's disease and may be a superior pharmacokinetic measure than concentration.

Key words: Crohn's disease; Adalimumab, pharmacokinetics, Clearance

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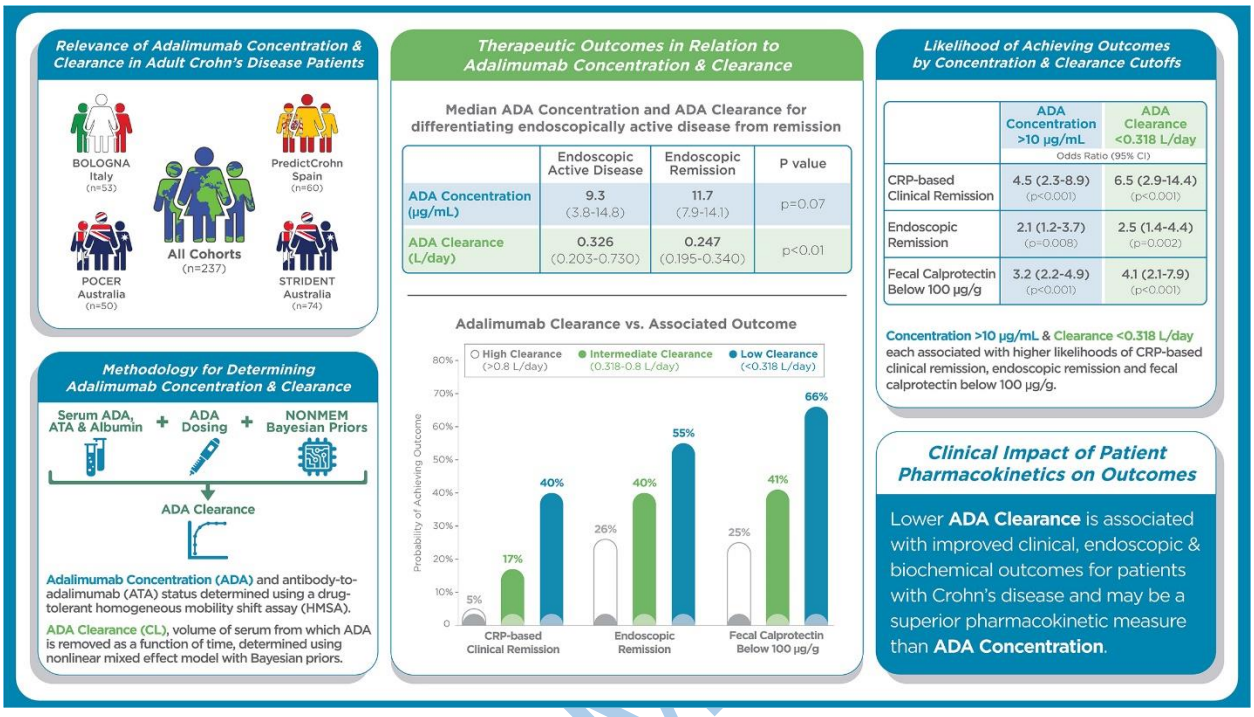
Graphical abstract



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INTRODUCTION

Therapeutic drug monitoring (TDM) is now routine for many patients with CD receiving anti-tumor necrosis factor-α (TNF-α) therapy and helps direct and improve drug management[1]. The measurement of adalimumab (ADA, a monoclonal antibody targeting TNF-α) blood concentration can guide clinicians about the potential need for dose escalation to achieve exposure commensurate with disease control. It can also provide reassurance regarding the absence of immune tolerance and formation of antibodies to adalimumab (ATA).

In order to maximize the clinical yield associated with ADA and availability to neutralize the inflammatory burden present, gastroenterologists have endorsed TDM of ADA, reactively, in the face of inadequate disease control,[2, 3] or proactively with maintenance of ADA concentration above a minimal effective concentration, associated with enhanced drug tolerance and sustained disease control[4, 5]. Reactive or proactive, the decision to increase or decrease the dose intensity requires careful implementation to maintain exposure above the desired concentration. To that end, model informed precision guided dosing (MIPD) tools that employ population PK with Bayesian priors have recently demonstrated their value in assisting with the achievement of desired exposure[6], with the potential to also fine tune the therapeutic window between minimal effective concentration and over-exposure where side effects may occur[7].

These MIPD tools are now becoming available in clinical practice[8] and have demonstrated value in anti-TNF treatment.[9] Both retrospective and prospective clinical utility studies support the value of this approach to improve outcomes[6, 10]. Population PK based tools now allow the determination of CL, a key predictive factor of pharmacokinetic (PK) origin that accelerates in the presence of immunization against

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the drug[11] and an increasing inflammatory burden[12]. As such, this PK outcome measure, which represents the volume of serum from which the drug is removed as a function of time, may perform equally well or better than ADA concentration in associating with outcome. This hypothesis was tested in this report.

METHODS

In this retrospective analysis, CD patients from 4 different cohorts started subcutaneous ADA treatment with the standard induction schedule (160 mg followed by 80 mg and 40 mg every other week) followed by 40 mg every two weeks during maintenance (Cohort 1 through Cohort 3)[13 14, 15] or on an intensive induction schedule (160 mg weekly for 4 consecutive doses followed by 40 mg every other week). Dose intensification based on dose increase or frequency was at the discretion of the clinician in three of the four cohorts (Cohort 1 did not allow for dose intensification) and based on the presence of symptoms or inflammation[16]

The first cohort (BOLOGNA cohort) was performed in the context of a one-year prospective observational clinical trial aimed at identifying biomarkers, and predictors of a failure response to commonly used biological therapy in patients with Crohn's Disease[13]. The second cohort (PredictCrohn) was a prospective multicenter cohort study in patients naïve to biologics and active luminal disease and followed for 14 weeks[14]. The third cohort (the POCER[15] cohort) examined a cohort of patients with ileo-colonic CD following intestinal resection of all macroscopic disease, with ADA used post-operatively to prevent recurrence. The fourth cohort (STRIDENT cohort) was from an open-label, single-centre,

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randomized controlled trial evaluating Intensive drug therapy versus standard drug therapy for symptomatic intestinal Crohn's disease strictures[16]. Patients from each cohort were followed longitudinally during their maintenance treatment.

For all studies, the sampling time for ADA PK were matched to the study visit assessment, occurring anytime relative to the last dose. Blood was collected in serum separator tubes at each of the sites, centrifuged and stored at -80°C before shipping on dry ice. Serum ADA concentrations and antibodies to ADA (ATA) were determined using a drug tolerant homogenous mobility shift assay in a central clinical laboratory (Prometheus Laboratories, San Diego, CA)[17]. Lower and upper limit of quantification of the drug assay was 1.6 µg/mL and 50 µg/mL, respectively. Cutoff associated with ATA status was 1.7 U/mL. Serum albumin and C-reactive protein (CRP) were determined from frozen serum using immunochemistry centrally at Prometheus Laboratories. Fecal calprotectin (FC) was determined using immunoassays at each of the sites, where a cut off below 100 µg/g consistent was consistent with endoscopic remission [18].

The population PK parameters were estimated from the first cohort[13] (model building cohort). These estimates were used as Bayesian priors to calculate the post-hoc individual CL estimates in all specimens available from the three other cohorts (independent validation cohorts). Details of these methods are provided in the Supplementary materials.

The outcomes consisted of CRP based clinical remission status (clinical remission in the absence of systemic inflammation), corresponding to CRP levels below 3 mg/L with the Crohn's Disease Activity Index < 150 points, determined at each study visit, and sustained CRP-based clinical remission

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throughout maintenance (corresponding to CRP based clinical remission status achieved at all evaluable time points for a given patient). Endoscopic remission (ER) corresponded to the Simple Endoscopic Score for CD (SES-CD<3 points) available during treatment in Cohorts 1, 3 and 4 with each ER assessment having a paired specimen collected on the day of procedure.

Statistical analysis consisted of univariate and multivariate logistic regression with odds ratio (OR, with 95% confidence interval and pseudo R^2 calculated and reflective of the proportion of variance explained). Mann-Whitney test and receiver operating characteristics (ROC) curves with calculation of area under the curve (AUC) were also used in this analysis (conducted using R,version 4.0.3). Results were expressed as median with interquartile ranges (IQR), as appropriate. The impact of PK parameters (measured ADA concentrations and CL estimates) on outcomes was estimated using categorical data analysis using logistic regression (odds ratio) using non-linear mixed effects modeling via Monolix (Lixoft, 2021R2). For each model tested the change in objective function value (ΔOFV, as assessed using -2 log likelihood [-2LL] by importance sampling) calculated with 5% level of significance to assess the value of the additional predictor where lower -2LL indicated better fit and performances in association with outcome.

RESULTS

The patient characteristics (n=237, with a total of 818 study visits and 211 endoscopic assessments during maintenance) are presented in **Table I**. A total of 219 patients had more than one PK measurement. The population PK parameter estimates are presented in **Table S1** and goodness of fit plots for the population model are provided in the supplementary materials (**Figures S1-S2**). The Population CL and V determined from the model building Cohort 1 were 0.317 L/day and 8.9 L respectively. These estimates are consistent with those reported in the literature (supplementary **Table**

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S2).These population estimates from model building cohort were used as Bayesian priors to calculate individual CL estimates in all three remaining cohorts. Correlation between CL and measured concentrations is highlighted in **Supplementary Figure S3**. A total of 41 patients (17%) had received prior biologics before starting ADA treatment and 33/168 patients (from Cohort 2, 3 and 4) had dose intensification to 40 mg weekly with further increase to 80 mg weekly in 13 of them. Less than half of the patients were in ER (46%). Sustained CRP based clinical remission and FC below 100 ug/g was achieved in 31% and 53%, respectively. Overall, the prevalence of ATA was 10% (81/818) of the specimens. ATA positive status was associated with lower ADA concentrations than ATA negative status (<1.6 µg/mL [IQR: <1.6-<1.6] vs 11.2 µg/mL [IQR: <7.8-<14.8], respectively) (p<0.01) and higher CL (1.264 L/day [IQR: 0.660-1.580] vs 0.263 L/day [IQR: 0.197-0.373], respectively) (p<0.01). ATA status was associated with a 33.8-fold (95%CI: 18.7 - 61.0) higher likelihood to have ADA concentration below 5 µg/mL.

Overall, there was a significantly lower likelihood of sustained CRP based clinical remission, FC below 100 µg/g, and lack of endoscopic remission in the group of PK measurements from patients who received prior biologics (n=700) as compared to those who did not (n=118; OR= 0.35 [95%CI: 0.21-0.59; p<0.01], OR= 0.27 [95%CI: 0.16-0.45; p<0.001], OR= 0.78 [95%CI: 0.42-1.44; p<0.01], respectively). Prior biologic exposure was used as adjusting covariate in multivariate logistic regression and repeated event analysis. There was also higher measured CL during treatment in the group of PK measurements from patients who received prior biologic exposure as compared to PK measurements from biologic naïve patients (median 0.362 L/day [IQR: 0.225-0.673] vs median 0.270 L/day (IQR: 0.199-0.403], respectively) (p<0.01).

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As presented in **Table 2**, lower CL was associated with ER in two of three cohorts tested (all cohorts: 0.247 L/day [IQR: 0.195-0.340 L/day] vs 0.326 L/day [IQR: 0.203-0.730 L/day] in the presence and absence of ER, respectively, p≤0.05). There was a non-significant higher ADA concentration in the presence of ER (median 9.3 µg/mL [IQR:3.8-14.8 µg/mL] vs 11.7 µg/mL IQR: 7.9-14.1 µg/mL] in the presence and absence of ER, respectively, p=0.07), with ADA concentrations significantly associated with ER in cohort 1 (p=0.05). Sustained CRP based clinical remission status and FC below 100µg/g were generally associated with higher ADA concentration and lower CL in all cohort tested (except that concentration was not associated with FC levels in cohort 1). ROC analysis revealed a higher AUC for CL than concentrations for each of the three outcomes tested (**Supplementary Table S3**).

Odds ratio analysis with low (≤5µg/mL), intermediate (>5µg/mL), and high (>10 µg/mL) ADA levels or CL (<0.318 L/day and <0.8 L/day, for low, intermediate and high CL, respectively) for each of the outcomes tested confirmed these findings (**Supplementary Tables S4 through S11**). The proportion of CD who achieved ER by ADA concentration (>5 µg/mL and >10 µg/mL) and CL (<0.8 L/day and <0.318 L/day) is presented in **Figure 1**. The proportions of those who achieved sustained CRP-based clinical remission and FC below 100µg/g are presented in **Figure 2** and **Figure 3**, respectively. Higher concentrations and lower CL yielded better disease control.

Multivariate analysis with ADA concentrations and CL revealed that ER was associated with CL (each unit change in CL: adjusted OR=0.15 95%CI: 0.05-0.45 ; p<0.01) while no significant association was detectable with ADA concentrations (p=0.143; **Table 3**). A total of 14.3% (pseudo R^2=0.143) of the variance in ER could be explained by CL and concentrations. Similar results were observed with sustained CRP-based clinical remission and FC below 100µg/g outcome measures with no significance of

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concentrations after adjusting for CL, while prior biologic exposure remained associated with outcome. Univariate analysis within each of the 4 cohorts, with low, intermediate, and high ADA concentration and CL with the three outcomes is presented in **Supplementary Table S12 to S14**.

Logistic regression analysis revealed that longer time under treatment associated with lower probability of ER (estimate=-0.025 [RSE 34%]) and there was also lower probability of achieving FC levels below 100µg/g status (estimate=-0.017 [RSE 45%]. The probability of CRP based clinical remission was not significantly associated with disease worsening (estimate = -2.14 [RSE 27%] and -2.21 (RSE 28%), respectively) (**Table 4**). For both FC levels below 100µg/g and CRP based clinical remission status, prior exposure to biologics significantly associated with lower probability to achieve the desired outcome (estimate = -0.003 [RSE 193%]). Higher concentrations were not associated with higher probability of ER (estimate: +0.039 [RSE 78%) while higher CL (estimate -1.99; [RSE=34%] resulted in lower probability of ER. Lower -2LL were achieved with CL than with concentrations with themselves as regressors (-2LL=271.6 vs 282.2, ΔOFV = -10.6; p<0.01) and these findings remained significant after adding time on treatment as second regressor (-2LL=266.7 vs 277.9, ΔOFV =-11.2; p<0.01). Repeated event analysis with CRP-based remission and FC below 100µg/g revealed that higher concentration and lower CL also associated better probability of having these improved outcomes with better performances of CL vs concentrations (-2LL =746.4 vs 782.6, ΔOFV =-36.2; p<0.01 and 476.5 vs 484.9, ΔOFV =-8.4; p<0,01, respectively. The probability of having the therapeutic outcome calculated from those estimates and stratified by prior biologic exposure are summarized in **Figure 4**. Supplementary Figure S4 presented Visual predictive checks.

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In the three cohorts where dose intensification was allowed, dose intensification was not associated with improved outcome (estimate = -0.04 [RSE: 79%], -0.019 [RSE 44%], and -0.010 [RSE 71%] for ER, CRP based clinical remission and FC levels below 100 µg/g) (**Supplementary Table S15).** In the group of patients with paired PK measurement available, there was significant increase in concentrations upon dose intensification to 40 mg weekly (median 18.3 µg/mL [IQR: 14.2-24.5] vs 10.1 µg/mL [IQR: 5.9-13.7], n=33) (Wilcoxon paired test, p<0.01), the CL remaining elevated (median 0.308 L/day [IQR: 0.230-0.393] vs 0.266 L/day [IQR: 0.201-0.434], n=33) (Wilcoxon paired test, p<0.01) upon dose intensification. Similar results were observed in the group of patients who had further dose intensification to 80 mg weekly (data not shown).

DISCUSSION

ADA drug CL is a recognised PK parameter, reflective of the volume of serum from which the drug has been removed as a function of time (expressed as L/day). It is well established that immunization to ADA and other monoclonal antibodies results in high CL[11] with the consequence of having lesser ADA available, a condition that worsens with inflammation[19]; and is potentially preventable with the use of concomitant immunosuppressant[20] or proactive achievement of exposure that promotes tolerance to the antigen fraction itself (CDR3 of the fragment antigen binding domain of the IgG1)[21, 22]. In this report we describe the associations and performance characteristics of CL as well as ADA concentration in four cohorts of patients starting ADA treatment. All outcomes were collected during maintenance treatment. Endoscopic assessment (SES-CD score) was routinely performed with data available longitudinally.

Overall, our data support the expert opinion that ADA concentrations have value[1], based on their association with outcomes in patients with CD. However, the portion of the clinical picture explained was modest (with pseudo R^2 consistently below 20% for each of the three outcomes tested) ADA

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concentrations above 5 and 10 µg/mL yield several fold higher likelihood of better outcome than levels < 5 µg/mL. The measurement of concentration is therefore likely to assist with clinical decision making with respect to treatment and monitoring to achieve exposure commensurate with disease control.

While highly correlated with one another, CL performed better than drug concentration alone with respect to outcome. It may be that CL is a better reflection of inflammatory burden than concentration. Difference in interdose intervals (7 vs 14 days) might also have contributed to differences in the PK outcome measured. Lower CL and better retention of ADA yielded better endoscopic outcome (median: 0.246 L/day vs 0.320 L/day vs, in the presence and absence of ER, respectively; **Table 2**), sustained clinical disease control and lower inflammation. Also, for each of the outcomes tested, multivariate analysis of CL and concentration as independent predictors revealed higher likelihood of ER, sustained CRP based remission and FC levels below 100 µg/g were all a function of lower CL, with contribution of concentrations after adjusting with CL. Nonlinear mixed effect modelling of the longitudinal data also confirmed these findings with lower -2 log likelihood for CL than concentration for each of the outcome tested. We also noticed that prior biologic exposure significantly associated with lesser disease control achieved (sustained CRP based clinical remission and lower FC, but not endoscopic remission) with higher CL measured using treatment in this group as compared to the group of patients who were naïve to biologics. We speculate that the higher CL in that group of patients who received prior biologic might have been one of the reasons for treatment discontinuation. In this study, a limited number of patients had dose intensification, and we observed that while the concentration increased, it did not yield a higher probability of having disease control achieved. This might suggest that these patients were refractory to ADA, and this contention might be indirectly supported by the observation that CL remained elevated upon dose intensification.

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Loss of response to anti-TNFα therapy remains a challenging clinical problem. Immunogenicity due to the formation of antibodies against the TNFα antagonists is a major cause for this and can be, at least in part, prevented by the adequate dosing of these drugs. Our data provide important insights into the pharmacokinetics of anti-TNFα therapy in patients with Crohn's disease. The calculation of clearance will allow for improved decision making with respect to dosing of anti-TNF drugs and may help prevent loss of response. More broadly clearance calculation may assist in identifying patients who will respond better to monoclonal antibodies (particularly anti-TNFα therapy) and may be an important step towards the personalization of drug therapy in an era of rapidly expanding therapies and the increasing use of small molecules in inflammatory bowel disease.

In this work we cannot address the causality of the association with outcomes, but it is tempting to suggest that two key characteristics converge toward lower CL. Firstly absence of immunization and efficient PK (reflected by adequate albumin levels) and secondly achievement of sufficient supply of anti-cytokine drug as a reservoir available for the neutralization of inflammatory burden present. We acknowledge that this analysis is retrospective and that these findings could be significant by chance, or due to type one error, and confirmation will be required. However, these data suggest that CL is PK predictive factor in its own right, potentially outperforming drug concentration.

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Contributions: study design and data collection: all authors; writing of first draft: TD; analysis of PK data: TD, CP; data interpretation: all authors; approval of final manuscript: all authors.

Conflict of interest: TD and AE are employed by Prometheus Laboratories. CP is a consultant for Prometheus Laboratories.

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REFERENCES

1. Cheifetz AS, Abreu MT, Afif W, et al. A Comprehensive Literature Review and Expert Consensus Statement on Therapeutic Drug Monitoring of Biologics in Inflammatory Bowel Disease. Am J Gastroenterol 2021.
2. Vande Casteele N, Herfarth H, Katz J, et al. American Gastroenterological Association Institute Technical Review on the Role of Therapeutic Drug Monitoring in the Management of Inflammatory Bowel Diseases. Gastroenterology 2017;153:835-857 e6.
3. Feuerstein JD, Nguyen GC, Kupfer SS, et al. American Gastroenterological Association Institute Guideline on Therapeutic Drug Monitoring in Inflammatory Bowel Disease. Gastroenterology 2017;153:827-834.
4. Assa A, Matar M, Turner D, et al. Proactive Monitoring of Adalimumab Trough Concentration Associated With Increased Clinical Remission in Children With Crohn's Disease Compared With Reactive Monitoring. Gastroenterology 2019;157:985-996 e2.
5. Papamichael K, Juncadella A, Wong D, et al. Proactive Therapeutic Drug Monitoring of Adalimumab Is Associated With Better Long-term Outcomes Compared With Standard of Care in Patients With Inflammatory Bowel Disease. J Crohns Colitis 2019;13:976-981.
6. Strik AS, Lowenberg M, Mould DR, et al. Efficacy of dashboard driven dosing of infliximab in inflammatory bowel disease patients; a randomized controlled trial. Scand J Gastroenterol 2021;56:145-154.
7. Landemaine A, Petitcollin A, Brochard C, et al. Cumulative Exposure to Infliximab, But Not Trough Concentrations, Correlates With Rate of Infection. Clin Gastroenterol Hepatol 2021;19:288-295 e4.
8. Primas C, Reinisch W, Panetta JC, et al. Model Informed Precision Dosing Tool Forecasts Trough Infliximab and Associates with Disease Status and Tumor Necrosis Factor-Alpha Levels of Inflammatory Bowel Diseases. J Clin Med 2022;11.
9. Dubinsky MC, Phan BL, Singh N, et al. Pharmacokinetic Dashboard-Recommended Dosing Is Different than Standard of Care Dosing in Infliximab-Treated Pediatric IBD Patients. AAPS J 2017;19:215-222.
10. Eser A, Primas C, Reinisch S, et al. Prediction of Individual Serum Infliximab Concentrations in Inflammatory Bowel Disease by a Bayesian Dashboard System. J Clin Pharmacol 2018;58:790-802.
11. Berends SE, Strik AS, Van Selm JC, et al. Explaining Interpatient Variability in Adalimumab Pharmacokinetics in Patients With Crohn's Disease. Ther Drug Monit 2018;40:202-211.
12. Kantasiripitak W, Wang Z, Spriet I, et al. Recent advances in clearance monitoring of monoclonal antibodies in patients with inflammatory bowel diseases. Expert Rev Clin Pharmacol 2021;14:1455-1466.
13. Rizzello F, Gionchetti P, Spisni E, et al. Dietary Habits and Nutrient Deficiencies in a Cohort of European Crohn's Disease Adult Patients. Int J Mol Sci 2023;24.
14. Chaparro M, Guerra I, Iborra M, et al. Usefulness of monitoring antitumor necrosis factor serum levels during the induction phase in patients with Crohn's disease. Eur J Gastroenterol Hepatol 2020;32:588-596.
15. De Cruz P, Kamm MA, Hamilton AL, et al. Crohn's disease management after intestinal resection: a randomised trial. Lancet 2015;385:1406-17.
16. Schulberg JD, Wright EK, Holt BA, et al. Intensive drug therapy versus standard drug therapy for symptomatic intestinal Crohn's disease strictures (STRIDENT): an open-label, single-centre, randomised controlled trial. Lancet Gastroenterol Hepatol 2022;7:318-331.

17. Wang SL, Hauenstein S, Ohrmund L, et al. Monitoring of adalimumab and antibodies-to-adalimumab levels in patient serum by the homogeneous mobility shift assay. J Pharm Biomed Anal 2013;78-79:39-44.
18. Wright EK, Kamm MA, De Cruz P, et al. Measurement of fecal calprotectin improves monitoring and detection of recurrence of Crohn's disease after surgery. Gastroenterology 2015;148:938-947 e1.
19. Colman RJ, Xiong Y, Mizuno T, et al. Antibodies-to-infliximab accelerate clearance while dose intensification reverses immunogenicity and recaptures clinical response in paediatric Crohn's disease. Aliment Pharmacol Ther 2022;55:593-603.
20. Ungaro RC, Colombel JF, Dubinsky MC, et al. Impact of Thiopurine Exposure on Immunogenicity to Infliximab Is Negligible in the Setting of Elevated Infliximab Concentrations. Inflamm Bowel Dis 2022;28:649-651.
21. Spencer EA, Stachelski J, Dervieux T, et al. Failure to Achieve Target Drug Concentrations During Induction and Not HLA-DQA1 *05 Carriage Is Associated With Antidrug Antibody Formation in Patients With Inflammatory Bowel Disease. Gastroenterology 2022;162:1746-1748 e3.
22. Sazonovs A, Kennedy NA, Moutsianas L, et al. HLA-DQA1*05 Carriage Associated With Development of Anti-Drug Antibodies to Infliximab and Adalimumab in Patients With Crohn's Disease. Gastroenterology 2020;158:189-199.

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TABLES

Table 1: Patient Characteristics

	Cohort 1 (BOLOGNA) Italy	Cohort 2 (PredictCrohn) Spain	Cohort 3 (POCER) Australia	Cohort 4 (STRIDENT) Australia	All cohorts combined
Number of patients	53	60	50	74	237
Gender (female)	36% (19/53)	43% (26/60)	46% (23/50)	51% (38/74)	45% (106/237)
Number of patients with > 1 PK measure	51	58	41	69	219
Age:	34 (26-44)	40 (30-49)	40 (29-47)	44 (20-51)	40 (29-48)
A1 below 16 years old	3/52	2/60	3/48	8/74	18/234
A2 17 to 40 years old	44/52	53/60	44/48	45/74	178/234
A3 above 40 years old	5/52	5/60	5/48	21/74	38/234
Location:					
L1 ileal	2/52	29/60	5/48	53/72	107/229
L2 colonic	13/52	8/60	36/48	2/72	27/229
L3 ileocolonic	37/52	20/60	7/48	17/72	95/229
L4 modifier	0/52	3/60	0/48	2/72	7/229
Behavior					
B1 non-stricturing, non-penetrating	51/52	31/60	4/48	0/74	86/234
B2 stricturing	1/52	13/60	9/48	74/74	97/234
B3 penetrating	0/52	1660	35/48	0/74	51/234
p perianal disease modifier	0/52	10/60	10/48	0/74	20/234
Prior biologic usage	32% (17/53)	0% (0/60)	30% (15/50)	12% (9/74)	17% (41/237)
Number of cycles with PK estimates	182	313	115	208	818
Follow-up time (weeks) from start	54 (52-56)	46 (30-54)	54 (54-54)	52 (51-53)	53 (49-54)
Dose per two weeks	40 (40-40)	40 (40-40)	40 (40-40)	40 (40-40)	40 (40-40)
Weight (Kg)	70 (62-70)	72 (60-80)	75 (62-70)	78 (66-87)	73 (62-82)
Albumin (g/dL)	4.0 (3.8-4.3)	4.0 (3.6-4.5)	4.1 (3.8-4.3)	3.9 (3.6-4.2)	4.0 (3.7-4.3)
ADA Concentration (µg/mL)	10.0 (5.2-12.8)	10.0 (7.1-14.0)	9.1 (4.7-14.1)	13.2 (8.2-17.7)	10.5 (6.8-14.4)
ADA concentration >5 µg/mL	76% (139/182)	86% (268/313)	72% (83/115)	88% (184/208)	82% (674/818)
ADA >10 µg/mL	49% (90/182)	50% (156/313)	43% (50/115)	65% (135/208)	53% (431/818)
ATA positive (>1.7 U/mL)	15% (28/182)	6% (18/313)	12% (14/115)	10% (21/208)	10% (81/818)
Clearance (L/day)	0.280 (0.220-0.539)	0.279 (0.196-0.420)	0.301 (0.204-0.520)	0.242 (0.174-0.377)	0.273 (0.194-0.434)
SES-CD below 3 points	57% (51/90)	--	41% (27/66)	36% (20/55)	46% (98/211)
CRP based clinical remission	47% (84/178)	51% (120/236)	54% (43/80)	50% (104/207)	50% (351/701)
Sustained CRP based clinical remission[a]	26% (14/53)	22% (13/60)	41% (13/32)	38% (27/74)	31% (67/219)
Fecal calprotectin below 100 µg/g	38% (45/119)	--	46% (39/85)	66% (134/204)	53% (218/408)
Dose intensification during treatment	Not applicable[b]	9% (5/58)	7% (2/41)	38% (26/69)	14% (33/168)

Results are expressed as median (IQR) as appropriate; [a] based on 2 or more CRP based clinical remission assessments; [b]Dose intensification was not allowed in study 1; [c]one patient from cohort 3 did not have standard dosing (40 mg every other week) PK measurement.

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Table 2: PK variables and Outcomes

Median ADA concentration and CL are provided (with IQR) for each outcome variable and cohort with p value. Top estimate corresponds to the median and IQR in the absence of the outcome. Bottom estimate corresponds to the median and IQR in the presence of the outcome.

	PK estimate	SES-CD remission (<3 points)	Sustained CRP based clinical remission	FC below 100 µg/g
Cohort 1	Concentration (µg/mL)	6.7 (<1.6-12.8) 11.0 (8.3-12.8) p=0.05	8.5 (3.6-12.5) 12.0 (10.1-14.0) p<0.01	8.5 (3.0-13.4) 10.8 (7.4-12.2) p=0.16
	CL (L/day)	0.490 (0.211-1.240) 0.247 (0.216-0.324) p=0.03	0.325 (0.226-0.699) 0.239 (0.194-0.277) p<0.01	0.339 (0.207-0.829) 0.264 (0.235-0.380) p=0.19
Cohort 2	Concentration (µg/mL)	Not available	9.5 (6.4-13.4) 12.3 (9.4-15.7) p<0.01	Not available
	CL (L/day)	Not available	0.290 (0.206-0.442) 0.231 (0.164-0.303) p<0.01	Not available
Cohort 3	Concentration (µg/mL)	8.6 (4.2-12.0) 10.3 (6.5-14.2) p=0.57	7.1 (3.1-12.1) 10.6 (8.5-14.9) p<0.01	7.5 (3.9-9.9) 11.5 (6.2-15.0) p=0.01
	CL (L/day)	0.312 (0.241-0.491) 0.256 (0.184-0.435) p=0.19	0.370 (0.223-0.761) 0.255 (0.173-0.319) p<0.01	0.348 (0.265-0.610) 0.252 (0.175-0.470) p=0.03
Cohort 4	Concentration (µg/mL)	13.2 (7.5-17.5) 14.8 (11.2-23.3) p=0.20	10.8 (6.0-15.5) 14.5 (12.1-20.5) p<0.01	9.9 (5.8-15.4) 13.8 (10.0-18.6) p<0.01
	CL (L/day)	0.320 (0.191-0.678) 0.213 (0.171-0.289) p=0.07	0.314 (0.205-0.524) 0.187 (0.143-0.235) p<0.01	0.361 (0.248-0.619) 0.197 (0.154-0.279) p<0.01
All Cohorts	Concentration (µg/mL)	9.3 (3.8-14.8) 11.7 (7.9-14.1) p=0.07	9.4 (5.4-13.6) 12.6 (9.8-15.8) p<0.01	8.6 (4.1-13.5) 12.3 (8.6-15.8) p<0.01
	CL (L/day)	0.326 (0.203-0.730) 0.247 (0.195-0.340) p<0.01	0.311 (0.213-0.552) 0.220 (0.168-0.281) p<0.01	0.353 (0.238-0.670) 0.230 (0.172-0.331) p<0.01

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Table 3: Multivariate logistic regression for outcomes with ADA concentration and CL adjusting for prior biologic exposure

Results are presented for all 4 cohorts combined. **Table S12-S14** provide results by cohort.

	PK estimate	Adjusted OR per unit change	P value	Pseudo R^2
ER	Concentration (µg/mL)	0.96 (0.91,1.01)	0.14	
	CL (L/day)	0.15 (0.05,0.45)	<0.01	0.143
	Prior biologics	0.81 (0.45,1.64))	0.52	
Sustained CRP based remission	Concentration (µg/mL)	0.98 (0.95,1.02)	0.24	
	CL (L/day)	0.02 (0,0.09)	<0.01	0.404
	Prior biologics	0.42 (0.24,0.73)	<0.01	
FC below 100µg/g	Concentration (µg/mL)	1.01 (0.98,1.04)	0.49	
	CL (L/day)	0.25 (0.12,0.54)	<0.01	0.179
	Prior biologics	0.30 (0.18,0.51)	<0.01	

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Table 4: Repeated event analysis for outcome measures in association with PK parameters and prior biologics with all cohorts combined.

Estimates are provided with relative standard error (<50% indicates significant association).

	Time alone	Conc. alone	CL alone	Time and conc.	Time and CL
Endoscopic remission					
Number of individuals	155	155	155	155	155
Number of observations	211	211	211	211	211
Population	1.05 (43.9%)	-0.72 (60.0%)	+0.66 (46%)	+0.61 (101%)	+2.01 (25%)
Prior biologics	-0.42 (134%)	-0.34 (178%)	-0.18 (294%)	-0.32 (179%)	-0.21 (272%)
Time regressor (weeks)	-0.025 (34%)	Not applicable	Not applicable	-0.024 (41%)	-0.025 (35%)
PK regressor	Not applicable	+0.039 (77.6%)	-1.99 (34.2%)	+0.035 (88)	-2.25 (27%)
-2LL	279.6	282.2	271.6	277.9	266.7
CRP based clinical remission					
Number of individuals	237	237	237	237	237
Number of observations	701	701	701	701	701
Population	+0.53 (62%)	-0.789 (44.3%)	+1.92 (15%)	-0.63 (55%)	1.67 (19.9%)
Prior biologics	-2.14 (27%)	-1.86 (30%)	-1.52 (34%)	-1.89 (30%)	-1.85 (30%)
Time regressor (weeks)	-0.0003 (193%)	Not applicable	Not applicable	-0.003 (183%)	+0.005 (113%)
PK regressor	Not applicable	+0.103 (23%)	-4.12 (13%)	+0.089 (23.2%)	-3.97 (16%)
-2LL	803.7	782.7	746.4	782.6	744.5
FC below 100µg/g					
Number of individuals	164	164	164	164	164
Number of observations	408	408	408	408	408
Population	+1.36 (29%)	+0.16 (203%)	+1.36 (23%)	+0.88 (43%)	+1.77 (19%)
Prior biologics	-2.21 (28%)	-2.01 (28%)	-1.81 (29%)	-2.08 (29%)	-1.86 (29%)
Time regressor (weeks)	-0.017 (45%)	Not applicable	Not applicable	-0.017 (43%)	-0.011 (63%)
PK regressor	Not applicable	+0.039 (53%)	-1.9 (28%)	+0.035 (56%)	-1.84 (26%)
-2LL	504.6	484.9	476.5	484.3	476.5

†<50% is significant regressor (p<0.05 different from zero) using Logit model; NA: -2LL: -2 log likelihood.

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FIGURES

Figure 1: **ADA concentration and CL in association with ER**

ER was defined as SES-CD score below 3 points.

Top panel: Overall, ADA concentration >5 µg/mL, and >10 µg/mL associated with 2.6-fold (95%CI: 1.3-5.2) (p=0.007; pseudo R^2=0.047) and 2.1-fold (95%CI: 1.2-3.7) (p=0.008; pseudo R^2=0.040) higher likelihood of ER respectively (**Table S2**).

Bottom panel: Overall, CL <0.318 L/day, and <0.8 L/day associated with 2.5-fold (95%CI: 1.4-4.4) (p=0.002; pseudo R^2=0.058) and 3.0-fold (95%CI: 1.3-6.7) (p=0.008; pseudo R^2=0.047) higher likelihood of ER, respectively (**Table S3**).

Figure 2 ADA PK parameter and sustained CRP based remission.

Top panel: Overall, ADA concentration >5 µg/mL, and >10 µg/mL associated with 9.7-fold (95%CI: 2.3-41.7) (p<0.001; pseudo R^2=0.181) and 4.5-fold (95%CI: 2.3-8.9) (p<0.001; pseudo R^2=0.146) higher likelihood of sustained CRP based clinical remission, respectively (**Table S4**).

Bottom panel: Overall, CL <0.318 L/day, and <0.8 L/day associated with 6.5-fold (95%CI: 2.9-14.4) (p<0.001; pseudo R^2=0.197) and 10.6-fold (95%CI: 1.4-80.4) (p<0.001; pseudo R^2=0.133) higher likelihood of sustained CRP based clinical remission (**Table S5**).

Figure 3 ADA concentration and CL in association with FC below 100µg/g

Top panel: Overall, ADA concentration >5 µg/mL, and >10 µg/mL associated with 3.3-fold (95%CI: 2.0-5.7) (p<0.001; pseudo R^2=0.064) and 3.2-fold (95%CI: 2.2-4.9) (p<0.001; pseudo R^2=0.094) higher likelihood of FC below 100µg/g, respectively (**Table S6**).

Bottom panel: Overall, CL <0.318 L/day, and <0.318 L/day associated with 3.2-fold (95%CI: 2.1-4.9) (p<0.001; pseudo R^2=0.093) and 4.1-fold (95%CI: 2.1-7.9) (p<0.001; pseudo R^2=0.062) higher likelihood of FC below 100µg/g, respectively (**Table S7**).

Figure 4 Probability of achieving each of the three outcomes by CL or concentration stratified by prior biologic exposure

All estimates are provided in **Table 4** with the PK parameter as the regressor. *Panel A*: probability of SESC-CD below 3 points and CL; *Panel B*: probability of CRP based clinical remission and CL; *Panel C*: probability of FC below 100 ug/g and CL; *Panel D*: probability of SESC-CD below 3 points and concentration; *Panel E*: probability of CRP based clinical remission and concentration; *Panel F*: probability of FC below 100 ug/g and concentration.

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Figure 1

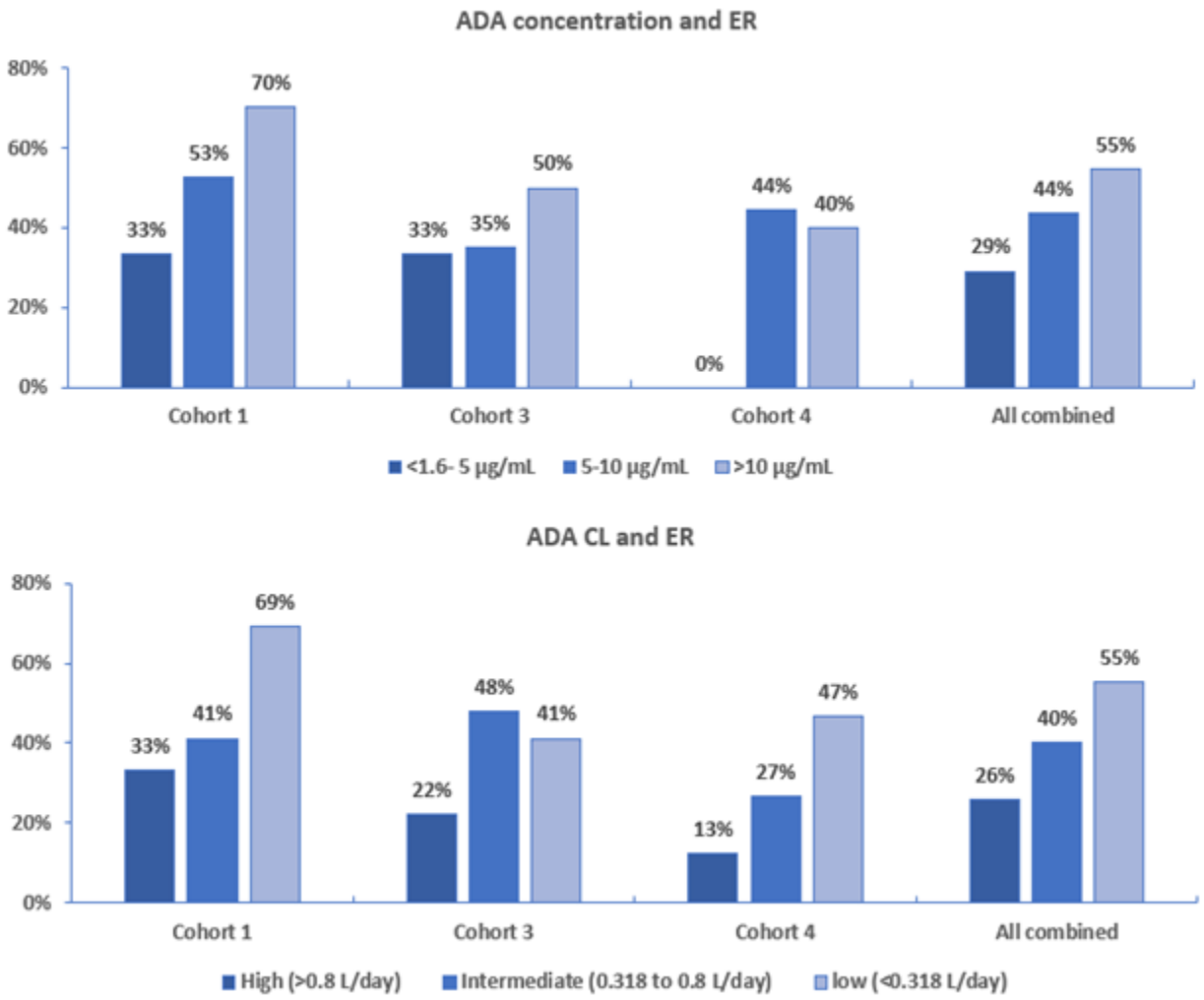

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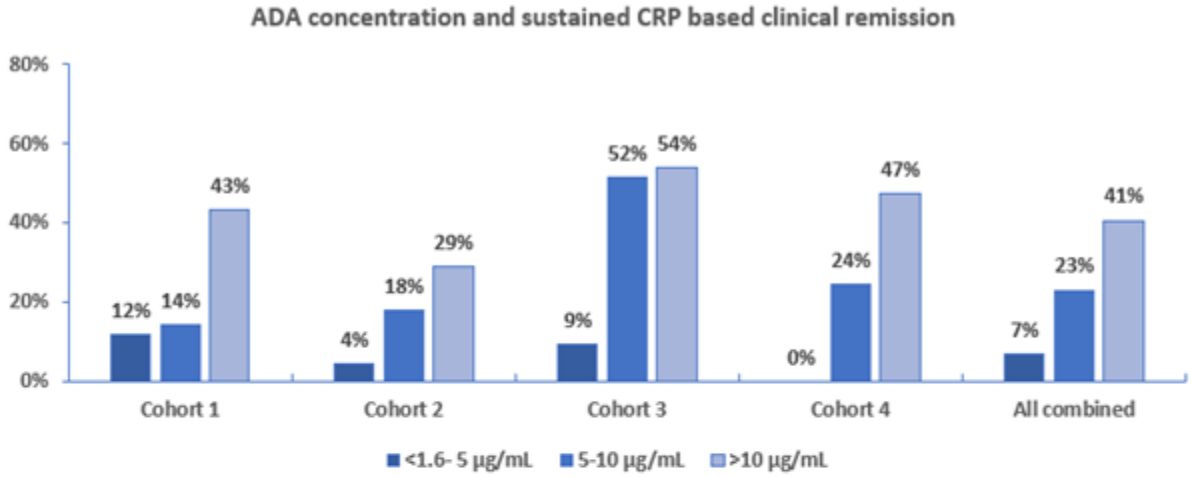
Figure 2



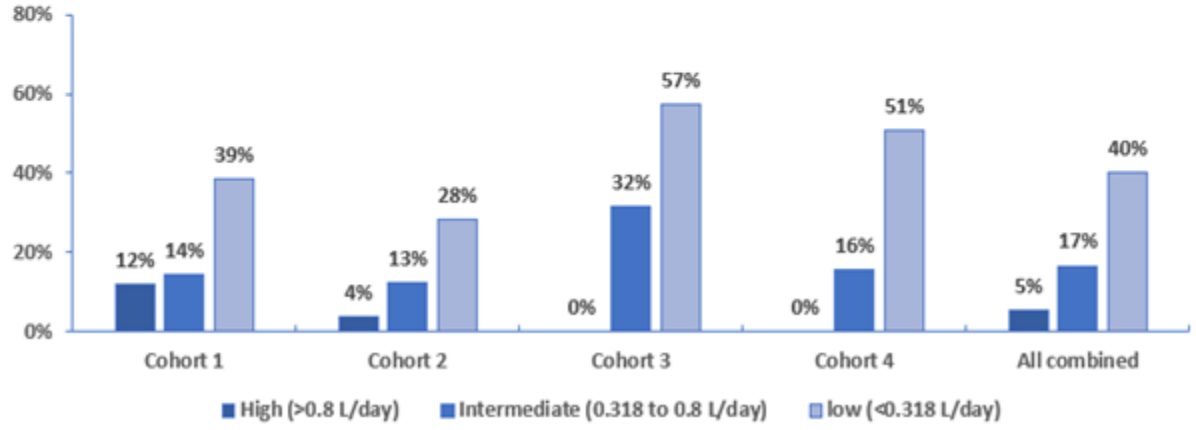


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Figure 3

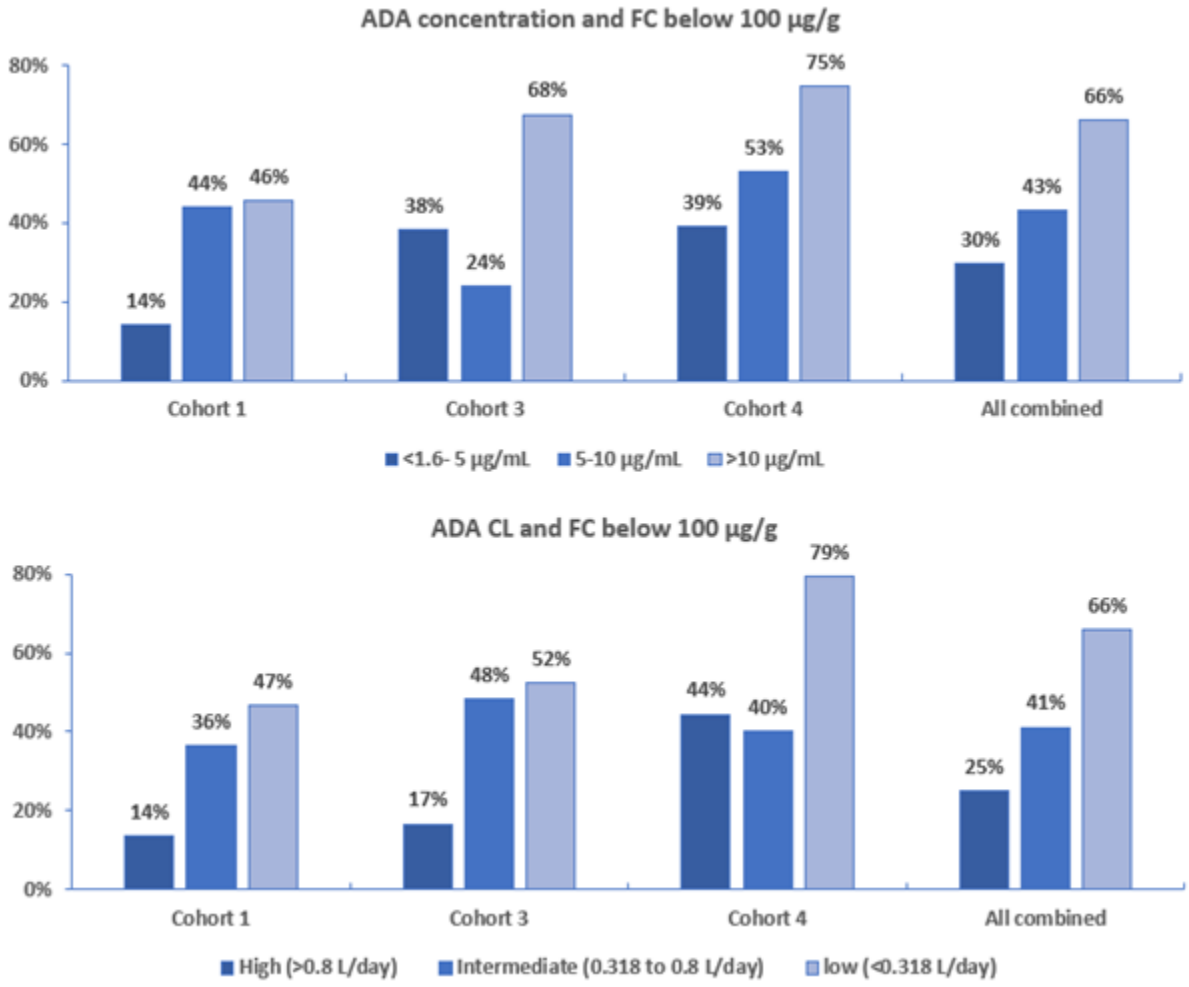

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Figure 4