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The Efficacy of Therapeutic Drug Monitoring of Rheumatoid Arthritis Patients on Infliximab

THESIS

submitted in partial satisfaction of the requirements
for the degree of

MASTER OF SCIENCE

in Biomedical and Translational Science

by

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LIST OF ABBREVIATIONS

TDM	=	therapeutic drug monitoring
TNF-α	=	tumor necrosis factor - alpha
CRP	=	C-reactive protein
RA	=	rheumatoid arthritis
IBD	=	inflammatory bowel disease
UC	=	ulcerative Colitis
CD	=	crohn’s disease
PA	=	psoriatic Arthritis
Abs	=	antibody
ATI	=	antibodies to infliximab
LOR	=	loss of response
PK	=	pharmacokinetics
PD	=	pharmacodynamic
TC	=	trough concentration
HBI	=	Harvey-Bradshaw Index
IQR	=	interquartile range
TAXIT	=	Trough Concentration Adapted Infliximab Treatment
ACR	=	American College of Rheumatology
DAS	=	Disease Activity Score
DMARD	=	disease-modifying antirheumatic drug;
MTX	=	methotrexate;
TNFi	=	tumor necrosis factor inhibitor;

TNFi biologics =	Adalimumab, certolizumab pegol, etanercept, golimumab, or infliximab.
Early RA =	RA patient has had symptoms of disease for 6 months or less
Established RA =	RA patient has had symptoms of disease for 6 months more

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ABSTRACT OF THE THESIS

The Efficacy of Therapeutic Drug Monitoring of
Rheumatoid Arthritis Patients on Infliximab

By

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Master of Science in Biomedical and Translational Science

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Professor Sherrie Kaplan, Chair

Background: Therapeutic Drug Monitoring is part of standard patient care and utilizes pharmacokinetic tests to measure trough drug levels in patients to guide patient therapy. Studies have suggested improved patient outcomes can be achieved by maintaining a target therapeutic trough concentration of drug through individualized patient dosage. However, the association between drug concentration in serum and patient outcomes is still poorly understood.

Objectives: Evaluate the association between serum drug levels, disease activity, and patient characteristics. Identify if an optimal therapeutic drug level in serum is associated with lower disease activity. Evaluate whether elevated drug levels in serum increase immune response against drug.

Design, setting, and participants: The study is a retrospective cohort study. Eligible study participants were identified through the arthritis internet registry from 2011 through 2016. The study included a total of 57 patients, 47 female and 10 males, 30 to 81 years of age, clinically diagnosed with rheumatoid arthritis and being treated with infliximab drug.

Methods: The study is a retrospective cohort study. Patient characteristics and disease outcomes were compared using independent sample t-tests for continuous variables and x^2 tests for

categorical variables. Regression analysis was conducted to establish the correlation between disease activity and drug level in serum.

Results: Therapeutic drug monitoring (TDM) guidelines for Infliximab trough levels for maintenance therapy did not show association with patient outcomes. Current Therapeutic drug monitoring (TDM) guidelines individualizing patient therapy show no difference in clinical or biological remission between patients maintaining recommended trough guidelines and those who do not. A significant association was observed between drug trough levels above 5 mcg/mL and anti-drug antibody levels. The results suggest that patients maintaining drug levels above 5 mcg/mL are less likely to develop anti-drug antibodies ($p=0.020$).

Discussion: TDM may benefit in individualizing patient dosage to maintain target drug levels to reduce immune response against anti-drug antibodies. However, a significant association between disease activity and trough drug levels was not observed.

CHAPTER 1:
INTRODUCTION

Rheumatoid arthritis affects 1% of the world population. In the past 25 years, the disease-modifying anti-rheumatic drug (DMARD) known as Methotrexate was the standard of care in the treatment of adult rheumatoid arthritis. Recently, the development of targeted biological therapies that block tumor necrosis factor alpha (TNFα), have flooded the market and replaced methotrexate as the standard treatment for rheumatoid arthritis as well as many other inflammatory diseases.

TNFi have been proven to be effective in the treatment of inflammatory diseases including rheumatoid arthritis (RA), inflammatory bowel diseases (IBD), Crohn's disease (CD) and ulcerative colitis (UC).[1] Currently Therapeutic Drug Monitoring is part of standard patient care and utilizes pharmacokinetic tests to evaluate trough drug levels in serum and guide patient therapy by individualizing drug dosage to maintain the recommended target drug trough levels. Studies have suggested improved patient outcomes can be achieved by maintenance of a target therapeutic drug level. Although an association between therapeutic infliximab trough concentrations and clinical or biochemical remission has not been clearly established.[42]

Additionally, studies suggest that drug levels in serum, in conjunction with anti-drug antibody levels can aid in the treatment of patients with no response to therapy do to immune mediated drug clearance. The American College of Rheumatology (ACR) as well as the American Gastroenterological Association (AGA) have published 'Guidelines on Therapeutic Drug Monitoring of Infliximab'. Nevertheless, 30% of patients show no clinical benefit, while another 50% lose response over time and need to escalate or discontinue anti-TNF therapy within one year of treatment.[22]

The purpose of this thesis is to evaluate therapeutic drug monitoring (TDM) of anti-TNF-alpha therapy as it applies to individualization of dosage and improved patient outcomes. This study will evaluate the recommended target drug trough levels in serum and the association with clinical remission, defined by physician and patient reported disease activity scores, and biological remission defined by rheumatoid arthritis biomarkers of disease activity.

CHAPTER 2
BACKGROUND

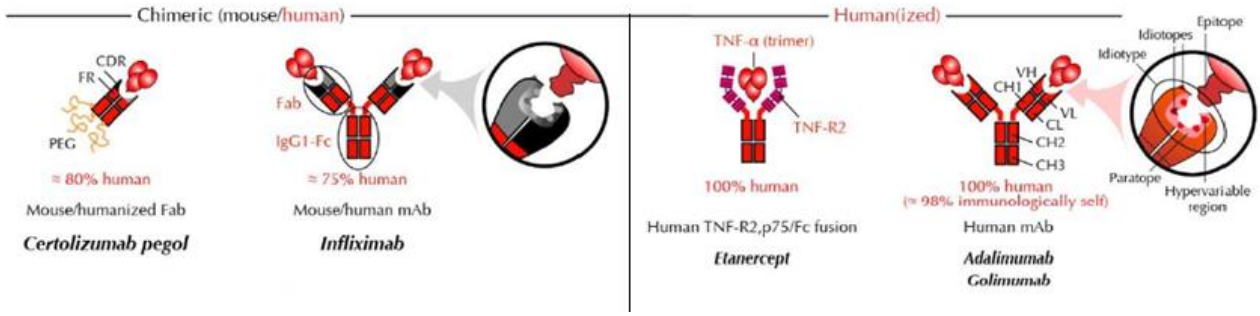
Anti-TNFα Therapy

Infliximab, also known by its trade name as Remicade®, was the first biologic TNF-alpha inhibitor to enter the US market in 1998, it has received FDA approval for the treatment of inflammatory diseases including rheumatoid arthritis, Crohn's disease, ulcerative colitis, psoriasis, psoriatic arthritis, and ankylosing spondylitis. To date, infliximab is approved for use in 88 countries and has been in over 37 clinical trials, evaluating its use in multiple inflammatory diseases.[3]

Infliximab is a chimeric antibody targeting tumor necrosis factor-alpha (TNF-α), figure 1. Infliximab binds both free and membrane bound TNF-α, inhibiting the activation of TNF receptors on cells, reducing inflammation, and stopping disease progression.[29]

The development of biologic drugs such as tumor necrosis factor (TNF) inhibitors has revolutionized the treatment of systemic inflammatory diseases such as rheumatoid arthritis (RA), psoriatic arthritis, ankylosing spondylitis, and inflammatory bowel disease.[25] Although TNFα inhibitors have been studied for over 15 years, little is known about the clinical significance of drug pharmacokinetics and pharmacodynamics.[29]

Figure 1: *Anti- tumor necrosis factor alpha (Anti-TNF) Biopharmaceuticals*



2017 ACR and AGA Guidelines

The American College of Rheumatology as well as the American Gastroenterology Association have released guidelines suggesting maintenance of target therapeutic drug trough concentrations are associated with superior clinical response and improved prognosis. The official AGA recommendation for therapeutic drug monitoring (TDM) in inflammatory bowel disease (IBD) is maintenance of a target infliximab trough level of 5 mcg/mL (Table 1). Whereas the ACR has recommended maintenance of target infliximab trough levels between 2 - 7 mcg/mL.

Table 1: *The American Gastroenterological Association (AGA) Suggested Target Trough Concentrations When Applying Reactive Therapeutic Drug Monitoring in Patients with Active Inflammatory Bowel Disease on Maintenance Therapy With Anti-Tumor Necrosis Factors.*[6]

Drug	Suggested trough concentration, μg/mL	Comments[b]
Infliximab	≥5	Six studies (929 patients) provided data on proportion of patients not in remission above predefined infliximab thresholds (1, 3, 5, 7, and 10 μg/mL). Based on these, proportion of patients not in remission decreased from 25% when using an infliximab threshold of ≥1 μg/mL, to 15% with an infliximab trough concentration of ≥3 μg/mL, to approximately 4% with an infliximab trough concentration of ≥7 μg/mL or ≥10 μg/mL

ACCENT Trial

In 2002, the ACCENT I trial was published, a randomized controlled trial to assess the benefit of maintenance infliximab therapy in patients with active Crohn's disease who respond to a single infusion of infliximab. 335/573 (58%) patients responded to a single infusion of infliximab within 2 weeks. Of these primary responders, patients were randomly assigned repeat infusions every 8 weeks of placebo (group I), 5mg/kg (group II) or 10 mg/kg (group III) of maintenance therapy until week 46. At week 30, 23/110 (21%) of group I, placebo, patients were in remission, compared with 44/113 (39%) group II, 5mg/kg maintenance therapy, (p=0.003) and 50/112

(45%) group III, 10 mg/kg maintenance therapy, (p=0·0002) patients. It was concluded patients in groups II and III combined were more likely to sustain clinical remission than patients in group I (odds ratio 2.7, 95% CI 1.6–4.6). Over the 54-week trial, the median time to loss of response was 19 weeks (10–45) for group I, 38 weeks (IQR 15 to >54) for groups II and more than 54 weeks (21 to >54) for groups III, (p=0.002 and p=0.0002, respectively). The ACCENT trails demonstrated the efficacy of infliximab therapy in IBD and set the precedence for infliximab treatment in IBD.

Although success of treatment was achieved in some patients, little has been gained in advancing infliximab therapy over that last 15 years. 30% of patients show no clinical benefit, while another 50% lose response over time and need to escalate or discontinue anti-TNF therapy within one year of treatment.[22] Recent evidence suggests that not only pharmacokinetics but also pharmacodynamics of TNF-alpha inhibitors differ significantly between individual patients, resulting in variable clinical outcomes. [30]

Non Responders and Loss of Response

The three causes identified in drug failure include mechanistic failure, non-immune-mediated pharmacokinetic failure, and immune-mediated pharmacokinetic failure. Mechanistic failure occurs when the patient does not respond to the drug despite optimal drug trough concentrations, inflammatory mediators driving the disease process are not inhibited by the drug.[36] Non-immune-mediated pharmacokinetic failure occurs when patients do not adequately respond to therapy in the setting of sub-therapeutic trough concentrations and absence of anti-drug antibodies, this results from rapid drug clearance.[30] Immune-mediated pharmacokinetic failure

occurs in patients who have low trough concentrations of drug and high titers of anti-drug antibodies, resulting from the immune-mediated formation of neutralizing anti-drug antibodies.[36]

Primary Nonresponse

One-third of patients treated with TNF inhibitor do not respond to the induction series and are classified as having primary failure.[42] The mechanisms underlying primary non-response to anti-TNF therapy are not yet clearly understood. [23]

Secondary loss of Response

About half of patients with initial response lose effect during the maintenance phase and experience secondary treatment failure.[7,30] Secondary loss of response can be caused by anti-drug antibody drug clearance or increased non-immunologic clearance of drug.

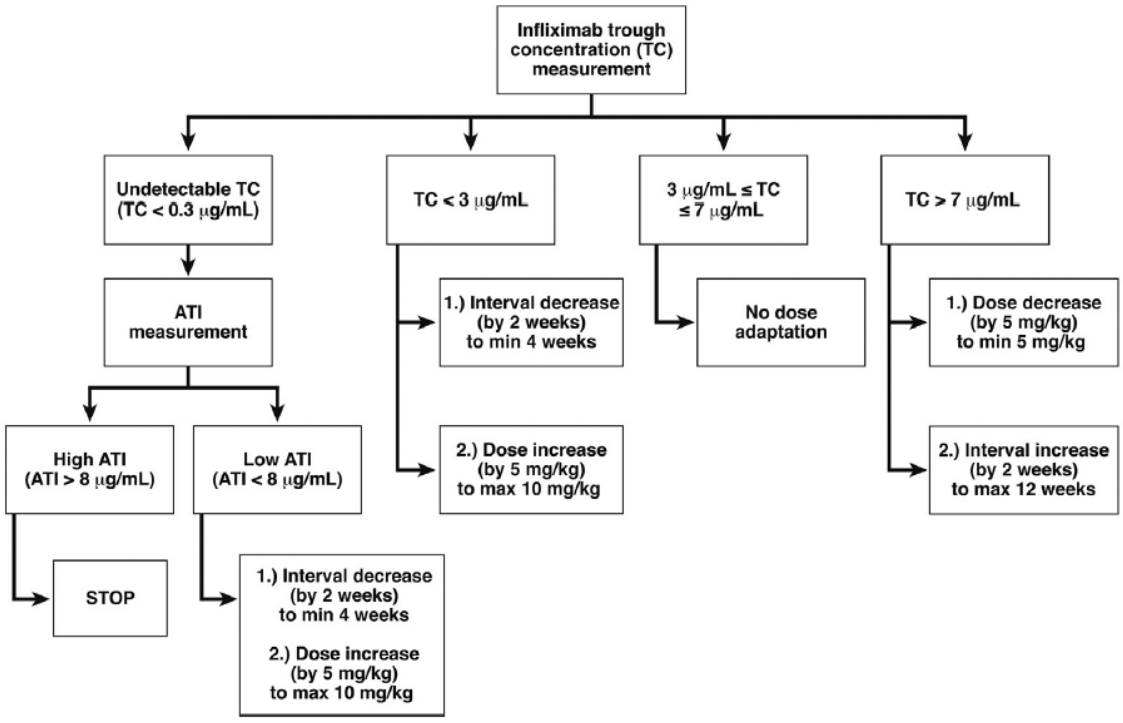
Anti-drug Antibodies

Anti-drug antibodies have been reported in approximately one third of IBD patients on infliximab maintenance therapy. [29] The therapeutic drug is recognized as foreign by the patients' immune system and T cell initiated clearance or direct activation of B-cells formation of anti-drug antibodies change the pharmacokinetics of TNF inhibitors and result in increased drug clearance. [29] Anti-drug antibodies are associated with undetectable or low drug trough levels and with decreased drug efficacy or treatment failure.[29] Furthermore, anti-IFX Abs are associated with acute infusion reactions to IFX.[89–91]

Therapeutic Drug Monitoring

TDM is used in clinical trials and management of patient therapy to determine drug efficacy and dosage, and is mandated for patients undergoing certain immunosuppressant therapy. TDM was introduced to clinical practice in the 1960s with the publication of initial pharmacokinetic studies correlating to patient outcomes. Measuring drug levels and anti-drug antibodies can aid in defining the underlying reasons for loss of response and allow an appropriate medication adjustment. It is used to optimize patient therapy by individualizing drug dosage in patients to maintain a drug concentration within a target therapeutic range thereby increasing the success of patient outcomes.[16] Unfortunately, measuring levels and anti-drug antibodies has several major limitations.[8] In TDM of Infliximab therapy, the relationship between low drug concentrations and patient outcome is poorly described, but some evidence suggests that below-normal drug concentrations may have potential impact on the proportion of patients with treatment failure, relapse and drug resistance.[5–10] However, there are several unresolved issues surrounding TDM of TNF inhibitors. Evidence has not address the optimal time for measuring trough concentrations, it is recommended trough level be drawn as close to the next dose as possible.[6] The lack of standardization creates variability in the data collected and makes evaluating outcomes difficult. Additionally, while the drug trough concentration is consistent across different commercial assays, assays for anti-drug antibodies are not readily comparable with each other.[6] When anti-drug antibodies are present, it is unclear what antibody level is clinically meaningful.[6] Low-titer antibodies may be non-neutralizing, in this setting shortening the drug-dosing interval or increasing the dose may optimize the trough concentrations and improve outcomes.[6]

Figure 2. *Therapeutic Drug Monitoring algorithm based on infliximab trough concentrations*



As seen in Figure 2, therapeutic drug monitoring involves initial optimization of patients to the therapeutic target of drug level in serum. Patients with low or undetectable drug levels without anti-drug antibodies will be managed by dose escalation, whereas patients with high titer anti-drug antibodies will be switched to another anti-TNF.

Further studies are needed to better define clinically meaningful versus insignificant anti-drug antibodies levels, indicating what concentration require changes to drug therapy. Additionally, well designed RCTs are needed that compare routine proactive TDM vs reactive TDM, and empiric dosing changes on patient relevant outcomes, and also the frequency and timing of proactive TDM. Finally, as newer biologic agents are approved, the use of TDM to optimize these drugs will need to be evaluated. [6] Currently, due to lack of relevant data, there are no

clinical recommendations and/or guidelines on how to handle IBD patients with PNR to anti-TNF therapy and management remains therefore empirical. The only available data derive exclusively from small, observational, non-controlled studies that focus on the short-term efficacy of adalimumab in CD patients with PNR to infliximab.[23]

For years, TDM has focused on pharmacokinetic studies, however more recent studies have shown many areas where pharmacokinetic studies are ineffective in predicting patient outcome and dosing fails to apply across populations. If the pharmacokinetic therapeutic drug monitoring does not prove to be efficacious in individualizing patient therapy and correlating to patient outcomes a more effective pharmacodynamics test should be sought after. analyzing cell function, have demonstrated high correlation between results and predicting patient outcomes.

Improved diagnostic methods can reduce ineffective medical practices, individualize drug dosing as well as improve therapeutic drug monitoring. Monitoring therapy and personalizing drug dosage based on functional analysis of immune response and not drug levels in serum can benefit patients by identifying the lowest dose effective in achieving suppression. Additionally non-responders can be quickly and accurately identified by lack of immune response, regardless of drug concentration in serum. Diagnostic testing which does not improve patient outcomes should not be mandated or advised as a part of patient therapy. Revisiting policy requiring ongoing pharmacokinetic TDM in the absence of proven improved patient outcomes provides little value to the patient.

CHAPTER 3
METHODS

Study Objective

The primary objective of the study was to evaluate the association between serum drug levels and disease activity. Patients will be categorized into infliximab tough levels. Disease activity will be measured by clinical outcome reported by DAS and biological outcome reported by levels of disease biomarkers CCP, CRP, and RF levels. Primary end point is defined as the proportion of patients in each group defined as low disease activity by clinical outcome DAS <3.2 and biological outcomes. Clinical remission was defined as a DAS <3.2. Biological remission was defined as having a CRP concentration of $\leq$ 8 mg/L. We hypothesized that the results of trough serum drug levels do not correlate with disease activity.

Aim 1: Evaluate the association between serum drug levels, disease activity, and patient characteristics.

Aim 2: Identify optimal therapeutic drug level in serum associated with decreased disease activity.

Aim 3: Evaluate whether elevated drug levels in serum increase immune response against drug.

Study Site

The study was conducted at Quest Diagnostics Nichols Institute Immunology Research and Development Laboratory in San Juan Capistrano.

Study Participants

Eligible study participants were identified and consented through the arthritis internet registry from 2011 through 2016. The study included a total of 57 patients, 47 females and 10 males, 30 to 81 years of age, clinically diagnosed with rheumatoid arthritis and being treated with infliximab. Patients not meeting the above criteria were excluded from the study. Study

participants were directed to the nearest Quest Patient Service Center for the blood draw. Serum was immediately separated from blood and shipped to the Quest Diagnostics Nichols Institute. Samples were received and stored frozen, below -60°C, within 48 hours of blood draw.

Study Design

The study is a retrospective cohort study. Patients clinically diagnosed with rheumatoid arthritis were tested for infliximab drug levels, anti-drug antibody levels, as well as three rheumatoid arthritis biomarkers CCP, CRP, and RF. Patients were categorized by serum drug levels and evaluated by disease activity.

Study Measures

The primary end point is disease activity. The primary end point was defined as the proportion of patients in clinical and biological remission or low disease activity after 6 months of treatment. Clinical remission or low disease activity was defined by having a DAS below 3.2 Biological remission was defined as having a CRP concentration below 8 mg/L, RF levels below 14 IU/mL, CCP levels below 20 IU.

Drug Level: Infliximab level drug level in serum will be quantified using commercial therapeutic drug level monitoring assay. Target drug trough levels of 2, 5, 7, and 10 mcg/mL will be evaluated for association with decreased disease activity.

Anti-drug antibody levels (ADA): Anti-infliximab antibody levels in serum will be tested using a commercial assay. Anti-drug antibody levels above 10 AU is considered abnormal or positive for antibodies against drug.

Disease Activity Score (DAS): Clinical outcome for disease activity, obtained from patient evaluation and questionnaire. The scoring used for the DAS is the following: dasequiv = 2.527148 + (.4192886 * stdhpg). Approximate DAS score from the mean of the HAQ, pain and global questions within the NDB patient surveys on a 0-10 scale. Following the rheumatology guidelines, DAS below 2.6 indicates disease remission, scores of 2.6 – 3.2 indicates low disease activity, 3.2 – 5.1 indicates moderate disease activity, and scores above 5.1 indicates high disease activity. This variable will be dichotomized to DAS above and below 3.2 and recommended by the ACR.

C-Reactive Protein (CRP): CRP is a biomarker for disease activity. CRP levels in serum will be quantified using commercial assay. CRP levels above 8 mg/L are considered abnormal

Rheumatoid Factor (RF): RF is a biomarker for disease activity. RF levels in serum will be quantified using commercial assay. RF levels above 14 IU/mL are considered abnormal.

Cyclic Citrullinated Peptide (CCP): Diagnostic and prognostic markers of rheumatoid arthritis. CCP levels above 20 IU are considered abnormal.

Covariates

Age: The data was dichotomized, above and below the mean, and analysis for each was performed.

Gender: The data was dichotomized, male and female, and analysis for each was performed.

Statistical Analyses

Statistical analyses were performed using SPSS v23. All variables were examined for normal distribution. Gender, age, and disease outcomes were compared using independent sample t-tests for continuous variables and X^2 tests for categorical variables. Regression analysis was conducted to establish the correlation between disease activity and drug level. A p-value less than 0.05 will be considered significant. The following guidelines provided by the ACR were utilized to recode the dependent variables into dichotomous outcomes and distinguish between normal and abnormal levels of DAS, CRP, CCP RF.

Table 2. Elevated levels of DAS, CRP, RF, CCP, and ADA.

	Low Disease Activity	Moderate/High Disease Activity
DAS	< 3.3	≥ 3.2
CRP	< 8	≥ 8
RF	< 14	≥ 14
CCP	< 59	≥ 59
ADA	<10	≥10

Missing data

All variables except one were 100% complete. The ADA outcome was incomplete, it included results for only 93% of patient. The 4 ADA levels were not included in the analysis.

CHAPTER 4
RESULTS

4.1. Patient Characteristics

Gender

We compared men and women's baseline demographic and disease characteristics using independent samples t-tests for continuous variables and x^2 tests for categorical variables. The mean age for the patient population was 61 years, females average 67 years and males average 60 years of age. The mean drug level for females and males was 13 mcg/mL and 4.5 mcg/mL, respectively. The independent samples t-tests was applied to evaluate the relationship between gender and levels of disease as continuous variables. The x^2 test was applied to understand the relationship between gender and outcomes of disease, dichotomized into low and moderate to high levels as indicated in Table 1. The mean disease activity score for females was 4.1 with 89% of patients having moderate to high disease activity. The mean disease activity score for males was 4.0 with 70% of patients having moderate to high disease activity. Gender did not have a significant association with moderate to high DAS (p-value 0.109), CRP (p-value 0.282), RF (p-value 0.257), CCP (p-value 0.088), or ADA (p-value 0.127). Patient demographics of the study are shown in Table 2.

Table 3. Patient Characteristics - Gender

	Gender		
	F	**M**	**sig. p-Value**
N	47	10	
Age	67.1 (7.0)	60.3 (12.01)	0.090
Drug	13.0 (13.59)	4.5 (2.90)	0.054
DAS	4.1 (0.88)	4.0 (1.11)	0.592
DAS ≥3.2	42/47 (89%)	7/10 (70%)	0.109
CRP	2.7 (4.29)	6.1 (9.15)	0.079

CRP > 8	4/47 (9%)	2/10 (20%)	0.282
RF	33.7 (49.76)	29.0 (20.93)	0.774
RF > 14	19/47 (40%)	6/10 (60%)	0.257
CCP	91.6 (100.34)	162.6 (114.43)	0.052
CCP >59	19/47 (40%)	7/10 (70%)	0.088
ADA	11.89 (37.5)	13.89 (19.25)	0.877
ADA >10	9/44 (21%)	4/9 (44%)	0.127
*Values presented as means with standard deviations in parentheses for continuous variables and as percentages for categorical variables. p-Values for group comparisons were computed using independent samples t-tests for continuous variables and x^2 tests for categorical variables.			

Age

We then compared demographic and disease characteristics by age. The data was dichotomized, above and below the mean, and analysis was performed. Independent samples t-tests for continuous variables and x^2 tests for categorical variables. The mean disease activity score for patients under 61 years was 4.3(0.81) with 96% of patients having moderate to high disease activity. The mean disease activity score for patients 61 years and older was 4.0(0.97) with 79% of patients having moderate to high disease activity. Age did not have a significant association with moderate to high DAS (p-value 0.067), CRP (p-value 0.679), RF (p-value 0.172), or ADA (p-value 0.696). However, a significant association was observed between age and CCP, patients under 61 years had a mean CCP level of 63.7 (82.72), whereas patients 61 years and older were observed to have a mean CCP level of 133.4 (111.49) (p-value < 0.012). It was observed that 29% of patients under 61 years had moderate to high levels of CCP, as compared to 58% of patients 61 years and older having moderate to high levels of CCP (Table 3). The results suggest that age has a statistically significant relationship with incidence of higher levels of CCP, specifically adults over the age of 61 are more likely to have a higher level CCP, and the null hypothesis must be rejected.

Table 4. Patient Characteristics – Age			
Age			
	<61	**>61**	**sig.**
N	24	33	
Drug	12.7 (13.35)	10.7 (12.52	0.564
DAS	4.3 (0.81)	4.0 (0.97)	0.189
DAS ≥3.2	23/24 (96%)	26/33(79%)	0.067
CRP	3.3 (4.11)	3.3 (6.38)	1.000
CRP > 8	3/24 (13%)	3/33(9%)	0.679
RF	28.4 (45.08)	36.1 (46.93)	0.540
RF > 14	8/24 (33%)	17/33 (52%)	0.172
CCP	63.7 (82.72)	133.4 (111.49)	0.012
CCP >59	7/24 (29%)	19/33 (58%)	0.033
ADA	21.2 (53.07)	5.8 (6.55)	0.113
ADA >10	6/22 (27%)	7/31 (23%)	0.696
*Values presented as means with standard deviations in parentheses for continuous variables and as percentages for categorical variables. p-Values for group comparisons were computed using independent samples t-tests for continuous variables and x^2 tests for categorical variables.			

4.2 Target Drug Trough Concentrations

We evaluated if maintenance of recommended therapeutic drug levels of 2, 5, 7, or 10 mcg/mL were associated with improved patient outcomes. Patients were dichotomized by serum drug levels, above or below the therapeutic recommendation and disease activity was evaluated.

Drug Trough Concentration above 2 mcg/mL

The mean drug level for trough levels above and below 2 mcg/mL was 13.3 (12.9) and 0.6 (0.8), respectively. The mean disease activity score for patients maintaining drug levels above 2 mcg/mL was 4.1 (0.9) with 84% of patients having moderate to high disease activity. The mean disease activity score for patients maintaining drug levels below 2 mcg/mL was 4.2 (0.7) with 100% of patients having moderate to high disease activity. Target Drug Trough Levels of 2

mcg/mL did not have a significant association with moderate to high DAS (p-value 0.218), CRP (p-value 0.844), RF (p-value 0.246), CCP (p-value 0.619), or ADA (p-value 0.226). Results are shown in Table 4.

Table 5. Target Drug Trough Concentration above 2 mcg/mL

	Infliximab Trough Concentration (mcg/mL)		
	<2	**≥2**	**sig.**
N	8	49	
Drug	0.6 (0.8)	13.3 (12.9)	
DAS	4.2 (0.7)	4.1 (0.9)	0.721
DAS ≥3.2	8/8 (100%)	41/49 (84%)	0.218
CRP	4.1 (5.8)	3.2 (5.4)	0.664
CRP > 8	1/8 (13%)	5/49 (10%)	0.844
RF	26.9 (37.0)	33.8 (46.7)	0.695
RF > 14	2/8(25%)	23/49 (47%)	0.246
CCP	82.0 (99.3)	107.6 (104.9)	0.529
CCP >59	3/8 (38%)	23/49 (47%)	0.619
ADA	16.0 (20.1)	11.7 (36.3)	0.762
ADA >10	3/7 (43%)	10/46 (22%)	0.226

*Values presented as means with standard deviations in parentheses for continuous variables and as percentages for categorical variables. p-Values for group comparisons were computed using independent samples t-tests for continuous variables and x2 tests for categorical variables.

Drug Trough Concentration above 5 mcg/mL

The mean drug level for trough levels above and below 5 mcg/mL was 17.4 (13.7) and 2.9 (1.5), respectively. The mean disease activity score for patients maintaining drug levels above 5 mcg/mL was 4.1 (0.9) with 85% of patients having moderate to high disease activity. The mean disease activity score for patients maintaining drug levels below 5 mcg/mL was 4.1 (0.9) with 87% of patients having moderate to high disease activity. Target Drug Trough Levels of 5

mcg/mL did not have a significant association with elevated DAS (p-value 0.859), CRP (p-value 0.611), RF (p-value 0.554), CCP (p-value 0.790) (Table 5).

A significant association was observed between drug trough levels above 5 mcg/mL and ADA levels. The mean ADA levels for patients maintaining drug levels below 5 mcg/mL was 14.0 (21.9) with 41% of patients having positive ADA results. The mean ADA levels for patients maintaining drug levels above 5 mcg/mL was 11.0 (41.3) with 13% of patients having positive ADA results. The results suggest that drug trough levels of 5 mcg/mL has a statistically significant relationship with incidence of ADA levels, specifically patients maintaining drug levels below 5 mcg/are more likely to have a positive ADA levels, and the null hypothesis must be rejected. Results are shown in Table 5.

Table 6. Target Drug Trough Concentration above 5 mcg/mL

	Infliximab Trough Concentration (mcg/mL)		
	<5	**≥5**	**sig.**
N	23	34	
Drug	2.9 (1.5)	17.4 (13.7)	
DAS	4.1 (0.9)	4.1 (0.9)	0.849
DAS ≥3.2	20/23 (87%)	29/34 (85%)	0.859
CRP	4.1 (6.3)	2.8 (4.7)	0.372
CRP > 8	3/23 (13%)	3/34(9%)	0.611
RF	37.7 (55.1)	29.6 (37.3)	0.516
RF > 14	9/23(39%)	16/34(47%)	0.554
CCP	107.3 (108.4)	101.8 (101.8)	0.984
CCP >59	10/23 (44%)	16/34(47%)	0.790
ADA	14.0 (21.9)	11.0 (41.3)	0.765
ADA >10	9/22 (41%)	4/31(13%)	0.020

*Values presented as means with standard deviations in parentheses for continuous variables and as percentages for categorical variables. p-Values for group comparisons were computed using independent samples t-tests for continuous variables and x^2 tests for categorical variables.

Drug Trough Concentration above 7 mcg/mL

The mean drug level for trough levels above and below 7 mcg/mL was 20.4 (13.7) and 3.5 (2.0), respectively. The mean disease activity score for patients maintaining drug levels above 7 mcg/mL was 4.0 (0.9) with 82%of patients having moderate to high disease activity. The mean disease activity score for patients maintaining drug levels below 7 mcg/mL was 4.3 (0.9) with 90% of patients having moderate to high disease activity. Target Drug Trough Levels above 7 mcg/mL did not have a significant association with moderate to high DAS (p-value 0.355), CRP (p-value 0.891), RF (p-value 0.933), CCP (p-value 0.866), and ADA (p-value 0.226) (Table 6).

Table 7. Target Drug Trough Concentration above 7 mcg/mL

	Infliximab Trough Concentration (mcg/mL)		
	<7	**≥7**	**sig.**
N	30	27	
Drug	3.5 (2.0)	20.4 (13.7)	
DAS	4.3 (0.9)	4.0 (0.9)	0.308
DAS ≥3.2	27/30 (90%)	22/27 (82%)	0.355
CRP	3.4 (5.7)	3.2 (5.2)	0.886
CRP > 8	3/30 (10%)	3/27 (11%)	0.891
RF	32.8 (49.3)	32.9 (40.9)	0.999
RF > 14	13/30 (43%)	12/27 (44%)	0.933
CCP	106.9 (105.9)	100.8 (102.9)	0.830
CCP >59	14/30 (47%)	12/27 (44%)	0.866
ADA	11.0 (19.8)	13.7 (46.6)	0.776
ADA >10	9/29 (31%)	4/24 (17%)	0.226

*Values presented as means with standard deviations in parentheses for continuous variables and as percentages for categorical variables. p-Values for group comparisons were computed using independent samples t-tests for continuous variables and x^2 tests for categorical variables.

Drug Trough Concentration above 10 mcg/mL

The mean drug level for trough levels above and below 10 mcg/mL was 26.4 (13.49) and 4.7 (2.64), respectively. The mean disease activity score for patients maintaining drug levels above 10 mcg/mL was 4.1 (0.87) with 94% of patients having moderate to high disease activity. The mean disease activity score for patients maintaining drug levels below 10 mcg/mL was 4.1 (0.94) with 82% of patients having moderate to high disease activity. Target Drug Trough Levels of 10 mcg/mL did not have a significant association with elevated DAS (p-value 0.211), CRP (p-value 0.922), RF (p-value 0.952), CCP (p-value 0.489), or ADA (p-value 0.630) (Table 6).

Table 8. Target Drug Trough Concentration above 10 mcg/mL

	Infliximab Trough Concentration (mcg/mL)		
	<10	**≥10**	**sig.**
N	39	18	
Drug	4.7 (2.64)	26.4 (13.49)	
DAS	4.1 (0.94)	4.1 (0.87)	0.990
DAS ≥3.2	32/39 (82%)	17/18(94%)	0.211
CRP	3.7 (6.38)	2.6 (2.75)	0.505
CRP > 8	4/39 (10%)	2/18 (11%)	0.922
RF	32.4 (45.50)	33.9 (48.11)	0.908
RF > 14	17/39 (44%)	8/18 (44%)	0.952
CCP	111.3 (109.10)	88.2 (98.136)	0.447
CCP >59	19/39 (49%)	7/18 (39%)	0.489
ADA	9.2 (17.91)	19.9 (60.01)	0.322
ADA >10	10/38 (26%)	3/15 (20%)	0.630

*Values presented as means with standard deviations in parentheses for continuous variables and as percentages for categorical variables. p-Values for group comparisons were computed using independent samples t-tests for continuous variables and x^2 tests for categorical variables.

CHAPTER 5
DISCUSSION

This study suggests that patients maintaining drug levels above 5 mcg/mL are less likely to develop anti-drug antibodies (p=0.020). Drug immunogenicity is the underlying factor in nearly 40% of patients who lose response, induction and maintenance therapy at drug concentrations of 5 mcg/mL may help to improve patient outcomes. Dose Escalation to therapeutic range of 5 mcg/mL may help to reduce the number of patients who have immune-mediated pharmacokinetic failure resulting from the formation of neutralizing anti-drug antibodies. Dose Reduction to therapeutic range may help patients by saving money.

The major limitation of the present study was the retrospective study design. The study limitations included a small sample size, large variance, and a large percent of female patients. This may be why we did not arrive at a statistically significant multivariate model for predicting disease activity.

Future study designs should include a stratified adaptive design to balance sample population by age and gender and a much larger sample size. Additionally, outcomes of disease activity should include the number of swollen joints by MRI. The MRI of swollen joints will provide a more comprehensive model of disease activity. The number of swollen joints by MRI could show a significant association with drug levels.

Considering the recent surge of biologics and biosimilars to the market and the higher cost of these drugs, therapeutic drug monitoring has huge implications for patient care. If it can

be shown that dosing based on therapeutic drug monitoring correlates with patient outcomes of disease activity, achieving target trough concentrations in patients would result in a more efficient use of the drug. Personalizing drug dosage and monitoring therapy based on drug levels in serum and immune response, can benefit patients by identifying the lowest dose effective in achieving remission and quickly identifying non-responders who will not benefit from the drug.

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