2016

# Incidence and Factors Associated With Nonalcoholic Fatty Liver Disease Among Patients With Rheumatoid Arthritis

Ani K. John
*Walden University*

# Walden University

College of Health Sciences

This is to certify that the doctoral dissertation by

Ani John

has been found to be complete and satisfactory in all respects,
and that any and all revisions required by
the review committee have been made.

Review Committee
Dr. Angela Prehn, Committee Chairperson, Public Health Faculty
Dr. Hebatullah Tawfik, Committee Member, Public Health Faculty
Dr. Namgyal Kyulo, University Reviewer, Public Health Faculty

Chief Academic Officer
Eric Riedel, Ph.D.

Walden University
2016

Abstract

Incidence and Factors Associated With Nonalcoholic Fatty Liver Disease Among Patients With Rheumatoid Arthritis

by

Ani Kattapuram John

MPH, University of Massachusetts, 2009

BSN, Azusa Pacific University, 1981

Dissertation Submitted in Partial Fulfillment

of the Requirements for the Degree of

Doctor of Philosophy

Public Health

Walden University

February 2016

## Abstract

Nonalcoholic fatty liver disease (NAFLD) has become one of the most common hepatic diseases worldwide, making the diagnosis and management of NAFLD an emerging public health issue. Theories associated with NAFLD surmise that inflammation may be the root cause, along with the complex interplay of other chronic conditions such as obesity, metabolic syndrome, diabetes, dyslipidemia, and cardiovascular disease (CVD). It is unknown if other inflammatory conditions such as rheumatoid arthritis (RA), along with the use of methotrexate (MTX), might confer increased risk for NAFLD. Longitudinal data collected from a retrospective cohort of 17,481 adult RA patients in the United States were used to determine the incidence and factors associated with the development of NAFLD using a noninvasive tool (Fibrosis-4 score). Results of the Kaplan Meier analysis showed that 31% of this cohort developed NAFLD, in about 7 years from baseline, with most having mild to moderate disease and only 1.4% with advanced disease. RA patients also had a prevalence of chronic conditions associated with NAFLD, as seen in the general population. In the Cox proportional hazard multivariate analysis, age (middle and elderly), hypertension, CVD, dyslipidemia, metabolic syndrome, exercise, use of MTX, and non-MTX antirheumatic drugs were independent predictors for the development of NAFLD. This research could improve early diagnosis of NAFLD using a novel noninvasive tool. Increase awareness of the prevalence and causes of NALFD inform clinical practice and management of the disease and influence policy about this chronic condition in patients with RA.

# Incidence and Factors Associated With Nonalcoholic Fatty Liver Disease Among Patients With Rheumatoid Arthritis

by

Ani Kattapuram John

MPH, University of Massachusetts, 2009

BSN, Azusa Pacific University, 1981

Dissertation Submitted in Partial Fulfillment

of the Requirements for the Degree of

Doctor of Philosophy

Public Health

Walden University

February 2016

# Dedication

I dedicate this dissertation to my family. To my parents, who gave me the gift of faith, love for life and the joy of learning. To my dad, a life long learner and my inspiration, his encouragement kept me going, never to give up and to always reach. To my mom, a woman of great compassion and kindness, she showed me how to live a life of gratitude. To my “favorite” son Phillip and “favorite” daughter Lisa, the joy and delight of my life, without their unfailing love and support I could not have made it. To my brother George for always being there for me. To our puppy Bentley, my constant and faithful companion, notably he spent almost as much time with me as I did on my PhD. I welcomed his ‘take a break’ interruptions, with his doggy smiles and enthusiastic woofs. Finally, I thank God, for giving me the strength and fortitude, I am truly blessed!

Acknowledgments

It is with great gratitude and pleasure; I would like to acknowledge and sincerely thank Dr. Angela Prehn for being my chair, for her responsiveness, thoughtful comments, thinking with and for me, and always insisting on quality work. I would like also to acknowledge Dr. Hebatullah Tawfik for her patience and kind words of encouragement along the way. A special thanks to George Reed my friend and mentor, he taught me so much, explaining difficult statistics in a simple and understandable way. Mighty big thanks to Dr. Joel Kremer, the founder and Chief Medical Officer of Corrona, a wonderful friend and human being whom I am truly blessed to know. To my "sister", Kate Nakfoor for her unfailing love and support. Anne Morris for encouraging me every step of the way. And finally to Del and Elaine Goehner, for "adopting" me when I was in nursing school, they provided the love and warmth of a home away from home. I am here today having completed my PhD because of Elaine, who many years ago planted the research bug.

I have done it …only by "Standing on the Shoulder of Giants"

Table of Contents

List of Tables ..... iv

List of Figures ..... vi

Chapter 1: Introduction to the Study ..... 8

Background ..... 10

Problem Statement ..... 12

Purpose of Study ..... 12

Research Questions and Hypotheses ..... 13

Theoretical Framework ..... 14

Conceptual Framework ..... 15

Nature of the Study ..... 16

Definitions of Terms ..... 16

Scope and Delimitations ..... 18

Limitations ..... 19

Significance ..... 20

Summary ..... 22

Chapter 2: Literature Review ..... 24

Theoretical Framework ..... 26

Conceptual Framework ..... 29

Background ..... 31

Tools and Measures for Nonalcoholic Fatty Liver Disease ..... 34

Epidemiology of Rheumatoid Arthritis (RA) ..... 36

Prevalence of NAFLD Related Risk Factor: RA Population ..... 37
Epidemiology of NAFLD: General Population ..... 43
General Population NAFLD Prevalence Rates: United States ..... 43
General Population NAFLD Prevalence Rates: Asia ..... 46
General Population NAFLD Prevalence Rates: Rest of the World ..... 50
NAFLD Incidence Rates ..... 52
Risk Factors Associated with Nonalcoholic Fatty Liver Disease ..... 56
Chapter 3: Research Method ..... 79
Research Design and Rationale ..... 79
Methodology ..... 83
Setting and Sample ..... 83
Population ..... 84
Sample and Sampling Procedures ..... 85
Data Collection ..... 90
Operationalization of Variables ..... 92
Dependent Variable ..... 93
Independent Variables ..... 94
Statistical Analysis and Tests ..... 99
Descriptive Analysis ..... 100
Bivariate and Multivariate Analysis ..... 104
Threats to Internal and External Validity ..... 107
Ethical Procedures ..... 109

Summary ..... 110
Chapter 4: Results ..... 113
Dataset Collection and Preparation ..... 115
Transforming Data ..... 116
Dataset Preparation ..... 120
Research Question 1 ..... 131
Research Question 2 ..... 143
Research Question 3 ..... 143
Research Question 4 ..... 153
Summary ..... 161
Chapter 5: Discussion, Conclusions, and Recommendations ..... 165
Discussion of Findings ..... 167
Overall NAFLD Incidence Rates ..... 168
Risk Factors Associated with Nonalcoholic Fatty Liver Disease ..... 169
Study Limitations ..... 175
Recommendations for Future Research ..... 177
Social Change Implications ..... 179
Conclusion ..... 180
References ..... 182

List of Tables

Table 1. NAFLD Prevalence Studies in the General Population: U.S. ..... 44

Table 2. NAFLD Prevalence Studies in the General Population: Asia ..... 47

Table 3. NAFLD Prevalence Studies in the General Population: Rest of the World ..... 51

Table 4. NAFLD Incidence Studies in the General Population ..... 55

Table 5. Power Analysis of NAFLD and Independent Risk Factors ..... 89

Table 6. Variable Characteristics ..... 93

Table 7. Relationship between NAFLD Score of Stages of Disease ..... 94

Table 8. Variables, Operational Definitions, and Codes ..... 95

Table 9. NAFLD Study Cohort Compared to Overall Registry Patients (sample) ..... 101

Table 10. NAFLD Rates: Incidence, Prevalence and by Risk Factors (Sample) ..... 102

Table 11. Comparison of Baseline Factors: Patients with vs. without Incident NAFLD 103

Table 12. Unadjusted Analyses of Baseline Predictors for Incidence of NAFLD ..... 105

Table 13. Adjusted Analyses of Baseline Predictors for Incidence of NAFLD ..... 107

Table 14. Overall Registry Patient Compared to NAFLD Study Cohort ..... 119

Table 15. Missing Variable Analysis ..... 122

Table 16. Descriptive Mahalanobis Distance ..... 125

Table 17. Mahalanobis Distance Summary ..... 126

Table 18. Factor Analysis Communalities[a] ..... 128

Table 19. Omnibus Tests of Model Coefficients[a] - Mean of Covariates ..... 129

Table 20. Test for Proportionality of Hazards- Time with All Covariates ..... 130

Table 21. NAFLD Rates: Overall Incidence Rates and Time to Event ..... 132

Table 22. NAFLD Rates: Incidence Rates by Demographic Factors ..... 134

Table 23. Incidence Rates and Time to Event by NAFLD Risk Factors ..... 137

Table 24. NAFLD Incidence Rates by RA Factors ..... 140

Table 25. Overall NAFLD Incidence by Comorbidities and Other Factors ..... 142

Table 26 ..... . Comparison of Demographic Factors: Patients with vs. without NAFLD Incidence ..... 145

Table 27 ..... . Comparison of Baseline RA Factors: Patients with vs. without NAFLD Incidence ..... 148

Table 28. Comparison of Baseline Comorbidities & Other Factors: Patients without and with NAFLD Incidence ..... 152

Table 29. Unadjusted Analyses of NAFLD Predictors: Baseline Demographic ..... 153

Table 30. Unadjusted Analyses of NAFLD Predictors: Baseline RA Factors ..... 154

Table 31.Unadjusted Analyses of NAFLD Predictors: Comorbidities & Other Factors 155

Table 32. Adjusted Analyses of NAFLD Predictors..... 159

List of Figures

Figure 1. Erickson's NAFLD conceptual model (2009)..... 31

Figure 2. Prevalence in the RA population (%):Obesity ..... 38

Figure 3. Prevalence in the RA population (%): Metabolic syndrome..... 39

Figure 4. Prevalence in the RA population (%): Hypertension ..... 40

Figure 5. Prevalence in the RA population (%): Dyslipidemia ..... 41

Figure 6. Prevalence in the RA population (%): Diabetes..... 42

Figure 7 NAFLD prevalence rates in the general population (%): U.S. ..... 46

Figure 8. NAFLD prevalence rates in the general population (%): Asia..... 49

Figure 9. NAFLD prevalence in the general population (%): Rest of the world..... 50

Figure 10. NAFLD incidence rates in the general population (%) ..... 52

Figure 11. NAFLD prevalence in the general population (%): Gender..... 59

Figure 12 NAFLD prevalence in the general population (%): Race/Ethnicity..... 62

Figure 13. NAFLD prevalence in the general NAFLD population (%):Obesity..... 67

Figure 14. NAFLD prevalence in the general population (%): Metabolic syndrome ..... 69

Figure 15. NAFLD prevalence in the general population (%): Diabetes ..... 71

Figure 16. NAFLD prevalence rate in the general population (%): Dyslipidemia..... 74

Figure 17. NAFLD prevalence: Hypertriglyceridemia and low HDL levels (%)..... 76

Figure 18. Sample size and power calculation for NAFLD incidence rate ..... 86

Figure 19. Sample size calculation for NAFLD prevalence rate ..... 87

Figure 20. Flow chart of study participants ..... 116

Figure 21. Event cases ..... 127

Figure 22. Censored cases..... 127

Figure 23. Survival function: Gender and age ..... 130

Figure 24. Survival function: Survival function ..... 130

# Chapter 1: Introduction to the Study

Nonalcoholic fatty liver disease (NAFLD) is predicted to be the next global epidemic of liver disorders, mostly as a result of obesity (Ray, 2013). Prevalent comorbid conditions rooted in inflammation such as metabolic syndrome, obesity, diabetes mellitus , cardiovascular disease (CVD), and dyslipidemia appear to accelerate the development of NAFLD (Argo & Caldwell, 2009; Lonardo, et al., 2015). Rheumatoid arthritis (RA) is a chronic inflammatory condition, also associated with similar risk factors such as obesity, metabolic syndrome, hypertension, hypertriglyceridemia, low levels of high density lipids (HDL), diabetes, insulin resistance and CVD (Chung et al., 2008; Crowson et al., 2011). What is unknown is if other chronic illnesses such as RA might confer a risk for NAFLD given the overlap of these factors associated with inflammation. Additionally, a known hepatotoxic drug, methotrexate (MTX) often used to treat RA has been associated with increased elevation of liver enzymes, and the development of NAFLD (e.g. fibrosis) of the liver (Arena et al., 2012; Sakthiswary, Chan, Koh, Leong, & Thong, 2014). In this study, I investigated whether NAFLD is present among patients with RA, given the overlap of several risk factors related to inflammation and exposure to MTX. This association has not been documented or well characterized in the literature for this potentially at risk population.

Increasing rates of obesity and metabolic syndrome and the anticipated parallel increases in the occurrence of NAFLD makes the diagnosis, management, and prevention of NAFLD an emerging public health challenge (Fabbrini, Sullivan, & Klein, 2010; (Vernon, Baranova, & Younossi, 2011). Diagnosing the condition using conventional

approach by biopsying the liver is a challenge, as it is an invasive and costly procedure associated with discomfort and even the risk of death (Shah et al., 2009a). Additionally, it is not always clear when a biopsy is necessary, hence there is a need to identify patients at risk using convenient and noninvasive tools (McPherson, Stewart, Henderson, Burt, & Day, 2010). Fibrosis 4 Score (FIB-4) is such a tool: an index is calculated corresponding to the various stages of NAFLD using commonly available laboratory measures such as alanine aminotransferase levels (ALT), aspartate aminotransferase levels (AST), platelet counts and age as shown in Table 7 (Shah et al., 2009a). Using such a tool makes the diagnosis of NAFLD more feasible.

There is a paucity of information about NAFLD for the population with RA. There is a need to disseminate information about the incidence, prevalence, and factors associated with NAFLD. To address this gap in knowledge, retrospective data collected from an observational cohort of patients with RA in the United States (U.S.) was used to determine the incidence and factors associated with NAFLD using FIB-4. This chapter provides a brief review of the background and statement of the problem, followed by the purpose of the study and the research questions. The next section in this chapter includes a brief review of the theories related to NAFLD, the design and methodology, definition of the study variables, the scope of the study and the related assumptions and limitations. Finally, this chapter describes the significance of this study and its potential contributions to advancing science, increasing clinical knowledge, and improving the lives of patients with RA.

## Background

The dependent variable for this study is the presence of NAFLD. NAFLD encompasses a spectrum of fatty liver disease that starts with the development of simple fatty liver, progressing to steatohepatitis, fibrosis, cirrhosis, and, for some, can lead to cancer. However, these disorders occur in the absence of significant alcohol use (Chalasani et al., 2012). Each stage of the disease is associated with histological changes in the liver (Kleiner et al., 2005). Liver biopsy is best able to elucidate these subtle pathological changes, however, its scalability in general practice is limited (Chalasani et al., 2012). The FIB-4 index is a validated noninvasive tool that corresponds with the various stages of NAFLD (Shah et al., 2009a; Vallet-Pichard et al., 2007). An FIB-4 score of $<1.3$ indicates the absence of advanced disease and the presence of NAFLD is denoted by a score of $\geq 1.3$ (McPherson et al., 2010; Shah et al., 2009a). More details about the validity and reliability of the tool are described in Chapter 3.

There were no studies in the literature that reported on the incidence rate of NAFLD in the RA population or the U.S. general population. However, outside of the U.S. studies reported NAFLD incidence of 15% to 20% as shown in Figure 5. There were a few studies addressing the prevalence of NAFLD in the U.S. Evidence of prevalence rates of NAFLD in the RA population was limited to one small study reporting a rate of 23% in the U.S. (Bhambhani, Amin, Gutierrez, Cuppari, & Disla, 2006). A second study reported lower rates (4.7%) but only included RA patients with elevated liver transaminase levels while on MTX (Sakthiswary et al., 2014). NAFLD prevalence in the U.S. general population ranged from 11% to 46% as shown in Figure 2.

Independent risk factors associated with NAFLD are age, gender, and race/ethnicity along with clinical features such as metabolic syndrome, obesity, diabetes, and dyslipidemia (Chalasani et al., 2012). These factors were used as the independent predictor or explanatory variables in this study. Other potential confounders associated with NAFLD but not considered as independent risk factors were MTX use, liver enzyme elevation, alcohol use, and liver disorders (Chalasani et al., 2012; 171; Nascimbeni et al., 2013). NAFLD rates appear to increase with age, peaking at the middle ages of life. Hispanics and men appear to be at higher risk for NAFLD. NAFLD rates steadily increased with age for women also, with higher rates reported in the latter years of life (Chalasani et al., 2012; Vernon et al., 2011). Also presented in Chapter 2 are the risk factors related to NAFLD in the general population, given the paucity of such information in the RA population. RA has some unique as well as common risk factors. The overlapping NAFLD related risk factors are related to high levels of systemic inflammation and are also prevalent in the RA population including: obesity, metabolic syndrome, hypertriglyceridemia, hypertension, dyslipidemia, and insulin resistance (Ahmed, 2006).

This study used retrospective data collected from an observational cohort of patients with RA in the U.S. to determine the incidence, prevalence, and the factors associated with NAFLD using FIB-4. This study has the potential to make significant contribution to the body of knowledge related to NAFLD, inform clinical practice and improve the lives of patients with RA, already burdened with challenges of the underlying disease due to inflammation.

## Problem Statement

In the last ten years, NAFLD has emerged as the leading cause of chronic liver disorders, notably unrelated to significant alcohol use (Preiss & Sattar, 2008). In the U.S. during the period between 1980 and 2010, about 30% of the population had NAFLD, rates paralleling the prevalence of metabolic syndrome and obesity (Browning et al., 2004; Lazo et al., 2013, Smits Ioannou, Boyko, & Utzschneider, 2013; Vernon et al., 2011). Patients with NAFLD are more likely to develop chronic liver diseases, such as cirrhosis of the liver, and are at increased risk for hepatocellular carcinoma (Farrell & Larter, 2006).

Patients with RA have higher rates of comorbidities similar to those with NAFLD, such as metabolic syndrome, and are also at increased risk for cardiovascular disease compared to those without RA (Chung et al., 2008; Crowson et al., 2011). MTX is used as the standard treatment for RA. It is a known hepatotoxic drug that can increase elevation of liver enzymes and has been associated with NAFLD and fibrosis of the liver (Arena et al., 2012; Sakthiswary et al., 2014). Given the overlap of several risk factors for NAFLD and RA, it can be presumed that NAFLD may be prevalent among patients with RA.

## Purpose of Study

The purpose of this quantitative research study was to determine the incidence and factors associated with NAFLD using observational data collected from a cohort of patients with RA. The primary objective of this study was to first establish the occurrence of NAFLD in this population by assessing the incidence rates. The second objective of

the study is, to determine if there are significant clinical and demographic factors that independently predict the development of NAFLD after adjusting for relevant confounders. These include several factors such as age, gender, race/ethnicity, metabolic syndrome, obesity, diabetes, dyslipidemia, MTX use, liver enzyme elevation, alcohol use, and liver disorders. All patients who met the eligibility criteria and were without NAFLD at the index baseline visit were grouped into the incident cohort. Since the incident cohort sample size was sufficiently powered to conduct the primary analysis for the study, the alternate plan for using the prevalent cohort analyses was dropped.

## Research Questions and Hypotheses

This was a quantitative, nonexperimental, retrospective cohort study that sought to answer the following research questions:

RQ1 Quantitative: What is the incidence rate of NAFLD among patients with RA from January 2001 - November 2014 in a RA registry in the U.S. using the FIB-4 test?

RQ2 Quantitative: What is the prevalence of NAFLD among patients with RA for one year (e.g. from January 2012-December 2012) in a RA registry in the U.S. determined by using the FIB-4 test?

RQ3: Among patients in the RA incident cohort, what are the baseline differences in clinical and demographic characteristics among those with NAFLD compared to those without?

RQ $H_0$: Among patients in the RA incident cohort, there are no significant baseline differences in the clinical and demographic characteristics among those with NAFLD compared to those without.

RQ3 $H_a$: Among patients in the RA incident cohort, there are significant differences in the baseline clinical and demographic characteristics among those with NAFLD compared to those without.

RQ4 Quantitative: For the RA incident cohort, what are the significant baseline factors (clinical and demographic) that predict the development of NAFLD after adjusting for relevant confounders?

RQ4 $H_0$: For the RA incident cohort, there are no significant baseline factors (clinical and demographic) that predict the development of NAFLD after adjusting for relevant confounders.

RQ4 $H_a$: For the RA incident cohort, there are significant factors (clinical and demographic) that predict the development of NAFLD after adjusting for relevant confounders.

## Theoretical Framework

The *two hit* theory postulated by Day and James (1998) is the most frequently referenced theory to explain NAFLD (Lim, Mietus-Snyder, Valente, Schwarz, & Lustig, 2010). This theory proposes that there is an accumulation of lipids in the liver that leads to the formation of triglycerides, insulin resistance, the onset of hyperglycemia, and eventually inflammation of the liver. These are thought to be precursors in the process for the development of NAFLD (Lim et al., 2010). Obesity and metabolic dysfunction are

central to the development of NAFLD. Specifically, as the result of high levels of intrahepatic triglycerides and the subsequent alteration in the metabolism of fatty acids, glucose, and lipoproteins leading to systemic inflammation (Fabbrini, et al., 2010). RA is also a disease associated with systemic inflammation and appears to have similar pathogenic changes that are associated with chronic inflammation (Ahmed, 2006). Additionally, liver enzyme elevation due to MTX use has been associated with an increased risk for NAFLD (Arena et al., 2012; Visser & Heijde, 2009). It is most likely that the development of NAFLD may be due to the confluence of several factors. All related to inflammation and altered lipid metabolism, metabolic syndrome, obesity, alteration of glucose metabolism, and for patients with RA, the use of MTX.

## Conceptual Framework

Theories, pathways, and relationships related to the development of NAFLD are still evolving (Erickson, 2009). NAFLD is a chronic disease with many complex interacting obesity and metabolic related pathways, as well as environmental and genetic factors (Erickson, 2009). According to Erickson's NAFLD conceptual framework, any and all factors related to the metabolic pathway play a significant role in the development of NAFLD as shown in Figure 1 and further explained in Chapter 2. Some theorists suggest that these relationships may be bidirectional, that NAFLD maybe in the causal pathway of inflammation, hyperlipidemia, obesity, and insulin resistance; however, this assertion lacks substantial evidence and is considered hypothetical (Azad & Quayum, 2007; Erickson, 2009; Vanni et al., 2010). The *two hit* theory further clarified by

Erickson's conceptual framework helps explain the relationships between factors associated with NAFLD and provides the theoretical context for this study.

## Nature of the Study

Retrospective data enabled access to a concurrent cohort of patients with data collected over many years, and also provided a large enough sample size, with specific data points, to reasonably answer the research questions. This approach accommodated an important element in the study: the ability to measure risk factors associated with NAFLD that may take years to develop and are key to identifying the predictors of NAFLD. The analysis for this study included determining the incidence and prevalence rates of NAFLD, descriptive analyses to characterize the study population, Kaplan Meier and adjusted Cox proportional hazard analysis to determine the incidence rate, time to event, and the predictors associated with the development of NAFLD.

## Definitions of Terms

Variables were defined for this study using guidelines for NAFLD and MetS (Fabbrini et al., 2010; Alberti et al., 2009; Grundy, et al., 2005) and were also based on the definitions used in the registry to capture the data. In this study the following variables were defined as follows:

*Age:* The amount of time a person has lived was categorized based on the age related prevalence rates of NAFLD reported in the literature (Chen, et al., 2006; Lazo et al., 2013; Yan et al., 2013). Age was operationalized into the following categories: younger, middle age, and, elderly.

*Alcohol use:* Defined as the use of alcohol in the following manner, if used on a daily, weekly or monthly basis (Corrona Rheumatoid Arthritis, 2015).

*Diabetes:* Refers to type 2 diabetes mellitus, the most common form of diabetes associated with NAFLD (Chalasani et al., 2012).

*Dyslipidemia:* Defined as alterations in the lipid panel; specifically elevations in total cholesterol, LDL and triglyceride and reduced levels of HDL as shown in Table 8. (Grundy et al., 2005).

*Gender:* Gender was defined as either as male or female.

*Liver Disorders:* History of liver disorder was defined as those with liver related disorders, hepatic events and also history of liver biopsy excluding NAFLD (Corrona Rheumatoid Arthritis, 2015).

*Metabolic Syndrome (MetS):* MetS was defined as the presence of any three of the following obesity related risk factors: elevated waist circumference, elevated triglycerides, blood pressure, glucose and reduced high density lipids (HDL)(Alberti et al., 2009; Grundy, et,al., 2005). Specific criteria for each of these measures are outlined in Table 8 (Grundy et al., 2005).

*MTX Use:* MTX was defined as current use of the drug, a standard treatment for patients with RA (Arena et al., 2012).

*NAFLD:* Was defined as the presence or absence of NAFLD, measured by using FIB-4 score corresponding to the various stages of fibrosis and was defined as: no advanced NAFLD, presence of NAFLD, mild/moderate NAFLD and advanced NAFLD (Kleiner et al., 2005; Vallet-Pichard et.al 2007; Shah et.al, 2009a)

*Obesity:* Obesity was measured using Body Mass Index *(*BMI). BMI is a ratio of an individual's weight (kg) / [height (m)]$^2$ adjusted for gender and are categorized into four groups: underweight, normal weight, overweight, and obese, criteria used by the Center for Disease Control (CDC, 2014). Elevated BMI score of ≥ 30 was used to determine the presence of obesity, a definition similar to studies conducted by Lazo et al. 2011, Lazo et al. 2013, Ong et al. 2008 and Williams, 2011.

*Race/ Ethnicity:* Information about race/ethnicity was collected from patients in the registry and was defined by the patients. Patients were asked to check all applicable categories: Caucasian (White), Hispanic or Latino, not Hispanic or Latino, African American (Black), American Indian, or Alaska Native, Asian, other (specify) and, multiracial.

## Scope and Delimitations

This study was limited to determining the incidence, prevalence, and factors associated with NAFLD among adults regardless of gender, or race/ethnicity in the U.S. using longitudinal data collected in a registry of patients with RA. Excluded from the analysis are those for whom the FIB-4 information was not available or those with secondary causes of NAFLD (e.g. Hepatitis C, B, polycystic ovarian disease), and other conditions that are not routinely collected in the registry. Factors associated with NAFLD are limited to those captured in the registry, such as age, gender, race/ethnicity, metabolic syndrome, obesity, diabetes, and dyslipidemia (Chalasani et al., 2012). A limited data set from patients meeting the study inclusion and exclusion criteria during the years of January 2001-November 2014 was extracted and used for the analysis. The scope of the

study outlined here and the use of a limited dataset based on the inclusion and exclusion criteria specific to this study yielded a highly selected sample, and thus threatens the generalizability of the results to the general RA population.

## Limitations

The main threat to internal and external validity for this study was the study design. This was a nonrandomized study that uses secondary data and utilizes a nonprobability sample of convenience (Szklo & Nieto, 2013, pp. 109-150). One of the inherent limitations of this study was the potential for systematic selection bias, since the cohort was primarily selected based on availability of laboratory parameters (liver enzyme and platelet counts) in the registry. The impact of this limitation was addressed in the analysis plan outlined in Chapter 3, where the comparability of NAFLD study cohort and the overall registry population was assessed and is reported in Table 9. Using real world data increased the external validity of the study; however, there remain many unmeasured confounders that threaten internal validity and thereby diminish the inferential power of the study ( Szklo & Nieto, 2014. pp. 153-182). Using a large sample size, as in this study, helped decrease overall variability and allowed assessment of the impact and adjustment of the measured confounders; these are factors that can help mitigate some of the threats to internal validity ( Szklo & Nieto, 2014. pp. 313-363).

Outlined below are other limitations using secondary data, however these cannot be addressed methodologically. Data for the registry were collected as part of routine clinical practice and may not have been a good fit for the research question. There may have been systematic missing variables, key variables may not have been available for

pertinent time points or patients may have been lost to follow up (Hofferth, 2005). Laboratory measures may not necessarily be captured for every patient on MTX in the registry. Since this was a retrospective study, misclassification of either the exposure or the outcome variable was also a potential risk. In this study misclassification of exposure was of greater concern, as there could be variations in RA practices and subsequently how the data was captured in the registry. However, this risk was less for the outcome variable as a uniform definition for NAFLD was applied to all cases in a similar manner.

While this was a large registry of RA patients across the U.S., there are some potential sources of bias. Patients can volunteer to participate in the registry and all patients at a site are not entered into the registry. There could be variation from site to site as to how participants are chosen and who is selected for the registry. Selection bias could pose a potential threat to the generalizability of the results. Regarding the incident analysis, another limitation may be that the observational time available in the registry may not be sufficiently long to identify incident cases of NAFLD. A third limitation was long term outcomes related to NAFLD were not available to corroborate the outcome findings of this study.

## Significance

It is well established that RA patients have lower life expectancy and are at increased risk of death, especially those patients with other comorbid conditions such as diabetes, metabolic syndrome, hypertension, and cardiovascular disease (Nurmohamed, 2009). In the general population NAFLD is an emerging problem associated with these

chronic conditions, thus it is important to identify if NAFLD may be prevalent among other related chronic diseases such as RA.

The overall burden from the confluence of many chronic conditions along with the emergence of NAFLD is yet unknown, though anticipated to significantly impact the health care system and the associated health care costs (Loomba & Sanyal, 2013). The results of this study may help inform future studies by identifying factors associated with increased risk for NAFLD among patients with RA, along with providing incidence rates, time to development of NAFLD, and rates of prevalence. This study is intended to help confirm the harmful effects of NAFLD and its interplay with many chronic conditions, including RA (Lazo & Clark, 2008).

Patients treated with MTX for RA are at risk for the development of NAFLD and fibrosis of the liver (Arena et al., 2012; Kneeman, Misdraji, & Corey, 2011). Biopsies, while a reliable approach to confirm NAFLD are not always feasible nor a sustainable strategy given the increased prevalence of NAFLD (McPherson et al., 2010). It is estimated that using non-invasive tools such as FIB-4 could reduce unnecessary liver biopsies by 50%, thereby saving the invasive procedures only for those with advanced disease thus reducing physical harm and overall costs (McPherson et al., 2010). Early identification and characterization of NAFLD is essential to improve long-term prognosis, potentially averting the development of chronic liver disease in this at risk population (McPherson et al., 2010). Prevention, diagnosis, and management of NAFLD have been identified as an emerging public health issue (Fabbrini et al., 2010; Ray, 2013; Vernon et al., 2011). There is a need for evidence and education to increase public

awareness of NAFLD, support public health practitioners and clinicians, and inform policy, particularly for chronic conditions such as RA associated with significant disease burden (Fabbrini et al., 2010; Ray, 2013; Vernon et al., 2011). Policies are needed to support surveillance programs and also those that foster interdisciplinary approaches for the management of chronic conditions. The potential for social change includes early diagnosis of NAFLD using a novel, less expensive, noninvasive tool and increased awareness of this chronic condition along with the other prevalent chronic diseases.

## Summary

NAFLD is a chronic condition that is emerging as a public health challenge in the U.S. (Argo & Caldwell, 2009). Obesity and metabolic dysfunction are central to the development of NAFLD and this is due to systemic inflammation (Fabbrini et al., 2010). The presence of these and other prevalent conditions associated with NAFLD, such as diabetes, CVD, and dyslipidemia appears to accelerate the development of NAFLD (Argo & Caldwell, 2009). Patients with RA also have a prevalence of these risk factors (Ahmed, 2006; Chung et al., 2008; Crowson et al., 2010). Use of MTX for the treatment of RA has been associated with increased elevation of liver enzymes and fibrosis of the liver (Arena et al., 2012; Sakthiswary et al., 2014). Hence, this study hypothesized that NAFLD may be prevalent in patients with RA, given the overlap of several NAFLD related risk factors and exposure to MTX. However, this association has not been documented or well characterized in the literature for this potentially at risk population. It is most likely that the development of NAFLD may be due to the convergence of several factors related to inflammation, including the use of MTX for patients with RA.

In the next chapter, I cover the research strategies used for the literature review, the theories related to NAFLD, and an extensive review of the literature pertaining to the incidence, prevalence, and factors associated with NAFLD in the general population.

# Chapter 2: Literature Review

NAFLD is described as the next global epidemic and poses an emerging public health challenge in the U.S. (Argo & Caldwell, 2009). NAFLD is a common chronic condition, primarily associated with other prevalent conditions such as obesity and metabolic syndrome, along with diabetes, cardiovascular disease (CVD), and dyslipidemia (Argo & Caldwell, 2009). Patients with rheumatoid arthritis (RA) also have a prevalence of many of these risk factors associated with inflammation (Chung et al., 2008; Crowson et al., 2011; Crowson et al., 2010 ). Additionally, MTX, a standard treatment for rheumatoid arthritis, is a known hepatotoxic drug that can cause increased elevation of liver enzymes and has been associated with the development of NAFLD and fibrosis of the liver (Arena et al., 2012; Sakthiswary et al., 2014). However, this association has not been documented or well characterized in the literature for this potentially at risk population.

This chapter covers research strategies used for the literature review. An extensive review of the literature was conducted pertaining to the theories, incidence, prevalence, and factors associated with NAFLD. In this review the primary focus of the search was for studies conducted in the U.S. however, if such information was not available or was inadequate, then studies from other countries were included to supplement the information. Similarly, NAFLD-related risk factors were presented mostly for the general population and also when this information was available for the RA population.

The purpose of this study was to determine the incidence, prevalence, and factors associated with NAFLD among patients with RA. Literature on NAFLD in the RA

population was limited, thus many of the constructs of this study are based on literature available in the general population. The information about the incidence, prevalence, and risk factors associated with NAFLD in the general population became the basis for this study. Peer-reviewed journal articles were retrieved using the Google Scholar search engine, the CINAHL & MEDLINE databases, and references from guidelines and review articles were also used.

The key search term used was *NAFLD*, along with several thematic terms such as *prevalence, incidence and risk factors related to NAFLD*. Other terms included were: *fatty liver and or NAFLD plus natural history, theories, epidemiology of NAFLD and RA, metabolic syndrome, obesity, diabetes, hyperlipidemia, CVD, and MTX associated laboratory abnormalities.* Studies conducted between 2009 and October 2015 was reviewed. Older publications were included if they were original works or were empirical or significant publications that were referred to in the more recent literature, or provided some information not available in the recent studies. Inclusion criteria for the literature review were publications related to NAFLD in the adult general population, RA population, clinical studies, observational studies, treatment guidelines, and review articles. Excluded from the review were studies related to NAFLD where alcohol use was eluded to but was not clearly defined, research in the pediatric population, and publications about other secondary causes of NAFLD as identified in the clinical guidelines for the diagnoses, treatment and management of NAFLD (Chalasani et al., 2012). Also excluded were studies in languages other than English, and laboratory (animal model, genetic, or basic science) studies.

## Theoretical Framework

Theories related to NAFLD are seated in principles of biological plausibility, of which the *two hit* theory (Day & James, 1998) is the most frequently referenced theory to explain NAFLD (Lim et al., 2010). This theory postulates that accumulation of fatty acids incites the development of fatty liver (first hit) which then sensitizes the liver (second hit) leading to inflammation and subsequently to hepatic injury and development of fibrosis of the liver (Day & James, 1998; Lim et al., 2010). The first hit is presumed to occur as a result of metabolism of fructose leading to the production of de novo lipogenesis and intrahepatic lipids, which inhibits the mitochondrial β-oxidation process of the long-chain fatty acids (Lim et al., 2010). The second hit proposes that the accumulation of these hepatic lipids interacts with hepatic antioxidants leading to molecular instability causing reactive oxidation and inflammation. The end result of this process is the formation of triglycerides, insulin resistance, the onset of hyperglycemia, and eventually liver steatosis (Lim et al., 2010).

Obesity and metabolic dysfunction are two factors central to the development of fatty liver, specifically, due to the increased levels of intrahepatic triglycerides (IHTG) (Fabbrini et al., 2010).The result of high levels of IHTG is theorized to alter the metabolism of fatty acids, glucose, and lipoproteins leading to systemic inflammation (Fabbrini et al., 2010). Development of NAFLD is complex and appears to be rooted in these processes, specifically in the second phase when the liver is overwhelmed with an overabundance of fatty hepatic lipids leading to inflammation (Lim et al., 2010). Thus, the association of obesity and metabolic dysfunction with the accumulation of

intrahepatic lipids and the subsequent cellular changes in the liver due to inflammation is central to the development of NAFLD. Similar pathogenic changes in the liver are seen in alcoholic patients, but with NAFLD, these changes are unrelated to alcohol use (Chen et al., 2011; Comar & Sterling, 2006; Lim et al., 2010). The pathophysiological alterations in the liver are thought to be due to the oxidative process due to metabolic syndrome and obesity along with the coexistence of other interdependent chronic conditions such as diabetes, dyslipidemia and CVD among others (Chitturi & Farrell, 2001; Lim et al., 2010; Utzschneider & Kahn, 2006). Thus, alterations in lipid and glucose metabolism, metabolic dysfunction along with many other chronic conditions are culprit pathways central to systemic inflammation associated with NAFLD.

RA is a chronic disease associated with systemic inflammation and has also been associated with NAFLD (Arena et al., 2012). Similar theories relating to inflammation have been hypothesized to explain the relationship between NAFLD and RA (Ahmed, 2006). Specifically proposed is the notion that the overlapping pathogenic changes seen in these two chronic diseases are due to inflammation (Ahmed, 2006). The result of these changes is manifested as hyperlipidemia, insulin resistance, as well as other altered inflammatory pathways unique to RA (Ahmed, 2006). Pathways unique to RA are also related to inflammation; these are fibrogenic and apoptotic responses expressed by specific inflammatory cytokines, such as tumor necrosis factor alpha (Ahmed, 2006). Such responses are presumed to contribute to the development of NAFLD (Ahmed & Byrne, 2005; Kitade, Yoshiji, & Kojima, 2008). An additional proposed link to NAFLD and RA is related to the altered glucose metabolism attributed to chronic inflammation

(Svenson, Lundqvist, Wide, & Hällgren, 1987). This alteration in glucose metabolism may be due to the intensity of the underlying inflammatory burden of chronic conditions such as RA resulting in insulin resistance and diabetes (Ahmed, 2006).

Another line of reasoning linking RA and NAFLD relates to treatment of RA with MTX. MTX is a standard treatment for patients with RA, and has been associated with liver enzyme elevation, and may also play a role in the development of NAFLD (Arena et al., 2012). Visser and Heijde (2009) conducted a systematic review of the literature from 1950 to 2007 and assessed the risk of liver toxicity associated with MTX use in patients with RA and psoriatic arthritis. During a period of three years the incidence rate was 13/100 per patient-years. About a third of the patients had elevated liver enzymes after initiation of treatment with MTX (Visser & Heijde, 2009) A more recent study of 978 subjects with long term exposure to MTX also supports these findings (Sakthiswary et al., 2014). This study reported that the cumulative MTX dose was associated with elevated liver enzymes and was an independent predictor for NAFLD. Visser and Heijde (2009) also reviewed studies that reported NAFLD results from liver biopsies for RA patients treated with MTX. During a period of four years, the risk of mild fibrosis was 15.3%, 1.3% for severe fibrosis, 0.5% for cirrhosis of the liver, and the risk prior to treatment was 9%, 0.3% and 0.3% respectively. For these reasons, it can be concluded that liver enzyme elevation due to MTX use was associated with an increased risk for NAFLD, and that there may also be an underlying risk for RA patients and this may be irrespective of MTX use.

Theories related to NAFLD are complex and appear to be linked to chronic inflammation affecting the liver. It is most likely that NAFLD may be due to a confluence of several factors related to inflammation and altered lipid metabolism, metabolic syndrome, obesity, alteration of glucose metabolism, and for patients with RA the use of MTX. These factors and relationships are supported by the *two hit* theory and provides the core theoretical framework for this research study.

## Conceptual Framework

Understanding of the theories, pathways, and relationships related to the development of NAFLD is still evolving (Erickson, 2009). NAFLD is a chronic disease with many complex interacting obesity and metabolic related pathways, as well as environmental and genetic factors. Erickson diagrammatically depicted these relationships in an attempt to summarize the current understanding of these complex interactions based on the strength of available evidence (see Figure 1). According to Erickson's framework, any and all factors related to the metabolic pathway play a significant role in the development of NAFLD. Some theorists suggest that these relationships may be bidirectional; that NAFLD is thought to be in the causal pathway of inflammation, hyperlipidemia, obesity, and insulin resistance; however, this assertion lacks substantial evidence and thus is considered hypothetical (Azad & Quayum, 2007; Erickson, 2009; Vanni et al., 2010).

Factors such as genetics, gender, diet, and environmental factors are illustrated in the diagram with unidirectional arrows as they have been uniquely linked to NAFLD (Erickson, 2009; Puppala, Siddapuram, Akka, & Munshi, 2013). Studies have reported on

the predisposition to NAFLD within families, certain ethnic groups, and some specific variant genes have been linked to NAFLD (Erickson, 2009; Puppala et al., 2013). The relationship between NAFLD and factors such as insulin resistance and diet are related to obesity, and are thought to be a result of diets rich in simple carbohydrates, saturated fats, and the consumption of highly processed foods, coupled with the sedentary lifestyle (Thoma, Day, & Trenell, 2012). Other factors associated with NAFLD such as inflammation, hyperlipidemia, insulin resistance, and obesity are thought to be bidirectional(Erickson, 2009). Erickson's conceptual model has been applied in several studies. Alisi et al. (2011), sought to identify determinants of diet-induced NAFLD and uses the Erickson's conceptual model to explain the complex interrelationships between NAFLD related factors. Similarly Chung et al. (2012) and Lin, Chou, Huang, & Chiou, (2011) assessed NAFLD rates and cited Erickson in their research studies.

This study was fundamentally based on the hypothesis that NAFLD and RA have shared risk factors associated with inflammation such as metabolic syndrome, hyperlipidemia and insulin resistance. Thus the interplay between these factors and NAFLD seen in the general population may also be present in the RA population. There is a gap in the literature establishing NAFLD in the RA population, the risk factors and the relationships between these risk factors. In summary, Erickson's conceptual model provides a framework to establish these relationships between the risk factors identified for this study and the nature of their relationships with NAFLD. Thus, the *two hit* theory further clarified by Erickson's conceptual framework helps explain these relationships with NAFLD and provides the theoretical context for this study.

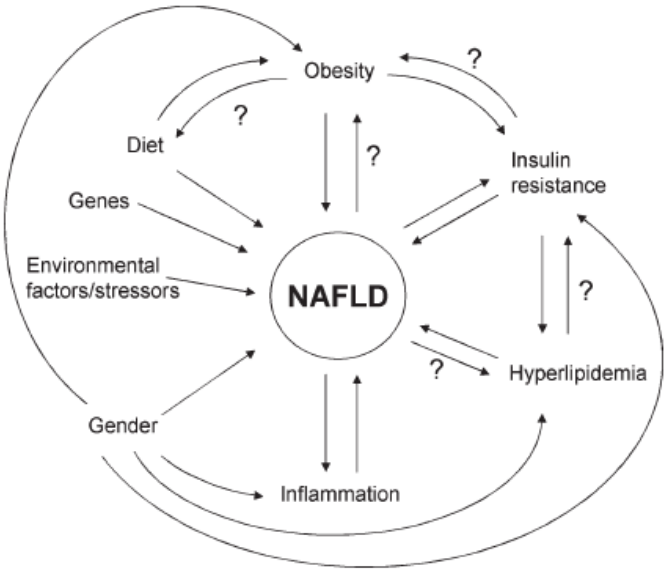



*Figure 1. Erickson's NAFLD conceptual model (2009). Reprinted with permission.*

## Background

The purpose of this study was to determine the incidence, prevalence, and factors associated with NAFLD among patients with RA. RA is a chronic disease associated with systemic inflammation. RA is characterized by joint swelling, joint tenderness, and destruction of the synovial joints (Aletaha et al., 2010). RA disease activity is measured by using several clinical measures such as swollen and tender joint counts, patient and physician global assessment of disease activity, patient assessment of pain, and also may include laboratory tests for acute phase response to inflammation (Aletaha, Funovits, Keystone, & Smolen, 2007).

In contrast, NAFLD is an asymptomatic condition, commonly associated with liver enzyme elevation (AST and ALT) and some may have non-specific vague right upper quadrant abdominal discomfort, fatigue, and malaise (Torres, Williams, &

Harrison, 2012). NAFLD is diagnosed using clinical and pathological criteria for liver injury or disease, that can range from the presence of fatty liver, simple steatosis, nonalcoholic steatohepatitis (NASH), liver cirrhosis to hepatocellular carcinoma (Torres et al., 2012).

RA and NAFLD have some shared risk factors such as age, gender, and race/ethnicity, along with clinical features such as metabolic syndrome, obesity, diabetes, and dyslipidemia (Ahmed, 2006; Chalasani et al., 2012). Several of these clinical risk factors are associated with inflammation (Lim et al., 2010). Additionally, patients using MTX may be at an increased risk for NAFLD. It is not known if NAFLD may be prevalent among patients with RA.

Liver biopsy is the conventional technique used to decisively confirm NAFLD. However, this is an invasive procedure associated with discomfort and even the risk of death (Shah et al., 2009a). Additionally, it is not always clear when a biopsy is needed. Given this inherent risk and the increasing prevalence of NAFLD, biopsies are not always feasible nor a long term sustainable strategy (Shah et al., 2009a).

Thus, there is a need to identify patients at risk for NAFLD using easily available non-invasive tools such as FIB-4 score (McPherson et al., 2010). FIB-4 is a noninvasive tool developed to identify NAFLD. It is validated to ascertain the various stages of NAFLD using laboratory liver function tests (AST, ALT levels) and platelet counts along with age (Shah et al., 2009a). The key feature of this composite score is that it uses commonly available measures, thus enabling clinicians to incorporate this tool easily into their routine clinical practice.

This study addressed this gap using this novel noninvasive tool (FIB-4) to determine the incidence, prevalence, and factor associated with NAFLD using retrospective data collected from a well established registry of patients with RA (Curtis et al, 2013; Curtis et al., 2010; Kremer, 2005). The rationale for using this registry includes its rich history of epidemiological studies conducted to address a variety of research questions that range from establishing prevalence rates to characterizing safety, effectiveness, and treatment patterns. As of April 2015, there have been over 50 manuscripts and 140 abstracts published using data from this registry (Corrona.org. 2015). There have been several studies conducted similar to this study. Furst et al. (2009) conducted a study to determine the prevalence of low hemoglobin. Whereas Greenberg et.al (2011) conducted a study to determine cardiovascular risk among patients with RA, and Fisher et.al (2012) validated malignancy rates by adjudicating the rates in the registry with the patient charts. Additionally, this is the only registry in the U.S. that has longitudinal data for close to 40,000 patients, where ascertainment of the incidence, prevalence, and risk factor for NAFLD is feasible (Corrona.org. 2015).

The literature was reviewed for the rates and factors associated with NAFLD and this review reflects information from studies conducted in diverse populations and countries using an assortment of tools to determine the presence of NAFLD. Aside from the challenges posed by the various tools used, a variety of standards were used to ascertain the presence of the disease. Some of these tools are more sensitive than others, for example, using the gold standard (biopsy) a more sensitive approach for the diagnosis of NAFLD compared to liver enzyme elevation alone. The heterogeneity of information

related to NAFLD proved to be a pervasive challenge throughout this literature review. Thus, to provide clinical perspective and ensure alignment with the standard of care, evidence and expert opinions provided for the diagnosis and management of NAFLD offered in the guidelines for clinicians was used as a guiding reference (Chalasani et al., 2012).

Given the gap in the literature for NAFLD in the RA population, information from the general population was reviewed. The following sections in this literature review describe the tools and measures used to stage NAFLD, followed by a brief review of the epidemiology of RA, prevalence of overlapping NAFLD risk factors in the RA population. An extensive review of the literature on the incidence, prevalence of NAFLD and NAFLD risk factors in the general population was conducted.

## Tools and Measures for Nonalcoholic Fatty Liver Disease

NAFLD fits into a spectrum of fatty liver disease that starts with the development of simple fatty liver, progressing to steatohepatitis (NASH), fibrosis, cirrhosis, and for some, leading to cancer (Chalasani et al., 2012). However, these disorders characteristically occur in the absence of significant alcohol use (Chalasani et al., 2012). Each stage of the disease is associated with histological changes in the liver (Kleiner et al., 2005). Fibrosis is staged from 0 to 4: with stage 0 = absence of fibrosis; stage 1 = peri sinusoidal or portal changes; stage 2 = peri sinusoidal and doorway/peri portal changes; stage 3 = septal or bridging fibrosis; and stage 4 = cirrhosis of the liver (Kleiner et al., 2005). A number of diagnostic tools can be used to identify the presence of NAFLD. These include using laboratory values (liver enzymes - ALT and AST); imaging tools

such as ultrasound (U.S.), computerized tomography (CT), magnetic resonance spectroscopy (MRS), and magnetic resonance imaging (MRI), biopsies or in a combination of these tools (Chalasani et al., 2012). There are significant variations in the rates of NAFLD depending on the tools used. Highest rates for NAFLD was reported using histological criteria from liver biopsies (20% to 51%), followed by liver ultrasound with rates that ranged 17% to 46%; and MRS was about 31% (Chalasani et al., 2012). The lowest rates [7% to 11%] were reported when liver enzyme levels were used alone without including imaging or histological criteria (Chalasani et al., 2012).

The FIB-4 index is another tool that is used to determine the NAFLD. It uses commonly available measures such as age, liver enzymes (AST, ALT), and platelet counts to determine the presence of NAFLD. FIB-4 score is calculated by using the following formulae: age [years] × AST [U/L]) / (platelet [$10^9$] × √ALT [U/L] (Shah et al., 2009a). FIB-4 score corresponds with the various stages of fibrosis as shown in Table 7 (Shah et al., 2009a; Vallet-Pichard et al., 2007).

Liver biopsy is best able to elucidate these subtle pathological changes in the liver (Chalasani et al., 2012). However, its scalability is limited and is associated with procedure related morbidity, mortality, costs and sampling error, and thus highlights the need to use reliable and valid non-invasive tools to diagnose NAFLD (Chalasani et al., 2012; Shah et al., 2009a). There are several noninvasive tools available; seven of these tools were compared for their reliability and validity to determine the presence of fibrosis (Shah et al., 2009b). Based on results of sensitivity, specificity, positive predictive value, and negative predictive value, the authors concluded that FIB-4 index was superior to the

other markers and deemed it as a valid and reliable tool to measure the absence or presence of advanced fibrosis. Specifically, FIB-4 index had an 80% positive predictive value for advanced fibrosis with FIB-4 score ≥ 2.67 and 90% negative predictive for FIB-4 index ≤ 1.30. Others have evaluated the accuracy of the FIB-4 index in other chronic liver conditions, corroborating the FIB-4 score to liver biopsies and deemed that the tool was reliable (Mallet et al., 2009; Vallet-Pichard et al., 2007).

The dependent variable for this study is the presence of NAFLD, determined by using the FIB-4 index. A FIB-4 score of <1.3 indicates the absence of disease and the presence of NAFLD with a score of ≥ 1.3. FIB-4 index uses commonly available clinical measures (ALT, AST, platelet counts, and age), and since these measures are collected in the RA registry, this was the tool used to determine the presence of NAFLD for this study.

## Epidemiology of Rheumatoid Arthritis (RA)

A review of the literature found only two studies that reported on the prevalence of NAFLD in the RA population, of which one was an abstract. This was a small study of 100 RA patients in the U.S. and the researchers used ultrasound to determine the presence of NAFLD (Bhambhani et al., 2006).The prevalence rate for the RA group was 23% compared to 15% among those without RA; the significance of this difference was not reported. The other study was conducted in Singapore during the years of 2006 to 2013 and also used ultrasound to determine NAFLD (Sakthiswary et al., 2014). The intent of this study was to determine the risk factors associated with MTX use and NAFLD among patients with RA. Only patients with elevated liver transaminitis while

on MTX use were included in the analysis ($N = 978$). The NAFLD prevalence rate was 4.7% in this study. This rate was much lower compared to the Bhambhani et al., (2006) study, which was conducted in U.S. and in a broader RA population. No studies were found that reported on the incidence of NAFLD in this population. Given the paucity of studies in RA, information about the prevalence of NAFLD in the RA population remains a gap in the literature.

**Prevalence of NAFLD Related Risk Factor: RA Population**

There are certain risk factors associated with NAFLD seen in the general population were also prevalent in the RA population. For example, some of the demographic factors such as age, gender, and ethnicity are also risk factors for RA. Likewise, there is increased prevalence of obesity, MetS, hypertension, dyslipidemia, hypertriglyceridemia, and insulin resistance in this population also (Chung et al., 2008; Crowson et al., 2011). Following is a review of the prevalence for each of these factors in the RA population.

Obesity a known risk factor for NAFLD is also prevalent in the RA population. Studies that reported on the prevalence of obesity in the RA population ranged from 21% to 65% and almost three times more among those with RA compared to those without the disease (Dessein et al., 2002) as shown in Figure 2.

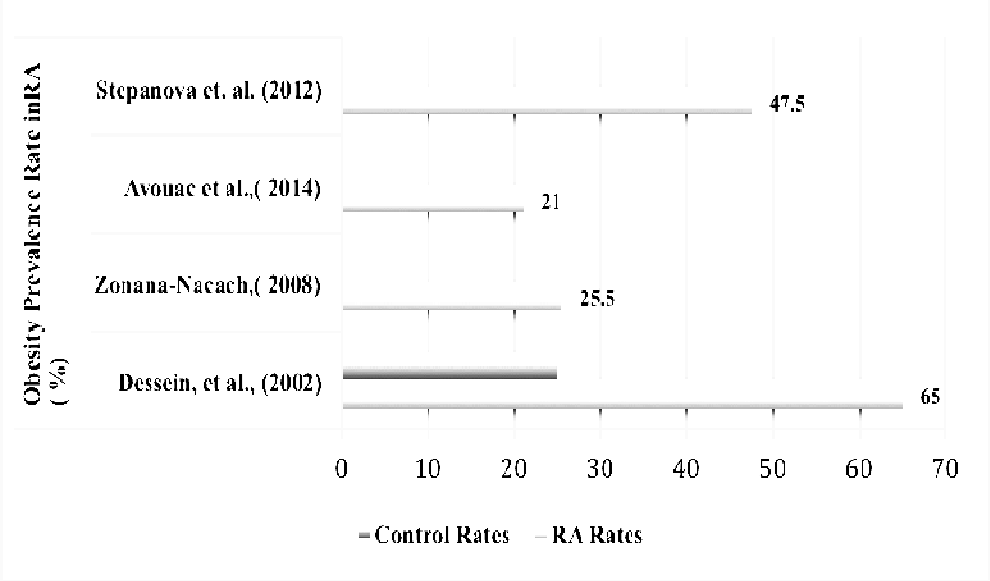



*Figure 2. Prevalence in the RA Population (%): Obesity*

Of the many NAFLD related risk factors, MetS plays a key role in the development of NAFLD (Chalasani et al., 2012). The presence of one or more of the many Mets related factors could contribute to the development of NAFLD (Erickson, 2009). Patients with RA appeared to have higher rates of MetS compared to those without the disease as illustrated in Figure 3. Prevalence rates of MetS ranged from 18 % to 45 % among those with a diagnosis of RA. Most studies reported higher rates in the RA group, with the exception of two studies. These studies reported higher rates for the control group, however, both were conducted outside of the U.S. (Karimi, Mazloomzadeh, Kafan, & Amirmoghadami, 2011; Sahebari et al., 2011). Studies carried out in the U.S. comparing RA patients to a control group reported higher rates in the RA group and these rates ranged from 33% to 42% (see Figure 3).

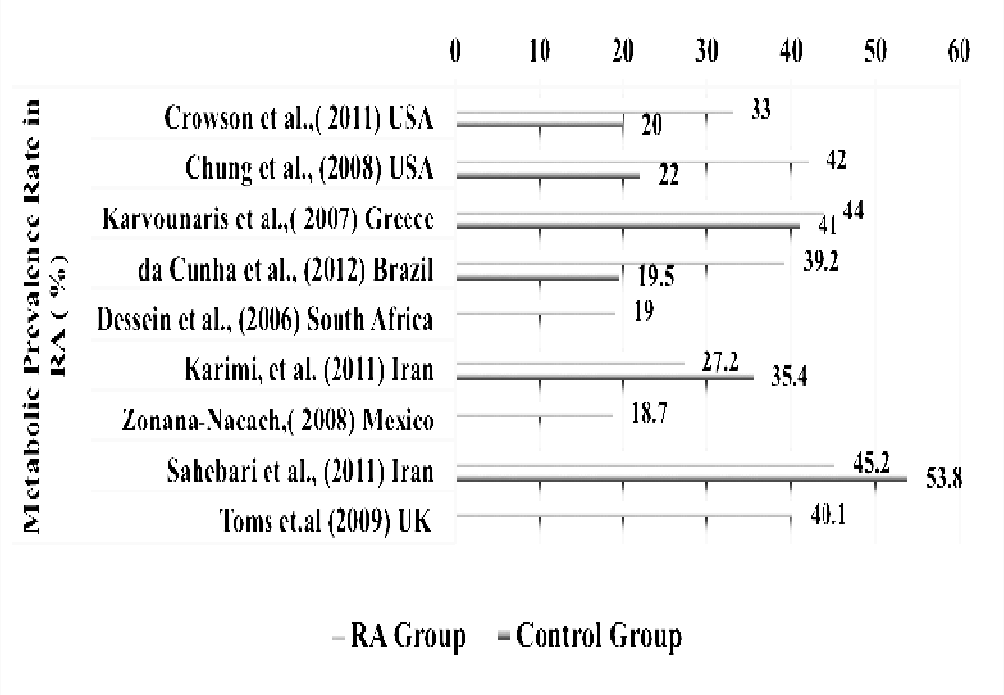



*Figure 3. Prevalence in the RA Population (%): Metabolic Syndrome*

Hypertension is a prevailing problem for patients with RA and is also a key contributing factor associated with MetS (Grundy et al., 2005). Rates were consistently higher for patients with RA compared to those in the control group as shown in Figure 4. Prevalence rates of hypertension ranged from 31% to 80% in this population.

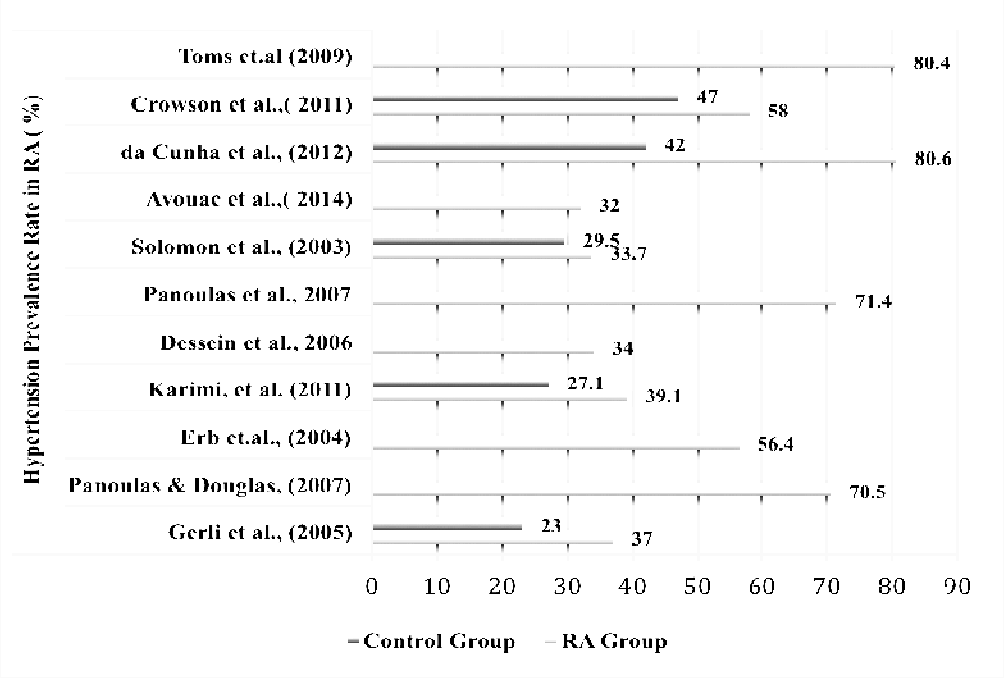



*Figure 4. Prevalence in the RA Population (%): Hypertension*

Dyslipidemia or hypercholesterolemia, terms often used interchangeably, is another risk factor associated with NAFLD and also prevalent in the RA population(Chalasani et al., 2012; Chung et al., 2008; Crowson et al., 2011). Gerli et al. in 2005 reported a dyslipidemia rate of 12.9% among patients with RA compared to 8% in the control group, however this difference was not significant. A second study reported a similar rate of 16% among patients with RA (Avouac et al., in 2014 ). Of the lipid parameters, hypertriglyceridemia and low levels of HDL are of interest as they are used to define the presence of MetS and also because they have been uniquely associated with NAFLD (Chalasani et al., 2012; Grundy et al., 2005).

Rates for hypertriglyceridemia in the RA population ranged from 16% to 38% as depicted in Figure 5. Two studies compared rates of hypertriglyceridemia for those with

and without RA (Crowson et al., 2011; da Cunha et al., 2012) . Even though the rates of hypertriglyceridemia were higher for patients with RA in both the studies, these differences were not significantly different compared to the control group. One study conducted in Iran showed that the control group had statistically significant higher rates of hypertriglyceridemia compared to the RA group (Karimi et al., 2011). This study also reported a statistically significant higher prevalence of low HDL levels in the control group. Prevalence rates for low HDL ranged from 24% to 44.6% (see Figure 5). Those with the RA group had higher prevalence of low HDL, but these rates were not significantly different from the control group (Crowson et al., 2011; da Cunha et al., 2012). Based on this review, most studies reported higher prevalence of hypertriglyceridemia and low levels of HDL among patients with RA when compared to patients without the disease.

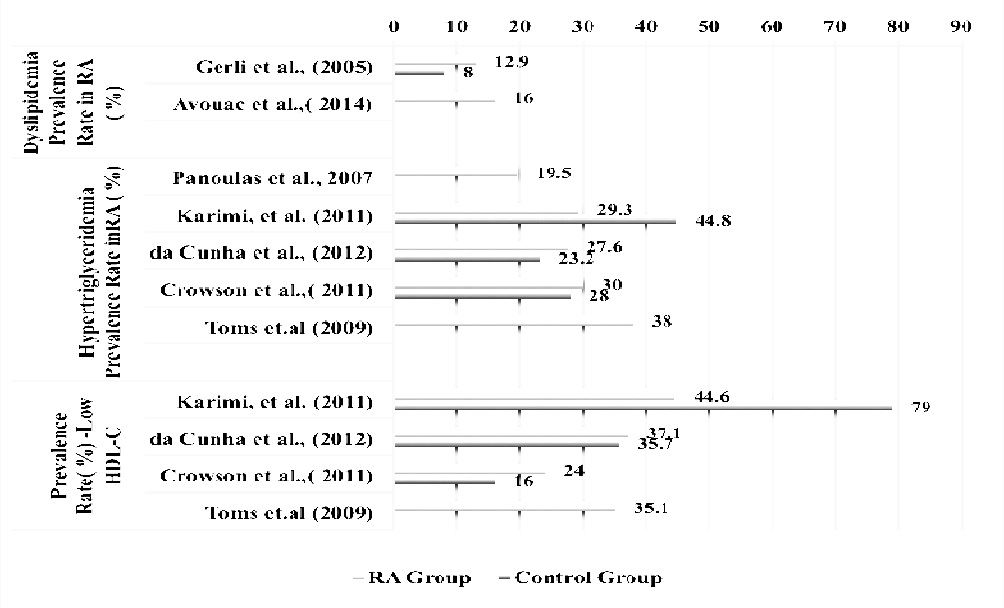



*Figure 5.* Prevalence in the RA Population (%): Dyslipidemia

Insulin resistance is another NAFLD risk factor related to high levels of systemic inflammation and is also associated with RA (Dessein, Woodiwiss, Joffe, & Norton, 2007). Several studies have reported higher rates of insulin resistance in the RA population, a factor associated with metabolic syndrome, and also thought to be a precursor for diabetes (Dessein, Joffe, Stanwix, Botha, & Moomal, 2002; Svenson et al., 1987). Despite the recognition of insulin resistance in the RA population (Dessein et al., 2006), more often the prevalence rates of diabetes was reported in most studies. Rates for diabetes ranged from 4.7% to 16.1% as shown in Figure 6. Two studies compared rates of diabetes for those with and without RA and reported mixed results. Del Rincón & Williams (2001) reported higher rates of 16.1% for RA compared 9.5% for those without ($p < 0.001$). However, Solomon et al., (2003) reported similar rates of diabetes for both groups (4.7% vs. 4.9%) respectively.

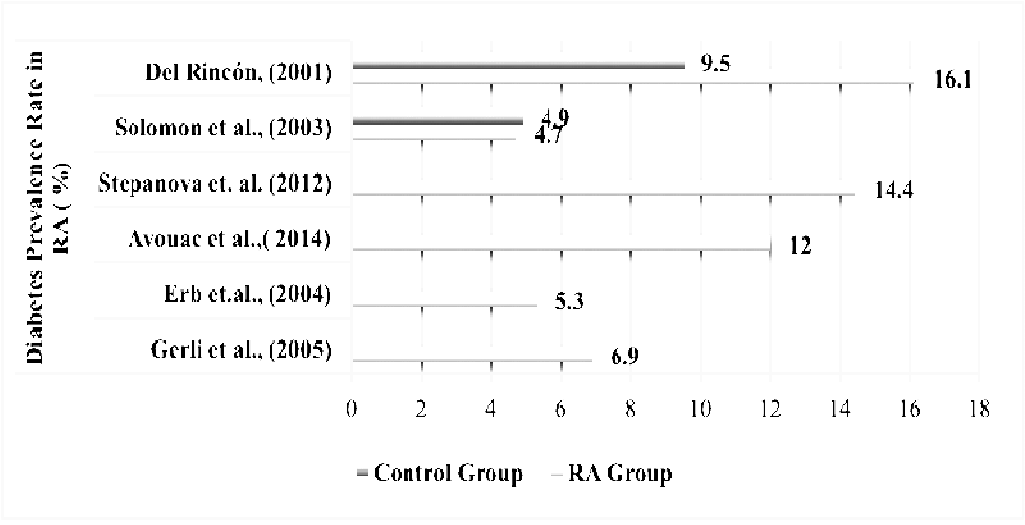



*Figure 6. Prevalence in the RA Population (%): Diabetes*

## Epidemiology of NAFLD: General Population

Unlike the review of literature related to NAFLD in the RA population, there was a wealth of information available in the general population. The majority of the studies reviewed were cross-sectional observational studies. Some were carried out in large populations, and others were smaller regional studies or studies conducted in select populations (e.g. patients from liver or lipid clinics). Tables 1 through 3 lists the studies that reported on the NAFLD prevalence rates in U.S. (Table 1), followed by Asia (Table 2) and then the rest of the world (Table 3). Table 4 covers studies that reported on the incidence rates and factors associated with NAFLD. The tables also include the details of the location where the studies were conducted, the study design, sample size, population, and the tools used to diagnose the presence of NAFLD. The results on the incidence and prevalence rates from these studies are graphically shown under each of the topic sections.

### General Population NAFLD Prevalence Rates: United States

Most of the studies conducted in the U.S. that reported NAFLD prevalence rates were cross-sectional observational studies with sample sizes that ranged from 70 to 20,050 (see Table 1). Most of the studies were conducted in the general population; several of the larger studies used nationally representative samples (e.g., Third National Health and Nutrition Examination Survey from 1988 -1994). As shown in Table 1, these studies included a variety of assessment tools: CT scan, MRI, MRS, ultrasound, liver enzymes, and biopsy to determine the presence of NAFLD.

Table 1

*NAFLD Prevalence Studies in the General Population*: U.S

| First Author | Year Published | Location | Study Design | Sample | Population | Age (years) | Diagnosis of NAFLD |
|---|---|---|---|---|---|---|---|
| Church | 2006 | U.S. Dallas | Prospective1996 - 2001 | 218 | Healthy Men only Non-Hispanic whites | Adults (30 –75) | CT, Liver Enzymes |
| Browning | 2004 | U.S. Dallas | Longitudinal Study, Prospective (duration not reported) | 2,287 | Heart Clinic Multiple Ethnic | Adults | MRS, Liver Enzymes |
| Lazo | 2011 | U.S. | Prospective cohort NHANES III: 1988-94; follow up to 2006. | 11371 | General population Multiple Ethnic | Adults (20 – 74) | U.S., Liver Enzymes |
| Lazo | 2013 | U.S. | Prospective cohort NHANES III: 1988-94; 2009 - 2010 | 12454 | General Population Multiple Ethnic | Adults | U.S. |
| Stepanova | 2012 | U.S. | Prospective cohort NHANES III: 1988-94 | 20,050 | General Population Multiple Ethnic | Adults | U.S. |
| Ong | 2008 | U.S. | Prospective cohort NHANES III: 1988-94 follow up to 2000 | 12,822 | General Population Multiple Ethnic | Adults (>17 years) | Liver Enzymes |
| Tran | 2006 | U.S. | May 2001 - June 2003 | 70 | Healthy Donors | Adults | CT or MRI |
| Williams | 2011 | U.S. Houston | Prospective Cohort Jan 2007 - March 2010 | 328 | General Population Multiple Ethnic | Adults (18-70) | U.S., Biopsy |
| Smits | 2013 | U.S. | NHANES III 1988 -1994 | 3846 | General Population Multiple Ethnic | Adults | U.S. |

NHANES –National Health and Nutrition Examination Survey; National MRI -Magnetic Resonance Imaging; CT-Computed Tomography; MRS -Magnetic resonance spectroscopy; Liver Enzymes –alanine aminotransferase levels (ALT), aspartate aminotransferase (AST); U.S. – Ultrasound

The prevalence rates for NAFLD for the general population in the U.S. are illustrated in Figure 7, and the rates ranged from 11% to 46%. Several of the larger more recent studies were conducted using data from Third National Health and Nutrition Examination Survey, data collected from 1988 to 1994 (NHANES III). Some of these studies also included additional follow up data through 2010 as shown in Table 1. The NAFLD prevalence rates were varied using the NHANES III data, most likely due to the varying definitions used to ascertain presence of NAFLD. For example, Lazo et al. in 2013 with a sample of 12,454 participants reported a rate of 19%, (95% CI [17.5, 20.6]). Smits et al. (2013) reported higher NAFLD rates of 30%. Some of the earlier studies reported similar rates to the Smits et al., (2013) study. For example, in 2004 from a large cross-sectional population-based sample of adults ($N = 2287$) using proton nuclear magnetic resonance spectroscopy reported a NAFLD rate of 31% (Browning et al., 2004). Tran et al., (2006) reported slightly higher rate of 38.5% in 2006; however this was a small study of living liver donors ($N = 70$) and used biopsy to determine NAFLD. Williams et al. (2011) reported the highest NAFLD prevalence rate of 46% in the U.S. Rates derived from a small prospective cohort study of middle-aged adults using ultrasound to determine NAFLD ($N = 328$). The lowest NAFLD prevalence rates of 11% was reported by Church et al., in 2006, however, this study only included non-Hispanic healthy men ($N = 238$). In summary as shown in Figure 7, prevalence of NAFLD was wide with about 10% to 50 % of the general population at risk for NAFLD in the U.S. It is not clear if this variation was due to the tools or criteria used to determine the presence of NAFLD or the populations that were studied.

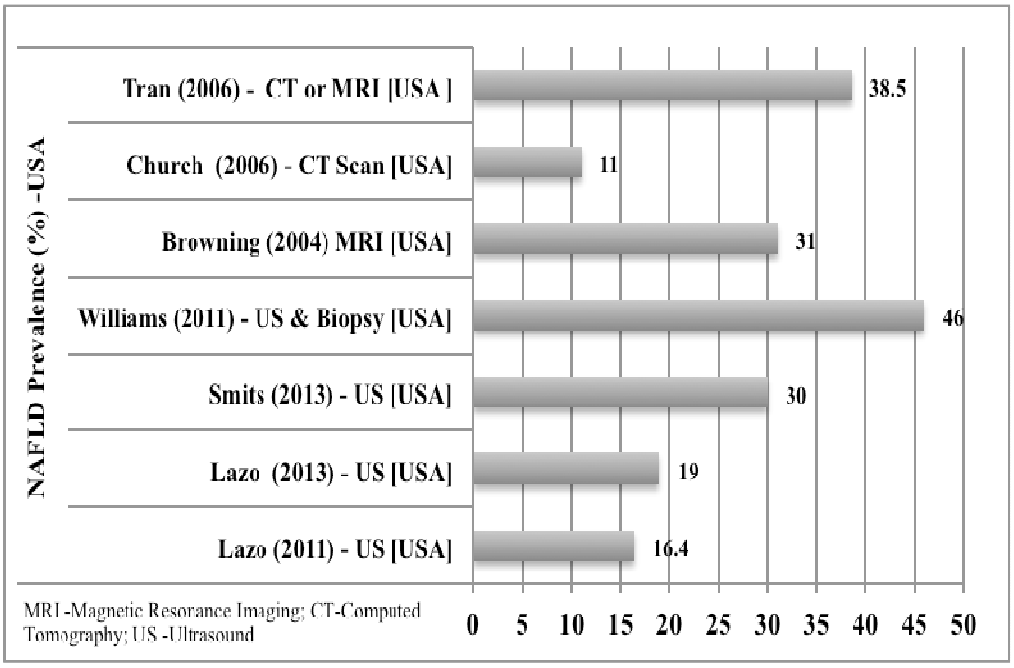



*Figure 7*. NAFLD Prevalence Rates in the General Population (%): U.S.

**General Population NAFLD Prevalence Rates: Asia**

Similar studies were conducted in Asia as shown in Table 2. However, there was a mix of large population and small regional level studies, with sample sizes that ranged from *N* = 768 to 29,994. Unlike the studies conducted in the U.S., most of these studies used ultrasound, including one study in Japan where FIB4-score was used to determine NAFLD (Eguchi et al., 2012).

Table 2

*NAFLD Prevalence Studies in the General Population: Asia*

| First Author | Year Published | Location | Study Design | Sample | Population | Age (years) | Diagnosis of NAFLD |
|---|---|---|---|---|---|---|---|
| Kim | 2004 | Korea | Apr 2001 - Jun 2001 | 768 | General population | Adults (>30) | Ultrasound |
| Kwon | 2012 | Korea | Retrospective Oct 2003 - Dec 2010 | 29,994 | General population | Adults (>18) | Ultrasound |
| Oh | 2006 | Korea | Jan - Dec 2004 | 40196 | General population | Adults | Ultrasound |
| Lee | 2010 | Korea | Population Cohort Mar 2006 - May 2007 | 3768 | Population | Adults (20–75) | Ultrasound |
| Zhou | 2007 | China | Cross-sectional Apr - Nov 2005 | 3543 | General Population | Adults (>18) | Ultrasound |
| Fan | 2007 | China | Prospective Cohort 1995 - 2002 | 14646 | Employee | Adults (>18) | Ultrasound |
| Fan | 2005 | China | October 2002 to April 2003, | 3175 | General population | >16 years | Ultrasound |
| Shen | 2003 | China | Sept 1 - Nov 30, 1999 | 4009 | Employee | Adults (20-81) | Ultrasound |
| Hu | 2012 | China | Cross-sectional | 7152 | Employee Population | Adults (18 - 65) | Ultrasound |
| Xu | 2013 | China | Prospective 5 Year follow up | 6905 | Employee Population | Adults | Ultrasound |
| Yan | 2013 | China | Cross-sectional | 3762 | General population | Adults (>20) | Ultrasound |

Table 2
*NAFLD Prevalence Studies in the General Population: Asia* (*table continues)*

| First Author | Year Published | Location | Study Design | Sample | Population | Age (years) | Diagnosis of NAFLD |
|---|---|---|---|---|---|---|---|
| Li | 2009 | China | Cross-sectional Jan - Dec 2007 | 9094 | Employed population | Adults (>18) | Ultrasound |
| Shi | 2011 | China | Cross-sectional | 6 043 | General Population | Adults | Ultrasound |
| Wong | 2011 | China | Cross-sectional | 1013 | General Population | Adults | MRS |
| Chen | 2008 | China | Jan 2005 to Jul 2007 | 26527 | General Population) | Adults | Ultrasound |
| Chen | 2006 | Taiwan | Cross-sectional | 3245 | General population | Adults | Ultrasound |
| Lin | 2005 | Taiwan | Jul to Sept 2003 | 2025 | Employee Men only | Adults (22 - 65) | Ultrasound |
| Hamaguchi | 2005 | Japan | Prospective observational Jan - Dec 2001 | 4401 | General population | Adults (21 - 80) | Ultrasound |
| Jimba | 2005 | Japan | Sept 2002 - Feb 2003 | 1950 | General population | Middle aged adults | Ultrasound |
| Eguchi | 2012 | Japan | 2009- 2010 | 8352 | General population | Adults | FIB-4 |

MRS -Magnetic resonance spectroscopy; FIB-4 - Fibrosis 4 Score

NAFLD prevalence rates for studies carried out in Asian countries ranged from 7% to 40%, as illustrated in Figure 8. More studies reported rates greater than 18%, the lowest rate was reported by Xu et al., (2013) of 7%. This prevalence rate is closer to the rate reported by the study conducted in Singapore in the RA population by (Sakthiswary et al., 2014) of 4.7%. Eguchi et al., (2012) study, used FIB-4 score to determine the presence of NAFLD and reported a prevalence rate of 29.7%, similar rates to several of the other studies that used ultrasound. It is not known if the wide variation in rates seen in the Asian countries were due to the tools and definitions used to measure NAFLD, regional differences or the underlying study populations. Nevertheless, the spread of prevalence rates in Asia was similar to the rates seen in the U.S. (see Figure 7). FIB-4 yielded results similar to other tools when used to determine the presence of NAFLD.

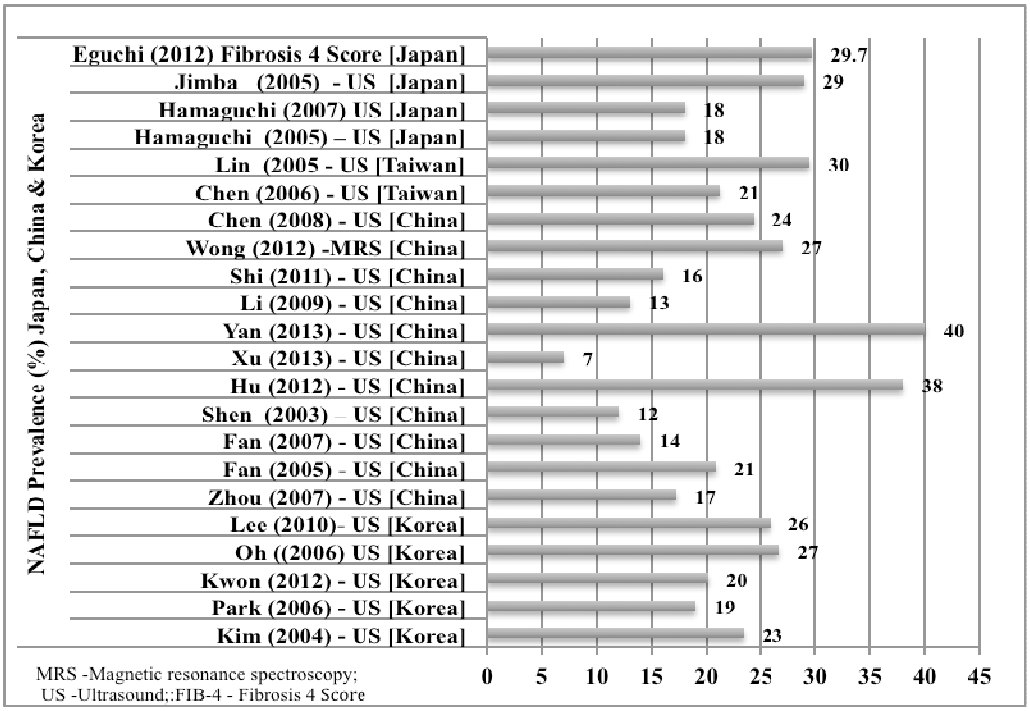



*Figure 8.* NAFLD Prevalence Rates in the General Population (%): Asia

**General Population NAFLD Prevalence Rates: Rest of the World**

Compared to the studies conducted in the U.S., studies from the rest of world were smaller *(N* of 134 to 3012) as shown in Table 3. NAFLD prevalence rates ranged from 17% up to 78.8% as depicted in Figure 9. The highest rate of NAFLD was 78.8% from a study conducted in Israel (Soresi et al., 2012). The authors attributed the rate to higher background prevalence rates of metabolic syndrome and hyperlipidemia in this population. The spread of prevalence rates for NAFLD in the rest of the world was mostly similar to the rates seen in the U.S.

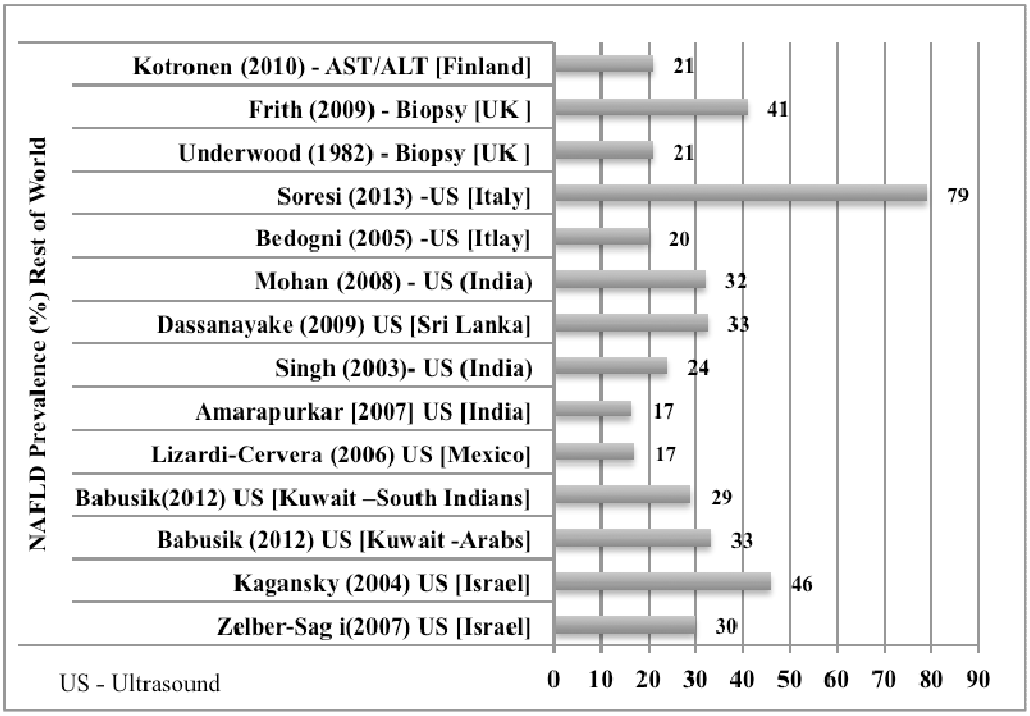



*Figure 9. NAFLD Prevalence Rates in the General Population (%): Rest of the World*

Table 3

*NAFLD Prevalence Studies in the General Population: Rest of the World*

| First Author | Year Published | Location | Study Design | Sample | Population | Age (years) | Diagnosis of NAFLD |
|---|---|---|---|---|---|---|---|
| Zelber-Sagi | 2007 | Israel | Prospective cohort 2003–2004 7 year follow up | 349 | General population | Adults (24–70) | Ultrasound |
| Kagansky | 2004 | Israel | Prospective study Aug 1, 2001 - Feb 28, 2002 | 134 | Non–liver related hospitalization | > 80 years | Ultrasound |
| Babusik | 2012 | Kuwait | Jan 2007 - Sept 2008 | 143 | Health Clinic | Adults (>18) | Ultrasound |
| Lizardi-Cervera | 2006 | Mexico | Retrospective Study | 2503 | General population | Adult | Ultrasound |
| Amarapurkar | 2007 | India | Cross-sectional 2003 - Jan 2004 | 1230 | General population | Adults (>20) | Ultrasound |
| Singh | 2003 | India | Cross-sectional | 159 | General population | Adults | Ultrasound |
| Mohan | 2008 | India | Cross-sectional | | | | |
| Dassanayake | 2009 | Sri Lanka | Cross-sectional | 3012 | Community Population | Adults (35–64) | Ultrasound |
| Underwood | 1892 | UK | 1955-1989 | 166 | Autopsy Men | Adults (18- 58) | Biopsy |
| Frith | 2009 | UK | Retrospective Cohort 2005 to 2007 | 351 | Liver Clinic | Adults (> 16) | Biopsy |
| Bedogni | 2005 | Italy | Cross-sectional Jan 2002 - Aug 2003 | 598 | General population | 12 to 65 years | Ultrasound |
| Soresi | 2012 | Italy | Prospective cohort 2007–2009 | 203 | Outpatient Clinic | Adults | Ultrasound |
| Kotronen | 2010 | Finland | Cross sectional Oct 2007 - Dec 2007 | 2766 | Population | Adults (> 45) | Liver Enzymes |

UK- United Kingdom; Liver Enzymes –alanine aminotransferase levels (ALT), aspartate aminotransferase (AST

In summary, a review of the literature from 1980 to 2010 reported an average NAFLD rate of 30 % in the U.S. (Vernon et al., 2011). This rate was similar to the range in rates from the review of the literature for this study, which also included more recent studies (Browning et al., 2004; Lazo et al., 2013, Smits et al., 2013)

**NAFLD Incidence Rates**

There were several studies that reported on the incidence of NAFLD in the general population as described in Table 4. All these studies were conducted outside of the U.S., and the follow-up periods were from 3 years up to 8.5 years. The reported incidence rates in the general population ranged from 9.3% to 36.7% as illustrated in Figure 10. All the studies used ultrasound to ascertain NAFLD except for one study that used liver enzyme elevations (Suzuki et al., 2004).

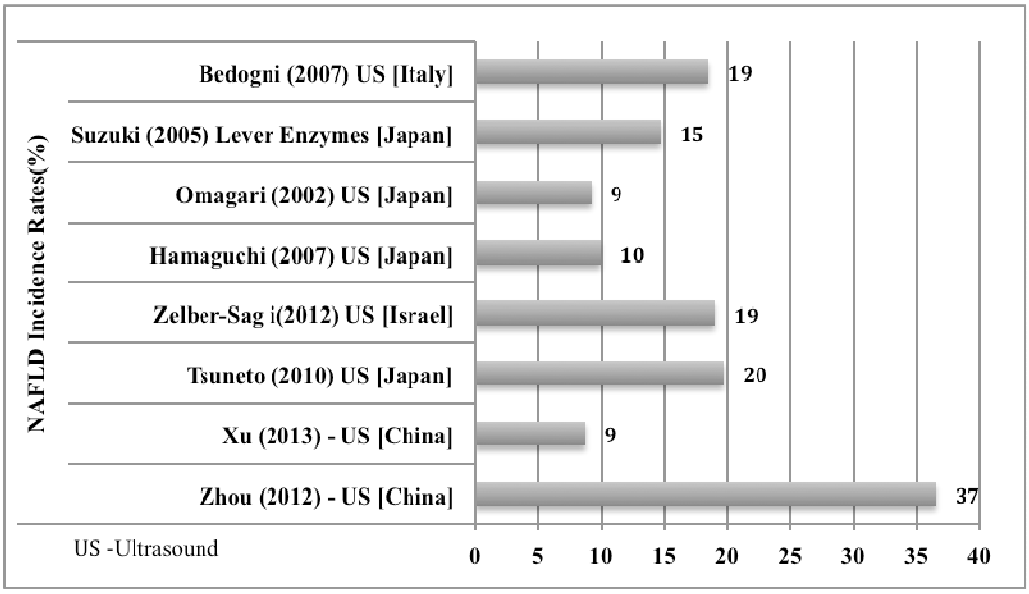



*Figure 10. NAFLD Incidence Rates in the General Population (%)*

There were four studies that reported NAFLD incidence rates that ranged from 15% to 20%, one reported a higher rate of 36.7% and three studies with lower rates of

around 10%; however there was variation in the follow-up period. Three of the four studies used ultrasound to determine the presence of NAFLD and had similar incidence rates, around 19%. Zelber-Sagi et al., (2012) reported an incidence rate of 19% with a follow-up period of 7 years. Bedogni et al., (2007) reported similar rates also using ultrasound, 18.5 % and 18.5 per 1,000 person-years (PY) developed NAFLD over the observational period of 8.5 years. Likewise, Tsuneto et al., (2010), also using ultrasound reported a rate of 20% and incidence rate of 19.9/1000 person-years (22.3 PY/1000 for men, 18.6 PY/1000 for women). In this review, the highest cumulative incidence rate of 36.5% using ultrasound to diagnose NAFLD was reported by Zhou et al., (2007); the participants in this study were followed up for 4.8 years. The lowest rate was about 10%, all using ultrasound to determine the presence of NAFLD (Hamaguchi & Kojima, 2007; Omagari et al., 2002; Xu et al., 2013). However, there was one study that used liver enzyme elevation as proxy for NAFLD and reported a rate of 15%; the follow-up period for this study was five years (Suzuki et al., 2004). This study also reported the highest incidence per patient year (PY) rate of 31 cases per 1000 PY. Similar rates were reported from a study in the UK that used retrospective data from a catchment area of 200,000 adults and reported 29 cases per 100,000 PY (Whalley, Puvanachandra, Desai, & Kennedy, 2007). However this study used a variety of parameters to attribute presence of NAFLD such as liver biopsy, liver imaging, laboratory blood tests, endoscopy for screening or management of esophageal varices, and paracentesis of ascites.

In summary for the general population, most studies reported rates between 15% to 20% and 18 to 31 per 1000 PY. Similar to the review of NAFLD prevalence rates, the

incidence rates varied by region, modality used to diagnose NAFLD, and cumulative incidence rates were also confounded by varied follow-up periods. It is not known if these rates are under-reported, given these issues and the absence of large long-term prospective population level studies; these are concerns also raised by others reviewing the literature for NAFLD incidence rates (Argo & Caldwell, 2009; Lazo & Clark, 2008; Vernon et al., 2011).

Table 4

*NAFLD Incidence Studies in the General Population*

| First Author | Year Published | Location | Study Design | Sample | Population | Age (years) | Diagnosis of NAFLD |
|---|---|---|---|---|---|---|---|
| Zhou | 2012 | China | Prospective cohort Nov 2005 - Nov 2009 (5 Year follow up) | 3543 | General Population | Adults (>18) | Ultrasound |
| Xu | 2013 | China | Prospective (5 Year follow up) | 6905 | Employee Population | Adults | Ultrasound |
| Zelber-Sagi | 2006 | Israel | Prospective cohort 2003–2004 (7 year follow up) | 349 | General population | Adults (24–70) | Ultrasound |
| Tsuneto | 2010 | Japan | Prospective 1990 – 2007 (8 year follow up) | 1635 | Atomic bomb Survivors | Adults | Ultrasound |
| Hamaguchi | 2007 | Japan | January 2001 -2003 (3 year follow up) | 3147 | Health Clinic | Adults | Ultrasound |
| Omagari | 2002 | Japan | Dec 2000 (not reported) | 3432 | Health Clinic | Adults | Ultrasound |
| Suzuki | 2004 | Japan | 1997 and 2002 | 1,537 | Employee Population | Adults | Liver Enzyme |
| Bedogni | 2007 | Italy | Prospective study (Over 8.5 years) | 480 | General population | 12 to 65 years | Ultrasound |

Liver Enzymes –alanine aminotransferase levels (ALT), aspartate aminotransferase (AST)

**Risk Factors Associated with Nonalcoholic Fatty Liver Disease**

This literature review focused on risk factors related to NAFLD in the general population, given the paucity of such information in the RA population. This review of the literature identified a plethora of risk factors associated with NAFLD, and reported prevalence rates from cross sectional studies. The process taken to derive the final list of risk factors was determined by identifying factors that were consistently reported in the literature. This list was triangulated against the list provided by the consensus guidelines for the diagnosis and management of NAFLD (Chalasani et al., 2012). Guidelines are an accepted source of information in the medical community, used to help standardize and inform clinical practice. The result of this exercise was a list of risk factors independently associated with NAFLD and these factors was used as independent predictor variables for this study.

Risk factors associated with NAFLD are age, gender, and race/ethnicity along with clinical features such as metabolic syndrome, obesity, diabetes, and dyslipidemia (Chalasani et al., 2012). However, there are other factors such as hypertension and liver enzyme elevations that have been associated with NAFLD but were not considered as independent risk factors and thus may act as potential confounders (Chalasani et al., 2012). These risk factors associated with NAFLD occur in the absence of significant alcohol use or other secondary causes of NAFLD, for example hepatitis C, Wilson's disease, and Reyes syndrome (Chalasani et al., 2012). The following sections in this review describe the prevalence and associations for each of these factors for the general population.

**Age.** NAFLD appears to affect people of all ages. Studies in the general population recognized age as a significant risk factor for NAFLD, noting that the risk increased as age increased (Chalasani et al., 2012; Vernon et al., 2011). Age brackets used to measure the prevalence of NAFLD varied by study. Frith, Day, Henderson, Burt, & Newton, (2009), Hu et al., (2012), Wong et al., (2011), and Yan et al., (2013), all reported that NAFLD rates increased with age and the risk peaked for those in the 50 to 65 year age group. Chen et al., (2007), reported a prevalence rate of 33.7% for those in the middle ages of 40 - 64 years of age with similar rates for those more than 65 years of age (32.6 %) and 17.7% for those 18-39 years. In the adjusted analysis, the risk for NAFLD was significant for the age bracket 40–64 years (odds ratio [OR] 1.58, 1.25 - 2.01 ($p < 0.000$) and also for those older than 65 years of age [OR 1.45, 1.08, 1.95, $p = 0.013$] (Chen et al., (2007). Similar patterns was also reported by Amarapurkar et al., (2007),with the highest prevalence rates in this study of 27.7% for the age of 40 to 49 years, followed by 24.5% (60 -69 years) and then 23.5% (50-59 years), 18.9% (>70 years) and lowest rates between 20-29 years of 8.8%. Lazo et al. (2013) also reported that those aged 40 years and older had higher rates compared to 20 to 39 years, and this was regardless of gender or race. Similar results were reported by Chen, et al., 2006 and Yan et al., (2013); these studies also reported statistically significant trends over time, the risk for NAFLD increased as age increased. Chen, (2006) in an adjusted analysis reported increase risk for those between 40-64 years with OR 1.59, 95% CI [1.25, 2.01] and similar risk for ages older than 65 years, OR 1.46, 95% CI [1.08, 1.96] compared to those less than 40 years of age.

However, some studies reported the reverse trend, with the younger group at increased risk for NAFLD. Ong, Pitts, and Younossi (2008) reported higher rates for the younger group, those less than 40 years of age versus those 40 years (50.4% vs. 34% respectively) and lower rates (15.6%) for those more than 60 years of age. The study did not report if there was significant differences between these age groups. Park et al., (2006) also reported similar patterns for NAFLD rates but used different age brackets; with those 30 years with a rate of 45.9%, followed by 33.4% for those 25-30 years and 9.8% for those less than 25 years of age.

Age was also associated with increased risk for progression of disease, poor hepatic related outcomes such as hepatic fibrosis, hepatocellular carcinoma, diabetes, and mortality (Adams et al., 2005; Ascha et al., 2010; Hashimoto et al., 2005; Kichian, Mclean, Gramlich, Bailey, & Bain, 2003; Ong et al.,2008; Vernon et al., 2011). Ong et al., (2008), identified age (older) along with other confounders (e.g. gender, race, higher body mass index [BMI] and metabolic syndrome) to be significantly associated with higher risk of overall mortality with OR 1.11, 95% [1.11, 1.12]) and for liver related mortality OR 9.22, 95% CI [9.11, 9.33]). Lee et al., (2007) used a combination of ultrasound and biopsy to diagnose NAFLD and reported similar results; age (>30 years) was an independent risk factor for significant hepatic disease (>30% stenosis of the liver). While most studies found age to be a significant risk factor, only one small study conducted in China followed 17 patients over a period of 6 years and compared those who progressed versus those who did not progress (Hui et al., 2005). In this study, age was not statistically significant ($p = 0.27$), when older individuals with a mean age of 46

years were compared to younger individuals with an average age of 36 years. In summary, most studies reviewed in the general population reported that the risk for NAFLD was associated with age and the risk increased over time, peaking in the middle ages of life.

**Gender.** Gender is another well recognized risk factor for NAFLD, however this association is related to age in the general population (Chalasani et al., 2012). NAFLD rates varied by gender, men were at higher risk for NAFLD and regardless of gender the risk increased with age (Chalasani et al., 2012; Vernon et al., 2011). As illustrated in Figure 11, all the studies reviewed in the general population showed that men were at higher risk for NAFLD except for three studies conducted by Dassanayake et al., (2009); Kotronen et al., (2010); and Zhou et al., (2007).

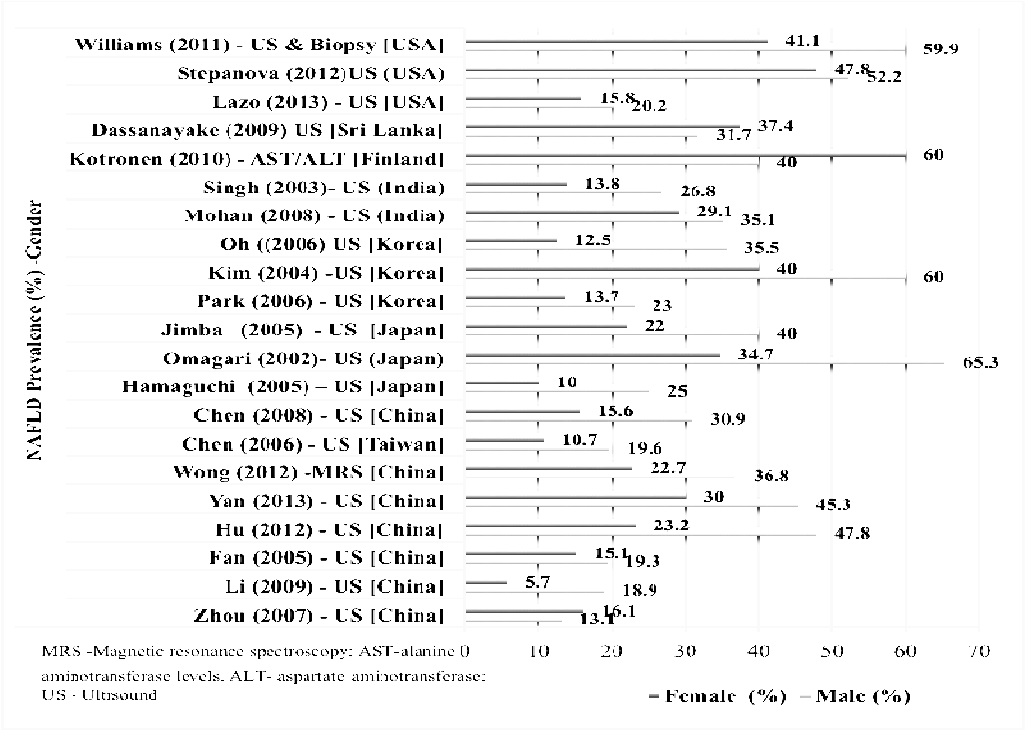



*Figure 11. NAFLD Prevalence in the General Population (%): Gender*

For studies conducted in the U.S., men appear to be at a higher risk for NAFLD than women as shown in Figure 11. Men had significantly higher rates of NAFLD 58.9% versus 41.1% for women, $p < .001$ (Williams et al., 2011). Other studies reported higher rates among men but did not report if these differences were significant (Lazo et al., 2011; Stepanova & Younossi, 2012). In adjusted analyses also, men were at significantly higher risk of developing NAFLD compared to women with hazard ratio (HR) of 1.26, CI 95% [1.264, 1.266], $p < 0.001$ (Ong et al., 2008). When rates was compared among men, the proportion of men with NAFLD compared to those without was significantly higher; 52.4 % versus 45.6%, $p <0.001$ (Lazo et al., (2011). Similar analysis of rates among men with NAFLD compared to those without was 58.8% versus 40.7% ($p < 0.001$) was reported by Williams et al., (2011).

There were many more studies conducted outside of the U.S. that detailed differences and relationship between gender and age. Hu et al., (2012) reported that the prevalence of NAFLD in males was significantly higher than females, and this risk was seen across all age groups ($p < 0.001$). When men were compared to women the rates were respectively: 25% versus 3.89% in the less than 30 year old age group; 50% versus 13.29% in 30-39 age group; 56.06% versus 24.27% in 40-49 age group, and 57.35% versus 45.79% in 50-64 age group. The prevalence increased steadily over time among these age groups for women. In a large study of 9094 adults conducted by Li et al., (2009), the prevalence rate was three times higher among males then women (18.9% vs. 5.7%, $P <0.001$). The prevalence rates among men gradually increased by age; 9.4% in less than 30 years, 24.4% up to the age of 50 and then decreased over the next several

decades of life. However, this trend differed for women. For women there was a gradual increase in prevalence rates over time, with a rate of 0.4% in those aged less than 30 years, and the highest rates of NAFLD were seen in the latter years of life, for those more than 70 years of age with a rate of 18.6% (Li et al., 2009). Park et al., (2006) also reported similar differences between men and women (23% vs.13.7% respectively, significance was not reported) and trend patterns of increased risk for women with age. Notably, in this study there was little variation in rates for men for the various age groups between 30 and those more than 70 years of age (about 25%) except for those in the age group of 20-29 had a lower rate of about 16%.

There were three studies conducted in the general population that reported higher rates of NAFLD in women. Zhou et al., (2007) reported higher overall NAFLD rates for women (16.1%) compared to men (13.1%) with a $p < 0.05$. The trends over time were similar for men and women with increasing rates up to the age of 60-70 years for both genders ($P < 0.01$). However, in this study when stratified by age the NAFLD prevalence rates for men less than 50 years, the rates was significantly higher than women (22.4 % vs. 7.1%, $P < 0.001$). Like other studies, rates increased for women aged older than 50 years of age and this rate was significantly greater for women 27.6% versus 20.6% for men ($P < 0.05$). Two other studies also reported lower rates among men, 31.1% versus 37.4% for women (Dassanayake et.al. 2009) and 40% versus 60% respectively for the study conducted by Kotronen et.al. (2010).

In several adjusted analyses, men were at higher risk for NAFLD. (Chen, et al., (2006) reported that men were at a 44% increased risk for NAFLD compared to women

(OR 1.44, 95% CI [1.09, 1.90], *P*= 0.011). This increased risk was also reported by Shi et al., (2011), with OR 1.04, 95% CI [1.03, 1.05]) for men. In a study by Lazo et al. (2013), after adjusting for age and other confounders, men remained at a significant higher risk for NAFLD 20.2%, 95% CI [18.0, 22.5]) compared with 15.8%, 95% CI [14.3, 17.2]) for women ($P < 0.001$). In summary, men appeared to be at higher risk for NAFLD in the general population. However, during the course of life, NAFLD rates steadily increased with age for women, with higher rates reported in the latter years of life.

**Race/Ethnicity.** Race and ethnicity is another independent NAFLD risk factor. This literature review focused on studies in the U.S., the country where this study was conducted. NAFLD prevalence rates by race and ethnicity for the general population are illustrated in Figure 12.

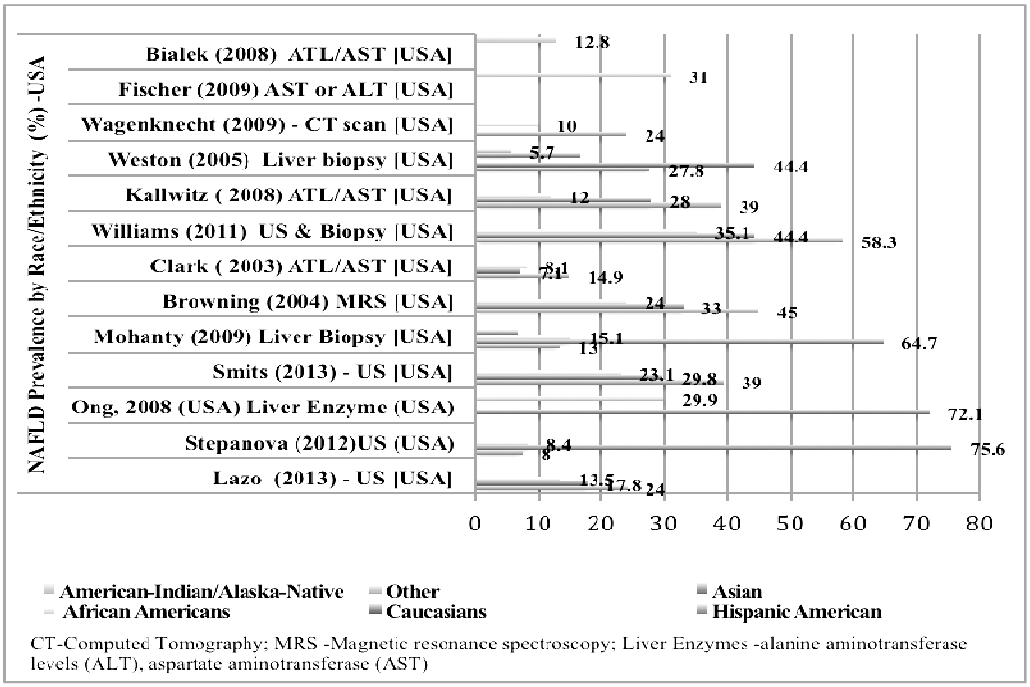



*Figure 12. NAFLD Prevalence in the General Population (%): Race/Ethnicity*

Most studies conducted in the U.S. reported differences in prevalence rates of NAFLD by race and ethnicity. The highest rates were reported among Hispanics followed by Caucasians with the lowest rates among African Americans (Stepanova & Younossi, 2012; Vernon et al., 2011). Williams et al., (2011), in a study conducted in Houston Texas of 328 individuals using liver biopsies to identify the presence of NAFLD reported the highest rates among Hispanics (58.3%), followed by Caucasians (44.4%) and then African Americans with 35.1%. There was a significant difference in the prevalence of NAFLD between Hispanics compared with Caucasians (19.4% versus 9.8%; P = 0.03). These race/ethnic differences were also confirmed by a study conducted by (Browning et al., 2004). This was a study of individuals with advanced NAFLD (cirrhosis of the liver) and used biopsy ICD-9 diagnosis code to determine the presence of NAFLD; they reported a rate of 68 % for Hispanic patients, followed by 22% for European Americans and 7% for African Americans. Similar patterns were reported by Browning et al., in 2004. This study assessed NAFLD (hepatic steatosis) using proton magnetic resonance spectroscopy and reported similar trends with 45% prevalence in Hispanics, 33% in whites, and 24% were blacks, and noted that there was higher underlying prevalence of obesity and insulin resistance among the Hispanics in this study.

However, when liver enzymes were used as a surrogate marker for NAFLD the differences in prevalence among the various races were similar to other studies but the overall rate was slightly lower. Kallwitz et al., (2008), conducted a study with a population of 547 obese individuals. Hispanics continued to have the highest rates (39%), 28% for Caucasians, and 12% for African Americans. These differences were significant

between the three groups. Hispanics had higher rates than African Americans ($P < 0.001$) and Caucasians were also at greater risk compared to African Americans [$P = 0.030$]. Two other studies also reported lower risk for NAFLD among African Americans (Caldwell, Harris, Patrie, & Hespenheide, 2002; Solga et al., 2005).

However in this literature review, there were several studies that reported higher NAFLD rates for Caucasians. Two studies were conducted using the NHANES database. Stepanova & Younossi, (2012), reported prevalence rates of 75.6% for Caucasians and 8% and 8.4% for Hispanics and African Americans respectively. The second study was conducted by Ong et al., in 2008, but included only two race/ethnicity categories (Non Hispanic Whites and other) and reported similar rates for Non Hispanic Whites (72.1%) and 27.9 % for all other races. However, another study also using NHANES data reported higher rates for Hispanic Americans (referred to in the study as Mexican Americans) by Lazo et al. (2013). In this adjusted analysis resulted in significantly higher rates for Mexican Americans of 24.1%, 95% CI [20.8%, 27.5%] compared to 17.8% 95% CI [6.1, 19.5] for Non Hispanic whites and 13.5%, 95% CI [1.3, 15.7] for Non Hispanic blacks (Lazo et al., 2013). Other studies that reported higher rates of NAFLD for Caucasians were small regional studies or conducted in select populations (e.g. liver clinic) and hence the rates may not be representative of the overall general population (Mohanty et al., 2009; Weston et al., 2005). For example, Mohanty et al., (2009) used biopsy samples from the University of Chicago hospital pathology database and reported NAFLD rates of 64.7% for Caucasians, 15.1% for African Americans and 13% for Hispanic Americans. Weston et al., (2005), also reported higher rates for Caucasians 64.7%, 13% and 15.1%

for Hispanics and African Americans respectively. However, this study only included patients from a regional liver clinic. In summary, this literature review of prevalence of NAFLD differed by race and ethnicity in the U.S. There were more studies that reported increased rates for Hispanics, followed by Caucasians, with African Americans consistently having the lowest risk for NAFLD.

**Obesity.** The link between NAFLD and obesity coupled with metabolic syndrome has been well established in the literature and has been accepted as central to the development of NAFLD in the general population (Chalasani et al., 2012; Lazo & Clark, 2008a; Vernon et al., 2011b). For this literature review, studies reviewed reported NAFLD rates related to obesity that ranged from 14.3% to 99.1% in the general population as depicted in Figure 13. The most recent guidelines recommends using waist circumference to evaluate the presence of obesity related to metabolic syndrome (Alberti et al., 2009). However, some studies used BMI if waist circumference was not available, for example Lazo et al. (2011), Lazo et al. (2013),Ong et al. (2008) and Williams et al. (2011). Rates of NAFLD varied by regions, populations, and also by the criteria used to measure obesity (e.g. with BMI > 30, or elevated waist circumference (≥40 inches in men and ≥35 inches in women). These rates are further confounded by the measure used to determine the presence of NAFLD (e.g., biopsy or ultrasound). Some of the studies conducted prior to 2005, reported higher NAFLD rates (>90%), and used biopsy results from severely obese patients undergoing bariatric surgery. Others were carried out in subpopulations with higher prevalence of metabolic syndrome or diabetes. These were unlike studies that were conducted in the general population, where ultrasound was used

most often to determine the presence of NAFLD, and which reported lower rates of obesity as seen in Figure 13.

In the U.S., NAFLD rates related to presence of obesity as measured by BMI or elevated waist circumference ranged from 25.8% to 99%, with most studies reporting prevalence rate greater than 65% (see Figure 13). The population level study conducted by Lazo et al. in 2013, compared all the various BMI categories to normal BMI. After adjusting for confounders there was a two-fold increase risk for NAFLD (OR 2.17, 95%CI [1.81, 2.60]) for those with a BMI of 25 –29.9 and the OR was 3.31, 95%CI [2.74, 4.0] for those with a BMI 30–34.9. However, there was a five-fold increase risk (OR 5.05, 95%CI [4.15, 6.14] for those with a BMI ≥35. Even those below normal weight with a BMI <18.5 had a two fold increase risk for NAFLD (OR 2.01, 95%CI [1.23, 3.27]. Similar results were also reported for those with abdominal obesity by Mohan, Farooq, Deepa, Ravikumar, & Pitchumoni, (2009); there was a two fold increased risk for NAFLD for those with a BMI ≥ 25 (OR 2.4, 95% C.I. [1.6, 3.5] $p < 0.001$), and a four-fold increased risk for those with BMI ≥ 30 (OR 4.0, 95% C.I. [1.9, 8.3] $p < 0.001$). Several other studies after adjusting for confounders also reported significant risk of NAFLD associated with increased BMI (Babusik, Bilal, & Duris, 2012; Chen et al., 2007; Jimba et al., 2005; Li et al., 2009; Shi et al., 2011).

Obesity is a significant risk factor for NAFLD and some studies suggest increased risk even among those with normal BMI. There appears to be a dose response in the prevalence of NAFLD associated with obesity, with the prevalence being highest among the severely obese. Most of the literature and recent guidelines recommend using waist

circumference as the measure for obesity when evaluating metabolic syndrome(Alberti et al., 2009). However, this measure is not collected in the RA registry, hence BMI was used in a similar manner to studies conducted by Lazo et al. (2011), Lazo et al. (2013),Ong et al.(2008) and Williams et al. (2011) where elevated BMI was used to determine the presence of obesity.

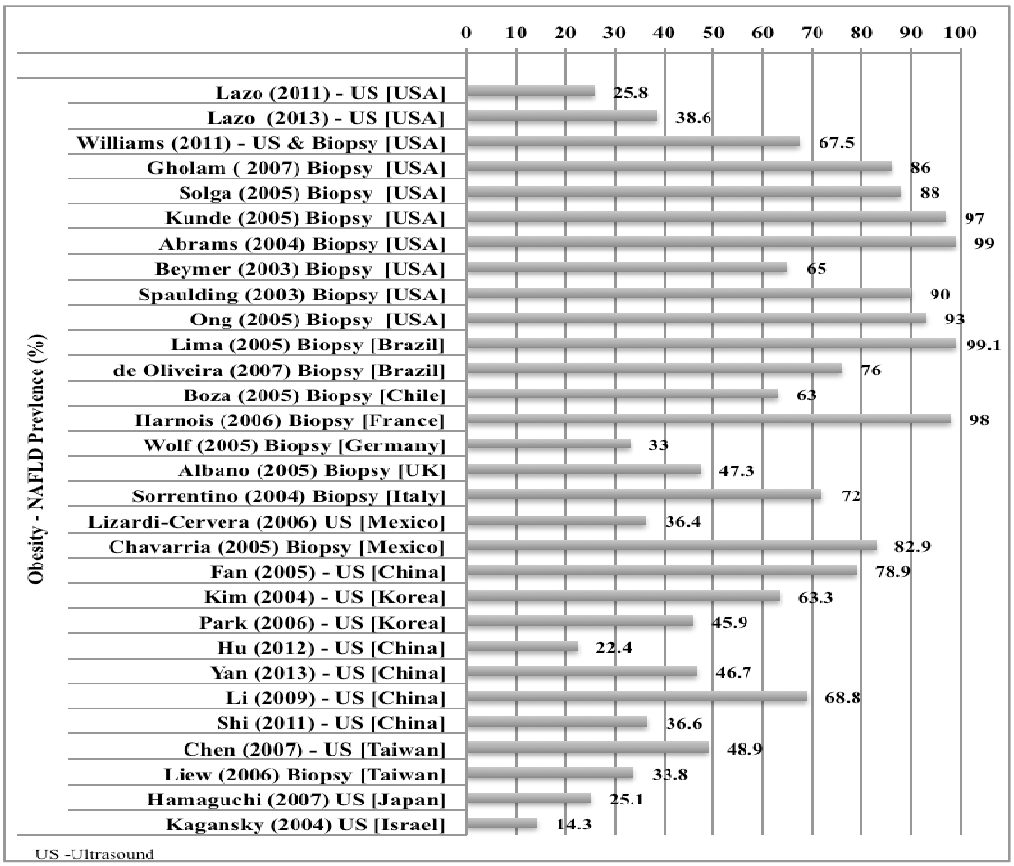



*Figure 13. NAFLD Prevalence in the General NAFLD Population (%):Obesity*

**Metabolic Syndrome.** Metabolic syndrome (MetS) is a significant risk factor for NAFLD (Chalasani et al., 2012). This syndrome is a collection of risk factors related to obesity, such as increased waist circumference, elevated triglycerides, blood pressure, and plasma glucose levels, and reduced HLD levels (Grundy et al., 2005). The most

recent consensus guideline defines MetS as the presence of any three of these risk factors (Alberti et al., 2009). There is a belief that the presence of any and all factors related to MetS could potentially place individuals at risk for NAFLD (Erickson, 2009). A variety of definitions were used in the literature and it appears that criteria used for MetS in the studies also evolved over time. For example, elevated blood glucose levels were a mandatory requirement according to the earlier guidelines. This was reflected in the earlier studies, while more recent studies used broader definition such as history of use or current use of diabetic medications. More studies reported on the rates of NAFLD using the individual components of MetS; only a few studies used the composite MetS definition.

Prevalence rates of NAFLD in the general population with MetS varied. Studies included diverse populations, sample sizes, and the criteria used to determine MetS and presence of NAFLD also varied by study. The overall MetS rates among those with NAFLD ranged from 15.7 % to 70%, with one study in the U.S. reporting a rate of 43.5% (Ong et al., 2008) and another of 20.5% (Smits et al., 2013). Studies included in Figure 14 reported MetS as a dichotomous variable that is the presence or absence of MetS.

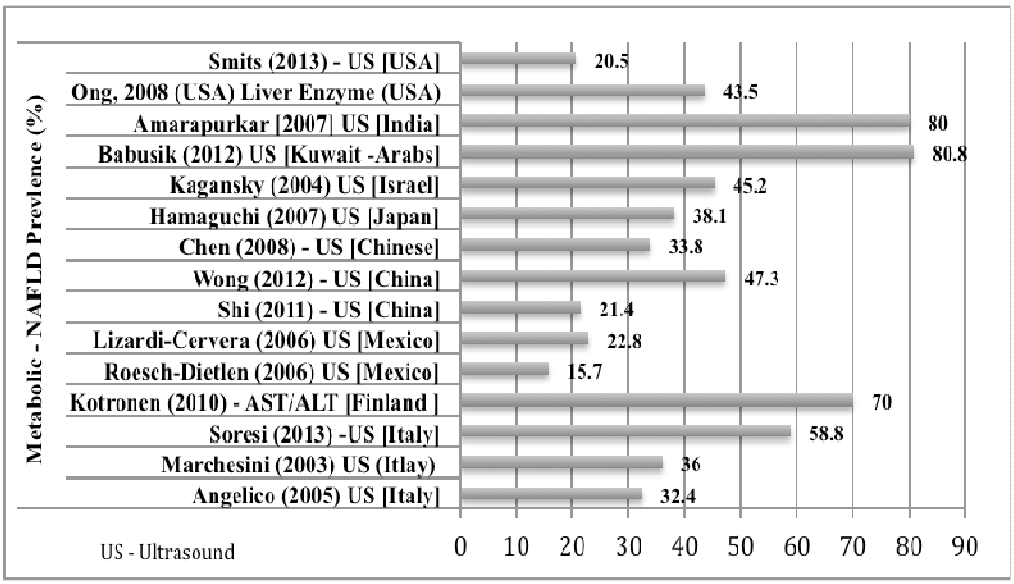



*Figure 14. NAFLD Prevalence in the General Population (%): Metabolic Syndrome*

There was a proportional increase in prevalence rates for NAFLD as the number of the individual criteria used to determine MetS increased. In a large U.S. study conducted by Smits et al. (2013) of 3846 individuals in the general population reflected this pattern. NAFLD rates were 90.8% for those who met all five criteria for MetS, and 58.8% for those with three criteria, however there was 17.2% of the population without MetS that had NAFLD. Marchesini et al. reported similar findings in an earlier but smaller study in 2003 ($N = 304$). This study evaluated the prevalence of NAFLD and MetS stratified by weight. NAFLD prevalence rate was 36% for the group with three or more MetS criteria, and about 18% among those within the normal weight group, 29% for the overweight group, and 67% in the obese group had NAFLD (Marchesini et al., 2003). In the group with at least one MetS criteria and normal weight, the rate for NAFLD was 70%; almost 90% in the overweight and nearly 99% for the obese group. In another study, 80% of individuals with NAFLD met all five of the MetS criteria, 63%

met three, with 5% of the population that did not have any of the criteria (Wong et al., 2011). However a study conducted by Chen, et.al. (2008) reported lower overall rates associated with the number of MetS criteria (0=11.22%, 1=29.40%, 2 =25.55% and ≥ 3 =33.83%).

Several studies showed MetS as an independent predictor for NAFLD. One study showed a two fold risk for NAFLD (OR 2.0, 95% C.I [1.3, 3.1] ($p < 0.001$) after adjusting for confounders (Mohan et al., 2009). However, another adjusted analysis reported a threefold increase risk for NAFLD-related diseases (OR 3.2, 95% CI [1.2-8.9] (Marchesni et al., 2003). In summary, the presence of any and all indications of MetS increased the risk for NAFLD in the general population. Secondly, there appeared to be a dose-response effect by the number of MetS component criteria and increased risk for NAFLD.

**Diabetes.** Diabetes is a well-established risk factor for NAFLD and often associated with insulin resistance (Chalasani et al., 2012). Insulin résistance is surmised to play a significant role in the pathogenesis of NAFLD (Jakobsen, Berentzen, Sørensen, & Overvad, 2007). Most studies accounted for it as one of the criteria for MetS (e.g. glucose levels, use of diabetes medication). Others measured it separately as an independent variable using measures such as homeostasis model assessment (HOMA) or insulin levels (Bajaj et al., 2009; da Cunha et al., 2012; Dessein, Christian, & Solomon, 2009).

Overall the prevalence of diabetes in the general population with NAFLD was wide and ranged from 4.8% to 87%. These rates reflect regional variations, with higher

rates in India and lower rates in studies conducted in China (see Figure 15). In the U.S., two studies were conducted in the general population with NAFLD; these studies reported rates of 15.8% to 26.3% for diabetes (Lazo et al., 2011; Williams et al., 2011). Likewise a study conducted by Mohan et.al (2009) in India reported a rate of 54.5% for diabetes. These were patients with NAFLD in the general population. In the multivariate analysis, diabetes was associated with almost a three fold increased risk for NAFLD (OR 2.9, 95% C.I [1.9–4.6], $p < 0.001$). However, the rates for NAFLD were significantly higher in studies conducted in the diabetic population, these rates ranged from 63% - 69.65% (Gupte et al., 2004; Kelley, McKolanis, Hegazi, Kuller, & Kalhan, 2003; Targher et al., 2007). Even though the overall diabetes-related prevalence might be lower in the general population in the U.S., those with a history of diabetes appear to be significantly at higher risk for NAFLD.

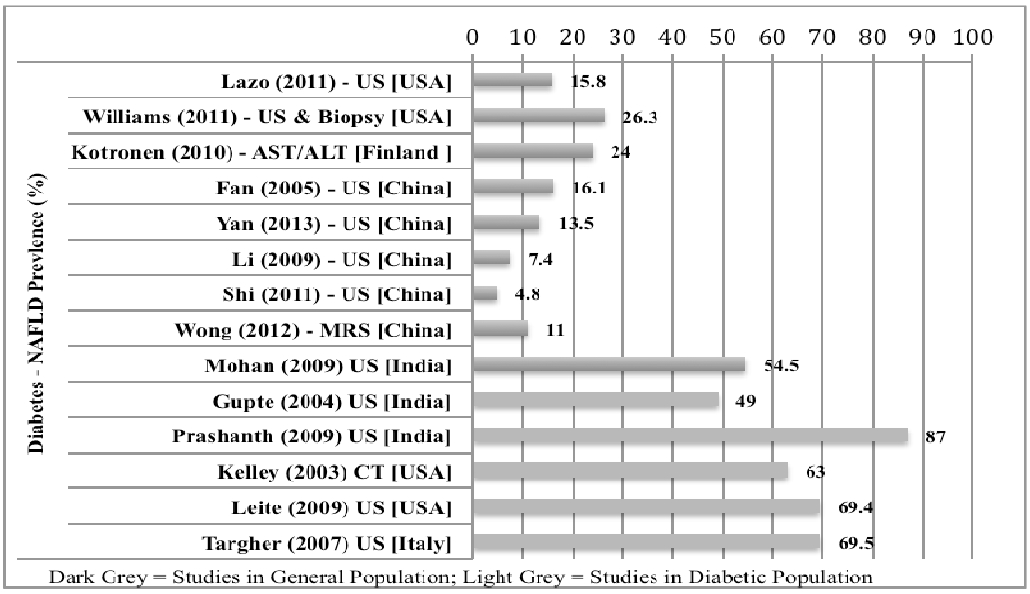



*Figure 15. NAFLD Prevalence in the General Population (%): Diabetes*

**Dyslipidemia.** Dyslipidemia or hypercholesterolemia is another risk factor associated with NAFLD (Chalasani et al., 2012). Dyslipidemia is defined as alterations in the lipid panel; specifically elevations in total cholesterol, low density lipid (LDL) and triglyceride (TG) and reduced levels of HDL (Grundy et al., 2005). Review of the literature found that dyslipidemia and hypercholesterolemia were used interchangeably. The definitions and or cutoff criteria to define dyslipidemia used varied by study. For example, Lazo et al. (2013), identified hypercholesterolemia as those with total cholesterol levels >240 mg/dL, while Hu et al., (2012), defined it as having either elevated total cholesterol level (≥5.2 mmol/L) or triglyceride level (≥1.7 mmol/). Consequently, a wide range of prevalence rates for dyslipidemia was seen in the literature. The focus of this literature review was limited to those studies that reported on the overall rates of dyslipidemia, hypertriglyceridemia and low levels of HDL, as these factors are among others are used to define the presence of MetS and also because of their unique association with NAFLD (Chalasani et al., 2012; Grundy et al., 2005).

Prevalence rates for NAFLD associated with dyslipidemia in the general population were varied in the U.S. Rates that ranged from 20% to 92% and similar rates were reported in other countries (see Figure 16). This wide range of prevalence rates maybe indicative of when these studies where conducted. Some of them were carried out before the 1990s and may reflect an era prior to increased prevalence of obesity and metabolic syndrome. Obesity and metabolic syndrome are two known key factors associated with the development of NAFLD (Erickson, 2009). About half of the individuals with NAFLD appear to have dyslipidemia, characteristically presenting with

high serum TG and low serum HDL levels (Chalasani et al., 2012). Since the recognition of high serum TG levels and low serum HDL levels as key predictors of NAFLD, some of the more recent studies reported on these two specific variables only and not the broad definition of dyslipidemia.

The review of literature yielded NAFLD rates from 19.8% to 75% among those with dyslipidemia. Li et al. (2009), in a large study compared those with NAFLD ($N$ = 1140) and without ($N$ = 7954) and showed a significant difference in the prevalence. The results showed that the rates among those with NAFLD were 74.7 % versus 35.7% respectively ($P < 0.001$) among those without NAFLD. Lazo et al. (2013) in a study conducted in the U.S., after adjusting for confounders, reported that the presence of dyslipidemia increased the risk for NAFLD by 26% (OR 1.26, 95% CI [1.11, 1.42]). Even higher risk for NAFLD associated with hypercholesterolemia of 70% was reported in a study conducted in India by Mohan et al. (2009), with OR 1.7, 95% C.I. [1.1–2.7] $p < 0.05$). Dyslipidemia is a well recognized risk factor for NAFLD in the general population despite the varying criteria used in the studies (Chalasani et al., 2012).

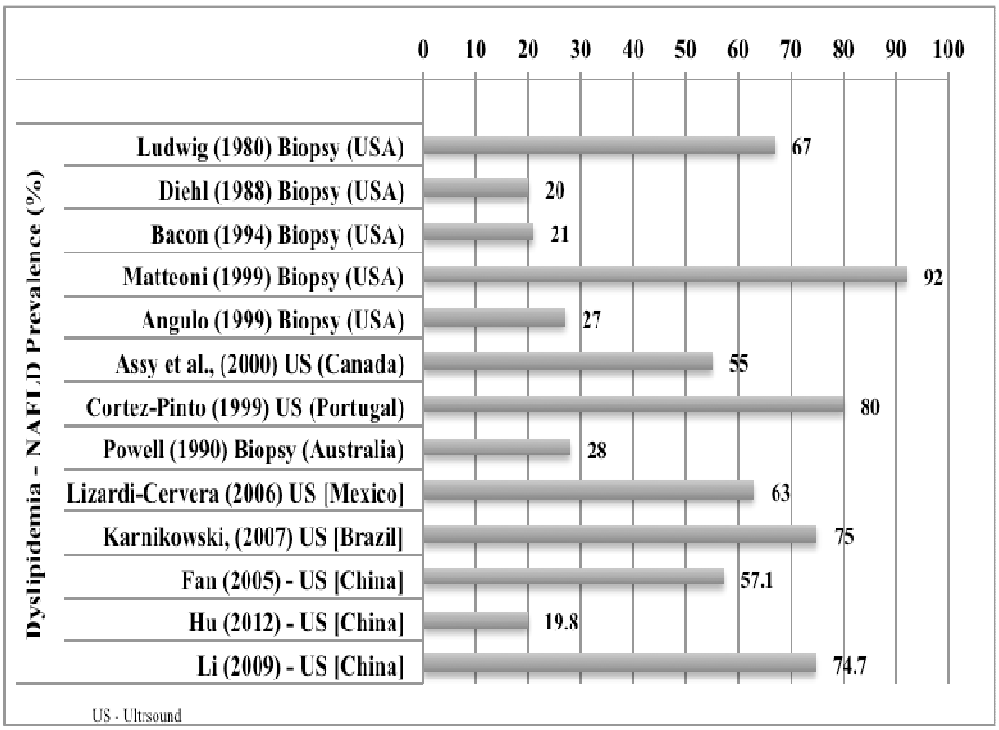



*Figure 16. NAFLD Prevalence Rate in the General Population (%): Dyslipidemia*

**Hypertriglyceridemia and Low HDL Levels.** High triglyceride levels along with low levels of HDL also are key predictors of NAFLD (Chalasani et al., 2012). Prevalence rates for NAFLD in the general population associated with hypertriglyceridemia ranged from 17% to 72% outside of the U.S. and a rate of 52% in the U.S. (see Figure 17). Mohan et al. (2009) reported that hypertriglyceridemia was associated with increased risk for NAFLD (OR 1.7, 95% C.I. [1.1–2.5], $p < 0.05$), after adjusting for age, gender and waist circumference. Dassanayake et al. (2009), also conducted a multivariate analysis and reported a significant association between elevated triglyceride and NAFLD (OR 1.33, 95% CI [1.08,1.63], $p$ 0.008). Similar findings were also reported by Chen, et al. in 2006; Jimba et al. in 2005; and Li et al. in 2009. Elevations in triglyceride levels is recognized in the guidelines as a significant risk factor

for NAFLD and remained a consistent finding in this literature review (Chalasani et al., 2012).

Low levels of HDL are also a significant risk factor associated with the development of NAFLD. Prevalence rates NAFLD associated with low HDL in the general population ranged from 20.7% to 71.4% (see Figure 17). In this review, studies reporting on the risk for NAFLD associated with low HDL after adjusting for confounders were mixed, though more studies reported an increased risk for NAFLD. Several studies indicated significant risk that ranged from 40% to 70% as shown in Figure 17. Mohan et al. (2009) reported OR 1.7, 95% C.I. [1.1, 2.6], $p < 0.05$); also by Kim et al. (2004) with OR of 1.41 95% C.I. [1.01, 1.97], $p < 0.05$); Park et al. (2006) reported OR 1.61 95% C.I. [1.14, 2.28], $p < 0.05$). Whereas, Li et al. (2009) after adjusting for confounders reported a 33% reduced risk for NAFLD associated with low HDL levels (OR 0.342, 95% C.I. [0.23, 0.50]). Dassanayake et al. also reported similar findings in 2009. Despite these mixed results, it is generally accepted that those with low HLD levels are at an increased risk for NAFLD (Chalasani et al., 2012; Grundy et al., 2005).

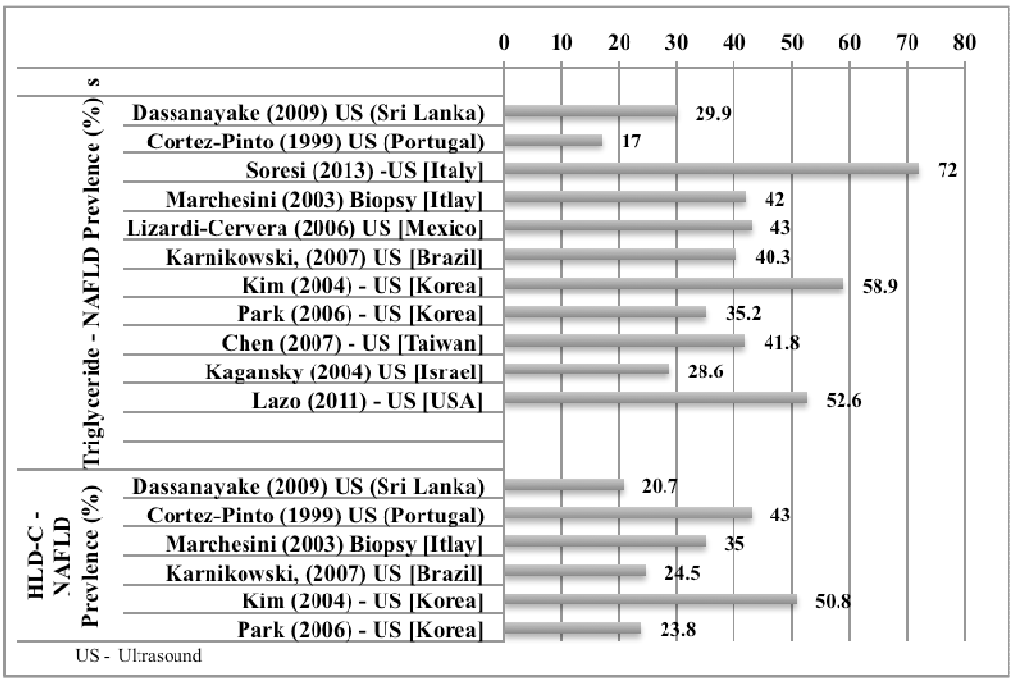



*Figure 17. NAFLD Prevalence Rate in the General Population: Hypertriglyceridemia & Low HDL Levels (%)*

In Summary, NAFLD is a chronic condition that is emerging as a public health challenge in the U.S. (Argo & Caldwell, 2009). The presence of other prevalent conditions associated with NAFLD, such as obesity and metabolic syndrome, diabetes, CVD, and dyslipidemia appears to accelerate the development of NAFLD (Argo & Caldwell, 2009). Patients with RA also have similar risk factors (Chung et al., 2008; Crowson et al., 2011). These factors are prevalent in the RA population with obesity rates as high as 65% (see Figure2), MetS rates up to 42% (see Figure 3); hypertension around 80% (See Figure 4); hypertriglyceridemia about 30% and low HDL of 35% (see Figure 5), and diabetes of 16% (see Figure 6). Use of MTX for the treatment of RA has been associated with increased elevation of liver enzymes and fibrosis of the liver ( Arena et al., 2012; Sakthiswary et al., 2014). Hence, this study hypothesized that NAFLD is also

prevalent in patients with RA, given the overlap of several NAFLD related risk factors and exposure to MTX.

Theories related to NAFLD are complex and appear to be linked to chronic inflammation affecting the liver. The *two hit* theory postulated by Day & James (1998) is the most frequently referenced theory to explain NAFLD (Lim, et.al., 2010). Obesity and metabolic dysfunction are central to the development of NAFLD due to systemic inflammation (Fabbrini et al.,2010). RA is also a disease associated with systemic inflammation with similar risk factors as NAFLD (Ahmed, 2006), along with liver enzyme elevation due to MTX (Visser & Heijde (2009). It is most likely that the development of NAFLD maybe due to the confluence of several factors related to inflammation and for patients with RA the use of MTX.

Only one small study in the U.S. reported on the prevalence of NAFLD in the RA population (Bhambhani, et.al., 2006). However, prevalence rate in the general population was as high as 46% in the U.S. (see Figure 7). No studies reported the incidence rates for NAFLD in the RA population, whereas the rate was about 20% in the general population (see Figure 10). Risk factors associated with NAFLD in the general population were age, gender, and race/ethnicity along with clinical features such as metabolic syndrome, obesity, diabetes mellitus, and dyslipidemia (Chalasani et al., 2012). In the general population, NAFLD increased with age and peaked in the middle ages of life (Chalasani et al., 2012; Frith, et.al 2009; Hu et al., 2012; Vernon et al., 2011; Wong et al., 2011;Yan et al., 2013). Men appeared to be at a higher risk than women for NAFLD in the general population (see Figure 11). Hispanics had a greater risk for NAFLD followed by

Caucasians, with African Americans having the lowest risk for NAFLD (see Figure 12). In the U.S. general population, more studies reported NAFLD rates >65% among the obese (see Figure 13) and for those with MetS the rate was as high as 80% (see Figure 14). NAFLD was reported for those with dyslipidemia (high as 92%), hypertriglyceridemia (high as 70%) and 50% for those with low HDL was seen in the general population (see Figure 16 and Figure 17).

Given the emerging prevalence of NAFLD, there is a need to identify patients at risk using non-invasive tools such as FIB-4 index (McPherson et al., 2010; Shah et al., 2009a).This study used retrospective data collected from an observational cohort of patients with RA in the U.S. to determine the incidence, prevalence, and factors associated with NAFLD using FIB-4. The next chapter details the methods used to determine the incidence, prevalence, and factors associated with NAFLD in this population.

# Chapter 3: Research Method

The primary objective of this study was to determine if there are significant factors that predict the development of NAFLD after adjusting for relevant confounders. All patients who met the eligibility criteria and did not have NAFLD at the index baseline visit were included in the incident cohort analysis. However, if the sample size was not adequate for an incident cohort analysis then the alternate objective was the prevalence analyses and a cross sectional cohort would be identified.

With these objectives in mind, this chapter covers the methodological approach taken to address the study objectives and includes: research design, the rationale for the approach, the criteria used to select the population, the setting for the study, assumptions used to estimate the sample size and power calculation, the statistical analysis plan, and description of the dataset. Also presented is the process that was taken to collect the data by the registry, specifications and procedures taken to access the study dataset, and operationalization of the study variables. Finally, the steps taken to protect and maintain the ethical standards for the participants in the registry are also included.

## Research Design and Rationale

This was a quantitative, nonexperimental, retrospective cohort study that sought to answer the following research questions:

RQ1 Quantitative: What is the incidence rate of NAFLD among patients with RA from January 2001 - November 2014 in a RA registry in the U.S. using the FIB-4 test?

RQ2 Quantitative: What is the prevalence of NAFLD among patients with RA for one year (e.g. from January 2012-December 2012) in a RA registry in the U.S. determined by using the FIB-4 test?

RQ3: Among patients in the RA incident cohort, what are the baseline differences in clinical and demographic characteristics among those with NAFLD compared to those without?

RQ $H_0$: Among patients in the RA incident cohort, there are no significant baseline differences in the clinical and demographic characteristics among those with NAFLD compared to those without.

RQ3 $H_a$: Among patients in the RA incident cohort, there are significant differences in the baseline clinical and demographic characteristics among those with NAFLD compared to those without.

RQ4 Quantitative: For the RA incident cohort, what are the significant baseline factors (clinical and demographic) that predict the development of NAFLD after adjusting for relevant confounders?

RQ4 $H_0$: For the RA incident cohort, there are no significant baseline factors (clinical and demographic) that predict the development of NAFLD after adjusting for relevant confounders.

RQ4 $H_a$: For the RA incident cohort, there are significant factors (clinical and demographic) that predict the development of NAFLD after adjusting for relevant confounders.

NAFLD was the dependent variable for this study. The presence of NAFLD was determined using FIB-4 score, a tool that measured the various stages of NAFLD. Based on the literature review presented in Chapter 2, the independent predictor variables included demographic characteristics such as age, gender, and race/ethnicity) as well as clinical risk factors such as metabolic syndrome, obesity, diabetes, dyslipidemia, and MTX use. Potential confounders associated with NAFLD, but not considered as independent risk factors, were liver enzyme elevation, alcohol use, and liver disorders. These were risk factors identified in the general population; as such information in the RA population was not available in the literature.

This was a non-experimental research study that used data collected from a historical, concurrent cohort of patients with RA and was designed to determine the incidence, prevalence, and factors associated with NAFLD. Thus, this study included a cohort with longitudinal data for the incidence analysis and a cross sectional data set to determine the NAFLD prevalence rate. The primary objective was the incident cohort analysis, allowing the opportunity to assess the development of NAFLD, and the ability to compare the prevalence of risk factors among those with and without NAFLD and also the opportunity to establish the temporal association between the risk factors and the onset of disease. A retrospective cohort and cross-sectional methodology was a rational approach to answer these questions as it permits one to evaluate the association between the environmental exposures, subject characteristics, and disease risk. Such data would have to be collected over many years and would not be realistically feasible or ethical

using alternative approaches, such as a randomized study or a prolonged prospective observational study (Forthofer, Lee, Hernandez, 2007, pp. 134-166)

There were other reasons for using a retrospective cohort study design. It allowed for the calculation of incidence rates, as information about temporality was available, specifically the time sequence between exposure and the outcomes (Szklo & Nieto, 2014, pp. 1-7). Additionally, a retrospective cohort study allowed the inclusion of many possible exposure measures of association with the outcomes. Taking this approach accommodated an important element in the study: the ability to measure risk factors that would take many years to develop and also the ability to measure the effect of them over a long period of time, key to identifying the predictors of NAFLD. Taking this longitudinal approach efficiently aligned with several research objectives of this study using the Kaplan Meier and Cox proportional hazard analysis. Information about the time to development of NAFLD is limited and therefore answering this question prospectively would be cost prohibitive and resource intensive due to the years and vastness of data needed to complete the study. Aside from the practical and logistical issues, it would not be ethical to knowingly allow those with risk factors associated with NAFLD to continue to be exposed without appropriate and timely interventions.

From an epidemiological perspective, a prospective cohort study is considered ideal, provided it is unbiased and reflects the real life temporal association of cause and effect for the event under study (Szklo & Nieto, 2014, pp. 1-7). This study was similar to several population level cohort studies that were presented in Chapter 2; for example, the studies conducted using NHANES data to determine the prevalence of NAFLD in the

general population (e.g. Lazo et al., 2011, Lazo et al., 2013, Stepanova & Younossi, (2012), and Smits et al., 2013). This study was similar to a study conducted by Xu et.al in 2013 to determine the prevalence and risk factors associated with the development of NAFLD in a non-obese cohort. Several epidemiological studies were also published using cohorts from the RA registry, by Fisher et.al (2012) and Greenberg et.al (2011).

Risk for NAFLD has not been documented or well characterized in the literature for this potentially at risk population with RA. Thus, determining the incidence, prevalence, and factors associated with the development of NAFLD was an important unanswered question. The approach of answering these questions was based on information learned from the literature review and also included important pragmatic consideration: the feasibility of conducting the study, availability of a concurrent cohort of patients with RA, with data collected over many years, and also having access to a large enough sample size with specific data points to reasonably answer the research questions.

## Methodology

### Setting and Sample

Data used for this study were from the Corrona registry. Corrona is an independent, prospective, observational cohort registry of adult patients with RA recruited from roughly 160 private and academic practice sites across 40 states in the U.S., with > 600 participating rheumatologists (Corrona Rheumatoid Arthritis, 2015). Approvals for data collection and research were obtained from local institutional review boards of participating academic sites and a central institutional review board for private

practice sites. Clinical, laboratory, imaging, medication, and drug toxicity data reflecting routine care has been collected since 2001(Corrona Rheumatoid Arthritis, 2015). Follow-up assessments are reported every four months and completed as part of routine clinical encounters. Data are collected from both patients and their treating rheumatologists using questionnaires and include physician and patient reported information on disease severity and activity measures, disease duration, medical comorbidities, use of medications, laboratory values, and adverse events (Corrona Rheumatoid Arthritis, 2015). ). It has been previously established that the results from studies conducted in this registry is generalizable to a national U.S. population of patients with RA (Curtis. J., Chen, L., Yun, H., et al, 2013). A sample of patients that met study requirements, as outlined below was selected for the study from this registry. A descriptive analysis was also conducted to determine comparability of NAFLD study population and the overall registry population (see sample Table 9).

**Population**

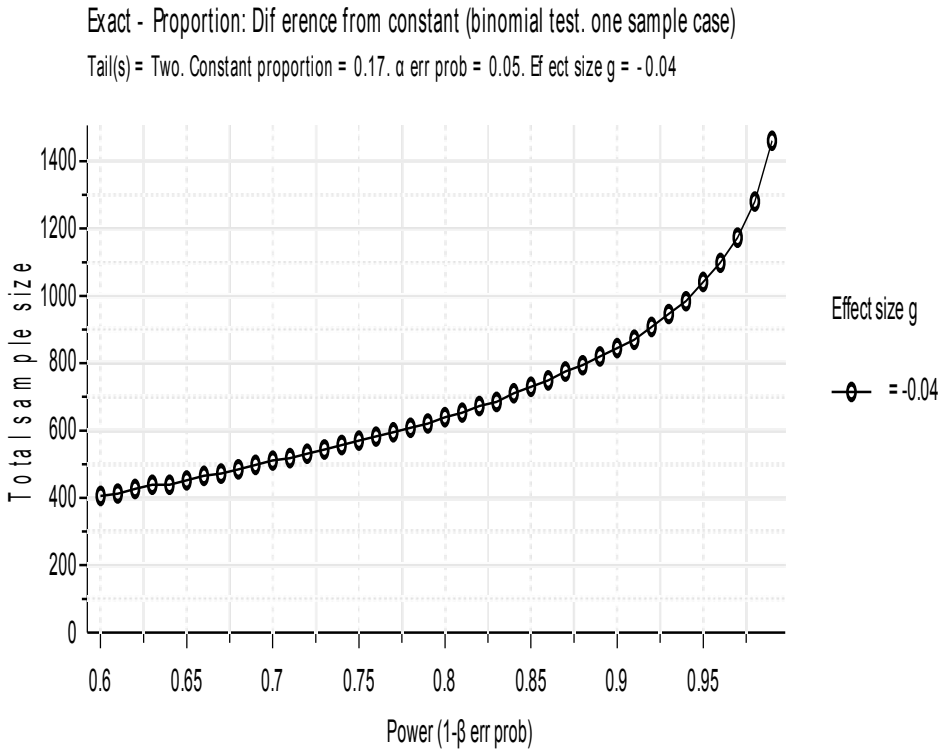
All adults with a diagnosis of RA and 18 years of age in the registry with information necessary to determine the presence of NAFLD (FIB-4 criteria - age, AST, ALT and platelets) were included in the study. Excluded were those for whom the FIB-4 information was not available or those with secondary causes of NAFLD collected in the registry, such as Hepatitis C, B, and polycystic ovarian disease. As of December 2014, data was amassed on more than 40,300 patients, with approximately 265,250 patient visits, and 107,650 patient years of follow-up observation time. Based on a preliminary feasibility assessment, approximately 20,000 patients were eligible for this study.

**Sample and Sampling Procedures**

Based on a retrospective analysis of a cohort of RA patients and the specific research questions, the only option was to use a purposive sample of convenience; only patients meeting certain inclusion and exclusion criteria were selected for the study (Frankfort-Nachmias, & Nachmias, 2008, pp. 213-24 8). To answer the specific research questions, an incident cohort with FIB-4 data to determine the presence of NAFLD was identified during the study period (January 2001 - November 2014). The incident cohort was defined as the group without NAFLD at the baseline visit; they also must have at least one or more follow-up visit. To increase the robustness of the analysis and reduce the possibility of misclassification those with at least two follow up visits with FIB-4 data were included in the analysis.

Sample size for the research questions was estimated using G*Power software and inputs for effect size were based on information from studies reviewed in Chapter 2. Research questions R1 and R2 were questions related to proportions specifically to determine the rates of incidence and prevalence rate. Incidence in the RA population in the U.S. was not available, however, cumulative rates in the general population ranged from 9.3 to 36.5% over a period of 5 to 8 years (See Figure 10). More studies reported cumulative rates of 15% to 20% (see Figure 10). Assuming that the rates in the RA population were similar, an average rate of 17% was used for the sample and power calculation for the incident cohort analysis. Using a two-sided test of significance with alpha of 0.05 and power of 80%, a sample of 640 patients was sufficiently large to detect a similar incidence rate of 17%. To increase precision and minimize chance, with 100%

power and two-sided alpha of 0.01, the study required a sample of 1913 patients. Thus, to detect an incidence rate of 17%, a minimum sample of 640 was sufficient and a sample of 1913 patients increased the power and robustness of the analysis given the fact that rates in the RA population were not known (See Figure 18).

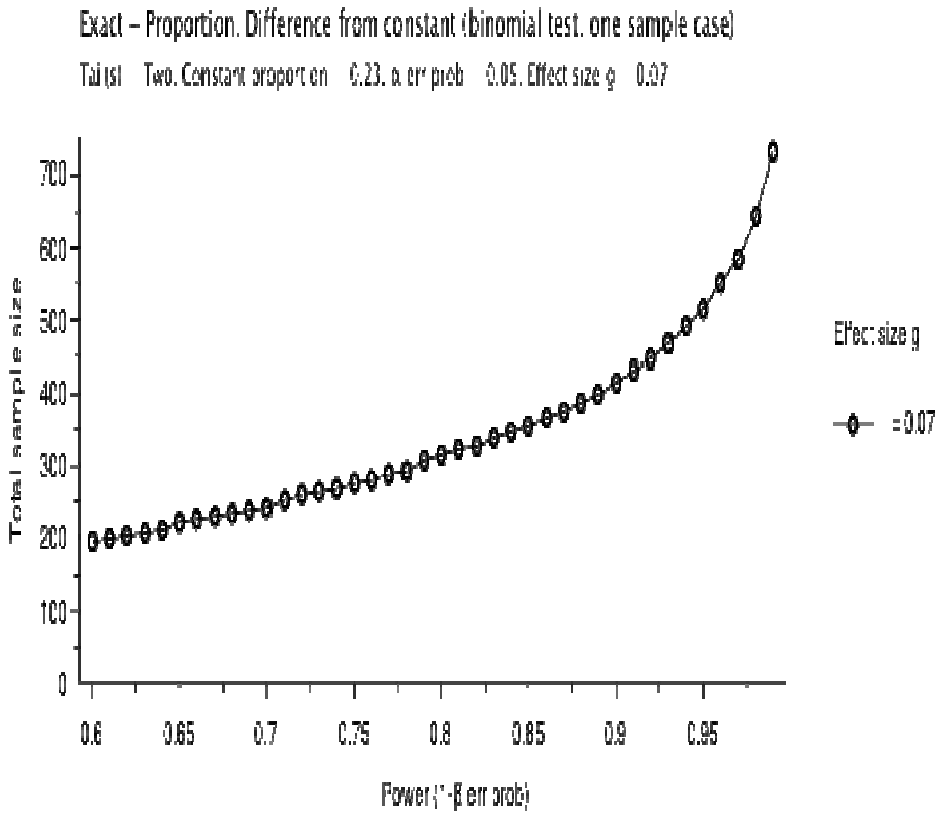



*G*Power estimated effect size based sample size assumptions*

*Figure 18. Sample Size and Power Calculation for NAFLD Incidence Rate*

Studies about NAFLD in the RA population are few, only one study reported prevalence rates in the RA population. This was a small study of 100 patients that reported a prevalence rate of 23% (Bhambhani, et.al., 2006), however slightly higher rates (30%) were reported in the general population (Vernon et al., 2011). Assuming a prevalence rate of 23%, using a two-sided test of significance with alpha of 0.05 and

power of 80%, a sample of 325 was sufficient to detect a similar prevalence rate of 23%. To increase the power and robustness of the analysis given the fact that rates in the RA population was unknown, sample size was calculated with a power of 100% and two-sided alpha of 0.01; a sample of 730 was sufficiently large (See Figure 19).




*G*Power estimated effect size based sample size assumptions*

*Figure 19. Sample Size Calculation for NAFLD Prevalence Rate*

A power calculation was also estimated for RQ4, which addressed the question: if there were significant factors that predict the development of NAFLD (incident cohort) after adjusting for relevant confounders. For the incidence cohort, Cox proportional hazard models yielded the hazard rates and hazard ratio for developing NAFLD. The

independent predictor variables were gender, age, race/ethnicity, metabolic syndrome, obesity, diabetes, hypertriglyceridemia and low HDL. There is limited information in the RA population and a wide range of rates was reported in the general population for the various independent risk factors. No single study reported all the relevant risk factors; hence information from several studies was used to determine the sample size. The effect size for each of the parameters was derived from several studies as outlined in Table 5. For each of the risk factors, power was calculated with 95% CI using the proportion of participants at risk for being exposed and unexposed. Assuming a sample size of 640 for the incident cohort, using a two tailed-test with alpha of 0.05, power was estimated for each of the factors. Overall all the variables considered were sufficiently powered for the bivariate analysis as shown in Table 5. For the multivariate analysis, a small effect size of 0.02 (Cohen, 1988, p. 412) was assumed to minimize chance for the eight key predictor variables in the model.

Table 5

*Power Analysis of NAFLD and Independent Risk Factors*

| Factor | Studies | | Risk Exposed | Risk Unexposed | Odds Ratio | % Power |
|---|---|---|---|---|---|---|
| Gender | Chen et.al., (2006) | Men | 30.4% | 66.3% | 4.5 | 100% |
| Age | Chen et al., (2007) | 40-64 yrs. | 33.7% | 66.3% | 3.9 | 100% |
| | | ≥ 65 yrs. | 32.6% | 67.4% | 4.3 | 100% |
| Race/ Ethnicity | Williams et al., (2011) | Hispanic | 58.3% | 41.7% | 0.5 | 98% |
| | | Caucasian | 44.4% | 55.6% | 1.6 | 80% |
| | | African American | 35.1% | 64.9% | 3.4 | 100% |
| Metabolic Syndrome | Zelber-Sagi et al., (2012) | | 29% | 8% | 3.51 | 100% |
| | Mohan (2009); | | 48.6% | 26.1% | 2.0 | 99% |
| | Ong 2008 | | 43.5% | 56.5 | 1.7 | 90% |
| Obesity | Dassanayake (2009) | | 38% | 62% | 2.7 | 99% |
| | Chen et al., (2007) | | 48.9% | 51.1% | 1.09 | 86% |
| Diabetes Mellitus | Mohan et al. (2009 | | 54.5% | 45.5% | 2.9 | 99% |
| | Lazo (2011), | | 15.8% | 5.4% | 0.3 | 99% |
| | Ong 2008 | | 12.2% | 87.8% | 51 | 100% |
| Hypertriglyceridemia | Chen et al., (2007) | | 41.8% | 58.2% | 1.9 | 98% |
| | Mohan (2009) | | 23.9 | 26.1 | 1.2 | 85% |
| | Dassanayake (2009) | | 30% | 70% | 5.4 | 100% |
| | Lazo 2011 | | 52.6 | 23.8 | 0.30 | 100% |
| Low HDL | Mohan (2009) | | 29.9 | 70.1% | 5.4 | 100% |
| | Kim (2004) | | 42.3 | 50.7 | 0.7 | 57% |
| | Dassanayake (2009) | | 20% | 80% | 16 | 100% |

Assuming a sample size of 640, two tailed-test with alpha of 0.05

Taking into consideration the known rates of NAFLD and the effect size of its associated risk factors, with a power of 80% and alpha of 0.05, a sample size of 759 was sufficiently large to detect the incidence rates and the study was also adequately powered for the multivariate analysis.

**Data Collection**

This was a retrospective study that used observational data. Patients with RA were recruited into the Corrona RA registry (Corrona Rheumatoid Arthritis, 2015; Kremer, 2005). Corrona RA is a disease-based registry that systematically collects and documents the effectiveness and safety of disease modifying anti-rheumatic drugs, biologic agents, and other treatments used in the management of patients with RA (Corrona Rheumatoid Arthritis, 2015; Curtis et. al, 2010). The information is maintained in a comprehensive database (the CORRONA Database) which then can be used for drug safety, clinical outcomes, and marketing related research. Corrona only provides de-identified aggregate reports to subscribers (Corrona Rheumatoid Arthritis, 2015).

According to Corrona RA registry (2015), registry patients must be ≥ 18 years of age with a diagnosis with RA, or started on approved biologic or small molecule medication for the treatment of RA. Patients must be able and willing to provide written consent for participation in the registry, collection of protected health information (PHI) and access to their medical record as needed. Health care providers recruit patients from their own practice and are encouraged to include vulnerable subject populations, such as elderly or economically disadvantaged persons reflective of disease and or population

demographics at the participating sites (Corrona Rheumatoid Arthritis, 2015). Patients are enrolled during regularly scheduled office visits. At the site, healthcare providers, as well as patients, complete the registry questionnaires during the course of regularly scheduled office visits, about 2 to 4 times per year (Corrona Rheumatoid Arthritis, 2015). Patients remain in the registry until they are lost to follow-up or withdraw consent, at which time a subject exit questionnaire is completed (Corrona Rheumatoid Arthritis, 2015). Protocol and case report forms may be requested directly from the registry (http://www.corrona.org).

A registry protocol is made available to the sites that outline the process for data collection During routine clinical care, providers perform assessments according to standard clinical practice; the registry does not mandate specific visits or tests (Corrona Rheumatoid Arthritis, 2015). The provider questionnaire form is used to collect routine physician related information, which includes laboratory, imaging or other clinical data. Patient recorded information, including demographic information, experience with prescribed treatments, functional status, and self- assessment of their disease activity is captured using the subject questionnaire forms (Corrona Rheumatoid Arthritis, 2015).

Subsequently, the data is entered into the registry database or alternatively, de-identified data can also be faxed to Corrona and then entered into the EDC system by the registry staff (Corrona Rheumatoid Arthritis, 2015). According to Corrona rheumatoid arthritis registry (2015), quality of the data is controlled by using edit checks that are built into the on-screen data entry forms to promote completeness and accuracy of the submitted data. Additionally, the registry conducts random checks and remote inspections

of the data received from sites to ensure consistency and completeness of the data, and, to ensure quality, on-site monitoring is conducted as needed to inspect source documentation, to ensure adherence to good clinical practice, protocol compliance, and accuracy of data reported (Corrona Rheumatoid Arthritis, 2015).

The Corrona Foundation is a non-profit entity whose primary purpose is to promote research in the areas of rheumatic diseases and other conditions (Corrona Rheumatoid Arthritis, 2015). Permission was granted to access a limited, de-identified data set required to answer the research questions for this study. The scope and terms of the data for research and publication was outlined in the foundation sublicense data agreement and available upon request.

## Operationalization of Variables

The NAFLD dataset retrieved from the RA registry for this analysis was limited to answer the specific objectives for this study. The NAFLD dataset included demographic information, RA specific information about the disease activity, duration of disease, and RA specific medications, along with data relevant to determine the incidence, prevalence, and factors associated with NAFLD. The dependent and key NAFLD related independent predictor variables are specified in Table 6. Operational definitions for each of the variables are outlined in a separate table (see Table 8).

Table 6

*Variable Characteristics*

| Variable Type | Variable Name | Data Source | Level of Measurement |
|---|---|---|---|
| Dependent | NAFLD | Clinical (laboratory) | Nominal |
| Predictor | Age | Demographic | Continuous<br>Ordinal |
| | Gender | Demographic | Nominal |
| | Race/ Ethnicity | Demographic | Nominal |
| | Metabolic Syndrome | Clinical | Nominal |
| | Obesity | Clinical | Nominal |
| | Diabetes | Clinical | Nominal |
| | Dyslipidemia | Clinical | Nominal |
| Potential Predictors | MTX | Clinical | Nominal |
| | Liver Enzyme Elevation | Clinical | Nominal |
| | Liver Disorders | Clinical | Nominal |
| | Alcohol Use | Clinical | Nominal |

MTX- Methotrexate

## Dependent Variable

The dependent variable was defined as the presence or absence of NAFLD using the FIB-4 score, a validated noninvasive tool described in detailed in Chapter 2. FIB-4 score was calculated by using the following formula: age [years] × AST [U/L]) / (platelet [$10^9$] × √ALT [U/L] (Shah et al., 2009a). FIB-4 score was divided into three categories to match the various progressive stages of NAFLD (see Table 7). FIB-4 score of <1.3 corresponds to fibrosis stage 0 and indicates the absence of fibrosis or advanced disease; FIB-4 score of ≥ 1.3 but < 2.67 = stage 1 with early changes in perisinusoidal or portal changes and also includes stage 2 – perisinusoidal, portal and periportal changes suggestive of mild to moderate disease; and score ≥ 2.67 encompasses advanced disease

with stage 3 with septal or bridging fibrosis or stage 4 which includes cirrhosis or advanced disease (Kleiner et al., 2005; Vallet-Pichard et.al 2007; Shah et.al, 2009a). As described above, there are four possible stages of NAFLD. For this analysis, the primary outcome of interest was to determine the presence or absence of NAFLD. A FIB-4 score of <1.3 indicates the absence of advanced disease and the presence of NAFLD with a score of ≥ 1.3. However, additional analyses was planned to ascertain the presence of the other two categories of secondary interest, mild/moderate NAFLD (score ≥ 1.3 but < 2.67) and advanced disease (score ≥ 2.67),

Table 7

*Relationship between NAFLD Score of Stages of Disease*

| NAFLD FIB-4 Score | NAFLD Stages | Disease Definition |
|---|---|---|
| <1.3 | Stage 0 | Absence of fibrosis or advanced disease |
| ≥ 1.3 but < 2.67 | Stage 1: with early changes in perisinusoidal or portal changes | Mild to moderate disease |
| | Stage 2: perisinusoidal, portal and periportal changes | |
| ≥ 2.67 | Stage 3 and Stage 4 with septal or bridging fibrosis, cirrhosis | Advanced disease |

**Independent Variables**

The independent predictor variables identified were a combination of demographic and clinical risk factors for NAFLD. These variables are collected in the registry and defined for the analysis was based on information learned from the review of literature presented in Chapter 2. Operational definitions for each of the variables are outlined in Table 8.

Table 8

*Variables, Operational Definitions, and Codes*

| Variable Type | Variable Name | Responses Categories/Code |
|---|---|---|
| Dependent | NAFLD | FIB-4 score<br>• <1.3 - no advanced disease (= 0)<br>• ≥ 1.3 - positive for NAFLD (=1)<br>• ≥ 1.3 but < 2.67 = mild/moderate (= 2)<br>• ≥ 2.67 = advanced disease (=3) |
| Predictor | Age at baseline | • Younger = ≥ 18 years < 39 years (= 1)<br>• Middle age =≥ 40 - 59 years (=2)<br>• Elderly ≥ 60 years (=3) |
| Predictor | Gender at baseline | • Male (=1)<br>• Female (=2) |
| Predictor | Ethnicity<br>Race at baseline | • Hispanic (yes=1, no=0)<br>• Caucasian (=1)<br>• African American (=2)<br>• Asian (=3)<br>• Other (=4)<br>• Multiracial (=5) |
| Predictor | Obesity at baseline | • BMI Underweight <18.5 (=1)<br>• BMI Normal weight 18.5–24.9 (=2)<br>• BMI Overweight 25–29.9 (=3)<br>• BMI Obesity 30 or > (=4) |

Table 8 *(table continues)*

*Variables, Operational Definitions, and Codes*

| Variable Type | Variable Name | Responses Categories/Code |
|---|---|---|
| Predictor | Metabolic Syndrome at baseline | Meets any three of the following criteria (yes=1, no=0)<br>• BMI >25 (yes=1, no=0)<br>• Elevated TG level is defined as levels ≥150 mg/dL (yes=1, no=0)<br>• HDL <40 mg/dL in men and <50 mg/dL in women (yes=1, no=0)<br>• Use of lipid lowering medications (yes=1, no=0)<br>• History of dyslipidemia (yes=1, no=0)<br>• Elevated blood pressure is defined as ≥130 mm Hg systolic blood pressure or ≥85 mm Hg diastolic blood pressure (yes=1, no=0)<br>• Use of antihypertensive drug treatment (yes=1, no=0)<br>• History of hypertension (yes=1, no=0)<br>• Use of drug treatment for elevated glucose (yes=1, no=0)<br>• History of diabetes (yes=1, no=0) |
| Predictor | Diabetes at baseline | Yes=1, No=0 |
| Predictor | Dyslipidemia at baseline | Yes=1, No=0 for any one of these criteria<br>Total cholesterol > 200 (mg/dL) or LDL > 130 (mg/dL) or HDL <40 mg/dL in men and <50 mg/dL in women, or TG >150 mg/dL |
| Predictor | MTX at baseline | Yes=1, No=0 |
| Predictor | Liver Enzyme Elevation* | Yes=1, No=0 |
| Predictor | Liver Disorders at baseline | Yes=1, No=0 |
| Predictor | Alcohol use at baseline | Yes =1, No =0<br>0=No, Daily = 2, Weekly =3,1 Daily = 2, Occasional 3, Monthly= 4 |

BMI- Body Mass Index; TG- Triglyceride; HDL- High Density Lipids: LDL- Low Density Lipids; *ULN > Upper limit of normal at baseline

Age, gender and race/ethnicity are self-reported demographic measures collected in the registry. Age is a continuous variable and reported in years in the registry. For this study, similar to studies conducted by Lazo et al. (2013), Yan et al. (2013) and Chen et.al. (2006), age was categorized into three groups: younger, middle age and elderly. Gender indicates the reported sex of the patient and captured as male or female. Race is another self -reported measure captured in the registry as Caucasian, African American, Asian, other or mixed race, and ethnicity grouped as Hispanic or non Hispanic.

Clinical risk factors for NAFLD are metabolic syndrome, obesity, diabetes, hypertriglyceridemia and low HDL, and are physician reported measures. Obesity for this study was assessed using BMI score, calculated for each patient using height weight, age and gender. BMI was then categorized into four groups: underweight, normal weight, overweight and obese using the Centers for Disease Center BMI criteria (CDC [BMI], (2014). Metabolic syndrome was defined as the presence of any three of the following obesity related risk factors: elevated waist circumference, elevated triglycerides (TG), blood pressure, glucose and reduced HLD (Alberti et al., 2009; Grundy et al, 2005). Specific criteria recommended for each of these measures were used in this analysis to determine the presence of metabolic syndrome (Grundy et al., 2005).

Waist circumference is the preferred measure to determine metabolic syndrome, however, it was not collected in the registry and was substituted with BMI score $\geq 25$ (overweight/obese) in this analysis. BMI is an appropriate substitute as it has been used in several studies and shown to be an independent risk factor for NAFLD (e.g. Smits et al. 2013, Wong, et al. 2011, and Mohan et al. 2009). Operational definitions for elevated

TG are: TG levels ≥150 mg/dL, the use of lipid lowering medications, history of dyslipidemia, elevated blood pressure of ≥130 mm Hg systolic blood pressure or ≥85 mm Hg diastolic blood pressure, the use of antihypertensive drug treatment, a history of hypertension and reduced high HDL levels <40 mg/dL in men and <50 mg/dL in women or the use of lipid lowering medication or history of dyslipidemia. Fasting glucose levels are not captured in the registry; hence the presence of elevated fasting glucose was defined as the use of drug treatment for elevated glucose or history of diabetes.

The variable diabetes was defined as those patients with a reported history of diabetes or use of drug treatment for elevated glucose. Dyslipidemia was defined as altered lipid levels (total cholesterol, low density cholesterol (LDL), HDL, and TG), as outlined in the ATP III guidelines, (NCEP, 2002) or taking lipid lowering medications. Potential predictors in the study are MTX use (as yes or no), liver enzyme elevation (as yes or no above upper limit of normal), alcohol use, and liver disorders. These variables are captured in the registry. For this analysis, presence of liver disorders included reported hepatic events, liver disorders and also those with history of liver biopsy. Alcohol use is captured in the registry as none, daily, weekly, or occasional use.

Statistical analyses for this study were conducted using the Statistical Package for Social Sciences (SPSS 22.0). The registry statistician extracted the dataset. Included in the dataset were adult RA patients who met the inclusion criteria and also had the measurements to calculate FIB-4 score (age, AST, ALT and platelets) during the period of January 2001 to November 2014. For the incident cohort analysis, the index baseline visit included those without NAFLD (calculated FIB-4 score <1.30) at or after date of

enrollment into the registry and must have had at least two or more follow-up visits. An incident case of NAFLD was defined when there was at least two follow up visits with FIB-4 ≥1.30. These were consecutive visits; the incident event occurred at the second visit with FIB-4 ≥1.30. However, should there not be sufficient sample size to conduct the analysis, then a more lenient criterion where patients with only one follow-up visit with FIB-4 ≥1.30 would be included for analysis. RA patients with one or more visits in the period of one year (e.g. from January 2012 - December 2012) would be included in the prevalent dataset. NAFLD (FIB-4 ≥1.30) must be present at two consecutive visits. Again, should sample size not be sufficient then only one follow-up visit would be used for the analysis.

**Statistical Analysis and Tests**

The following section outlines the statistical plan for each of the research questions and, wherever appropriate, shell output tables are provided, for example see table 9. This was a quantitative study that included descriptive and multivariate analysis. In preparation for these analyses using SPSS, measures of central tendency, distribution, and dispersion of data was assessed. The impact of missing data was evaluated along with assumptions for parametric tests and the appropriate use of nonparametric tests. Kaplan Meier survival and Cox proportional hazard analysis was used to determine the factors that predict the development of NAFLD. This approach allows one to estimate risk of an event (i.e. development of NAFLD) after adjusting for predictor or explanatory variables (Tabachnick & Fidell 2012, pp. 510-570) While satisfying assumptions of distribution for descriptive analyses and assessing for linearity, normality, and

homoscedasticity among covariates, are not necessarily required for survival analysis, however, assessing them would enhance the robustness of the analysis, additionally there are other factors and assumptions that must be evaluated and addressed when conducting survival analysis (Tabachnick & Fidell (2012, pp. 510-570). I used the checklist provided by Tabachnick & Fidell (2012) as my guide for conducting survival analysis. While satisfying assumptions of distribution for descriptive analyses and assessing for linearity, normality, and homoscedasticity among covariates, are not necessarily required for survival analysis, however, assessing them would enhance the robustness of the analysis, additionally there are other factors and assumptions that must be evaluated and addressed when conducting survival analysis (Tabachnick & Fidell (2012, pp. 510-570). I used the checklist provided by Tabachnick & Fidell (2012) as my guide for conducting survival analysis. I evaluated the adequacy of sample size, missing data, normality of distribution, proportionality of hazard assumption and multicollinearity. I also addressed issues with missing data, outliers, and difference between withdrawn and remaining cases, and changes in survival experience over time. The details and results of these evaluations are presented in the following sections. For inferential testing, a two- sided test with 95% confidence interval, and hazard ratios were used. Ratio analysis with results that do not include 1 or analysis with p-value $< .05$ was considered significant unless otherwise specified.

**Descriptive Analysis**

Several descriptive analyses were conducted pertaining to each of the research questions: continuous variables means, medians, and interquartile ranges were

determined. A descriptive analysis was conducted to determine comparability of NAFLD study population and the overall registry population (see sample Table 9). This information was provided by the registry and enabled the comparison of the NAFLD study cohort with core registry population.

Table 9

*NAFLD Study Cohort Compared to Overall Registry Patients (sample*)

| Variable | All Corrona Patients (N) | NAFLD Study Cohort (N) | *P* value |
|---|---|---|---|
| Age (years) | | | |
| Gender (female) (n, %) | | | |
| Race (n, %) | | | |

RQ1 Quantitative: What is the incidence rate of NAFLD among patients with RA from 2001 -2015 in a RA registry in the U.S. using the FIB-4 test?

The incident cohort dataset was used to determine the incidence rates and time to using Kaplan Meier survival analysis. The results of the analysis included time to event, person years, absolute number and percent of incident cases, rate per 1000 patient years with 95% CI, and the overall cumulative incidence rates during the study period. Rates of NAFLD by the key significant risk factors were identified in the study, and were also analyzed and reported (see Table 10).

RQ2 Quantitative: What is prevalence rate of NAFLD among patients with RA for one year (e.g. from January 2012 - December 2012) in a RA registry in the U.S. determined by using the FIB-4 test?

Similar analysis was conducted to determine the prevalence rates using the prevalent cohort. The results of the analysis include absolute number, percent of

prevalent cases, and prevalence per 1000 patients with 95% CI during the study period. Prevalence of NAFLD by the key significant risk factors identified in the study are also summarized and reported (see Table 10).

Table 10

*NAFLD Rates: Incidence, Prevalence and by Risk Factors (Sample)*

| Variable | N (%) | Prevalent Case (N, %) | Prevalence Rate (1000 PY) 95% CI | Incident Cases (N, %) | Incidence Rate (1000 PY) 95% CI |
|---|---|---|---|---|---|
| Overall NAFLD | | | | | |
| Mild/Moderate NAFLD | | | | | |
| Advanced NAFLD | | | | | |
| Age | | | | | |
| Younger | | | | | |
| Middle Aged | | | | | |
| Elderly | | | | | |
| Gender | | | | | |
| Male | | | | | |
| Female | | | | | |
| Race/ Ethnicity | | | | | |
| Caucasians | | | | | |
| Hispanic | | | | | |
| African American | | | | | |
| Asian | | | | | |
| Obesity (BMI) | | | | | |
| Metabolic Syndrome | | | | | |
| Diabetes | | | | | |
| Dyslipidemia | | | | | |
| MTX Use | | | | | |
| Liver Enzyme Elevation | | | | | |
| Liver Disorders | | | | | |
| Alcohol use | | | | | |
| Daily | | | | | |
| Weekly | | | | | |
| Monthly | | | | | |

PY= Patient Year; CI- Confidence Interval; BMI- Body Mass Index; MTX- Methotrexate

RQ3: Among patients in the RA incident cohort, what are the baseline differences in clinical and demographic characteristics among those with NAFLD compared to those without?

RQ *Ho*: Among patients in the RA incident cohort, there are no significant baseline differences in the clinical and demographic characteristics among those with NAFLD compared to those without.

RQ3 *Ha*: Among patients in the RA incident cohort, there are significant differences in the baseline clinical and demographic characteristics among those with NAFLD compared to those without.

Baseline characteristics for those with NAFLD were compared to those without for the incident cohort in the format shown in the sample table (see sample Table 11). Chi-square and independent t-test were used to determine if there are statistically significant differences between these two groups.

Table 11

*Comparison of Baseline Factors: Patients with vs. without Incident NAFLD*

| Variable | All Cohort Patients (N) | With Incident NAFLD (n) | Without Incident NAFLD (n) | *P*-value |
|---|---|---|---|---|
| Age (mean, years) | | | | |
| Gender (female) (n, %) | | | | |
| Race (n, %) | | | | |

*P* value = With Incident NAFLD vs. Without Incident NAFLD

RQ4 Quantitative: For the RA incident cohort, what are the significant baseline factors (clinical and demographic) that predict the development of NAFLD after adjusting for relevant confounders?

RQ4 *Ho*: For the RA incident cohort, there are no significant baseline factors (clinical and demographic) that predict the development of NAFLD after adjusting for relevant confounders.

RQ4 *Ha*: For the RA incident cohort, there are significant factors (clinical and demographic) that predict the development of NAFLD after adjusting for relevant confounders.

Cox proportional hazard analysis was used to determine the factors that predict development of NAFLD for the incident cohort analysis. The results were reported as hazard rates and ratios. The following known predictors of NAFLD were included in the model apriori regardless of the significance, including: age, gender, race/ ethnicity, obesity, metabolic syndrome, diabetes, dyslipidemia, MTX, liver enzyme elevation, liver disorders, and alcohol use.

**Bivariate and Multivariate Analysis**

Prior to conducting the multivariate analysis, a bivariate analysis was conducted to determine the effect of each of the factors associated with the outcome variable (see sample Table 12). The results from the unadjusted analysis were used to inform appropriate variables to be included in the multivariate analysis as described below.

Table 12

*Unadjusted Analyses of Baseline Predictors for Incidence of NAFLD*

| Variable | Hazard Ratio | 95% CI | *P*-value |
|---|---|---|---|
| Overall NAFLD | | | |
| Mild/Moderate NAFLD | | | |
| Advanced NAFLD | | | |
| Age | | | |
| Younger | | | |
| Middle Aged | | | |
| Elderly | | | |
| Race/Ethnicity | | | |
| Caucasians | | | |
| African American | | | |
| Asian | | | |
| Other | | | |
| Hispanic | | | |
| Obesity BMI | | | |
| Underweight | | | |
| Normal Weight | | | |
| Overweight | | | |
| Obesity (BMI ≥ 25) | | | |
| Metabolic Syndrome | | | |
| Diabetes | | | |
| Dyslipidemia | | | |
| MTX Use | | | |
| Liver Disorders | | | |
| Alcohol use | | | |
| Daily | | | |
| Weekly | | | |
| Monthly | | | |

BMI- Body Mass Index; MTX- Methotrexate

A multivariate analysis was conducted to determine if there are significant baseline factors that predict the development of NAFLD after adjusting for relevant predictors or explanatory variables among patients with RA (see sample Table 13). Cox proportional hazards analysis was used to identify factors associated with the development of NAFLD (Tabachnick, B. & Fidell, L., 2012. Ch. 11). An unadjusted and adjusted Cox proportional model was run to determine the effect of the confounders on the outcome variable. While *p*-values can assess differences, however, it is also important to ascertain the impact or magnitude of the change so as to avoid overestimation or

underestimation of the association of these factors and the outcome variable (Grayson, 1987). Factors with ratios that changed by 10% or more were included in the multivariate model as potential confounders (Grayson, 1987; Hernán, et al., 2002).

All factors identified apriori and significant factors from the bivariate analysis were entered into the model simultaneously. Several models were run to identify the most parsimonious model, and also evaluate the effect of the predictor variables: age, gender, race/ ethnicity, obesity, metabolic syndrome, diabetes, dyslipidemia, MTX use, liver enzyme elevation, liver disorders, and alcohol use as defined and categorized in Table 8. For example, one of the models would be adjusted for key demographic variables (e.g. age, gender, race/ethnicity). Then a second model could be adjusted for these key demographic variables as well as for factors associated with MetS. The risk over time was assumed to remain proportionally constant; this assumption was checked using Kaplan Maier analysis. Should the survival curves for each of these combinations cross, then the assumption of proportionality would be violated (Tabachnick, B. & Fidell, L., 2012. Ch. 11). Should this assumption be violated then diagnostic analysis would be conducted to determine the causes, such as outliers, highly influential variables, or inclusion of appropriate covariates in the model (Wilson, 2013). Incidence (hazard) rates and ratios were reported with 95% confidence interval and factors that do not include 1 and a p-value $< .05$ are considered significant.

Table 13

*Adjusted Analyses of Baseline Predictors for Incidence of NAFLD*

| Variable | Hazard Ratio | 95% CI | *P*-value |
|---|---|---|---|
| Overall NAFLD | | | |
| Mild/Moderate NAFLD | | | |
| Advanced NAFLD | | | |
| Age | | | |
| Younger | | | |
| Middle Aged | | | |
| Elderly | | | |
| Race/Ethnicity | | | |
| Caucasians | | | |
| African American | | | |
| Asian | | | |
| Other | | | |
| Hispanic | | | |
| Obesity BMI | | | |
| Underweight | | | |
| Normal Weight | | | |
| Overweight | | | |
| Obesity (BMI ≥ 25) | | | |
| Metabolic Syndrome | | | |
| Diabetes | | | |
| Dyslipidemia | | | |
| MTX Use | | | |
| Liver Disorders | | | |
| Alcohol use | | | |
| Daily | | | |
| Weekly | | | |
| Monthly | | | |

BMI- Body Mass Index; MTX- Methotrexate; Adjusted for age, gender, race/ethnicity, BMI, metabolic syndrome, diabetes, dyslipidemia, MTX use, Liver Disorder, Alcohol use

## Threats to Internal and External Validity

The main threat to internal and external validity for this study was the design. A valid study is unbiased, one that is designed and data collected fit for purpose in a reliable and valid manner such that the overall results of the study are closer to the truth ( Szklo & Nieto, 2014. pp. 109-150).

As this was a non-randomized study that used secondary data and utilized a non-probability sample of convenience there were inherent threats to both internal and

external validity (Szklo & Nieto, 2014, pp. 109-150). Systematic selection bias was a potential issue in this study, as the sample was selected primarily based on availability of laboratory parameters (liver enzyme and platelet counts); there is a potential for differences among this group and the rest of RA patients in the registry. Comparing the study cohort and the registry population assessed this threat of potential bias. Significant differences could be indicative of systematic selection bias (Szklo & Nieto, 2014, pp. 109-150).

There are other limitations of using secondary data, as the collected data may not be a good fit for the research question, there maybe systematic missing variables, or key variables may not be available at certain time points (Hofferth, 2005). Hence this analysis, using secondary data, may not be conducive to establishing causality (Smith et al., 2011). Given that this was a retrospective study, misclassification of either the exposure or the outcome was also a potential risk. This limitation was addressed during the data collection phase of the study, however, since this was a retrospective study. Conducting sensitivity and specificity analysis was not feasible; hence the magnitude of misclassification is unknown. Alternatively, additional sensitivity analysis was conducted under varying plausible assumptions to estimate the influence on the effect size. When these results are directionally consistent one can be assured that the results are from the effect of treatment rather than due to systematic errors (Szklo & Nieto, 2013, pp. 391-426). While this was a large registry of RA patients across the U.S., patients volunteered to participate in the registry, also not all patients at a site were entered into the registry;

leading to variation as to how sites select patients for the registry and may pose a potential threat to the generalizability of the results (Curtis, et al., 2013).

Using real world data increases the external validity, however, there remain many unmeasured confounders that threaten internal validity and thereby diminishing the inferential power of the study (Frankfort-Nachmias & Nachmias, 2008). This was an exploratory hypothesis-generating study and some of the limitations of observational data can be addressed by adjusting for confounders and assessing interactions along with sub group analysis; these are potential options to consider in order to increase the robustness of the study results (Frankfort-Nachmias & Nachmias, 2008). Thus, results can only be interpreted under the conditions study was conducted, as alternative explanations cannot be ruled out or cannot be adequately explained through statistical analysis (Szklo &Nieto, 2013, pp. 391-426).

**Ethical Procedures**

This was a retrospective study where a limited dataset with pertinent information for the analysis was retrieved from the registry. To ensure that the registry is operating in an ethical and compliant manner, it has institutional review board (IRB) approval in place and also has obtained participants' consent for the use of their clinical data for research. The registry has safeguards in place that includes obtaining the appropriate consent for accessing the data from the participants as well as from the sites for the use of their data in a Health Information Portability and Accountability Act (HIPPA) compliant manner (Corrona Rheumatoid Arthritis, 2015). Thus, the use of data for this study retrieved from the registry was within the scope of the consent (i.e. for research). To ensure

confidentiality was maintained, only the needed de-identified data was extracted and subsequently stored in a secure and protected place on my computer. A mutual agreement about rights, use, and storage of the data is specified in a data use agreement and available upon request. To protect against potential human rights violation and to ensure confidentiality was maintained, the study protocol, original IRB approval for the registry, and data sharing agreement was approved by Walden University's IRB (# 06-19-15-0335302).

**Summary**

This was a retrospective cohort study of patients with RA to determine the incidence, prevalence, and factors associated with NAFLD. Secondary data from an established RA registry was used to identify a cohort with longitudinal data for the incidence analysis. The primary objective of the study was to determine incidence rates, time to event and the factors that predict the development of NAFLD after adjusting for relevant confounders such as age, gender, race/ ethnicity, obesity, metabolic syndrome, diabetes, dyslipidemia, MTX, liver disorders, and alcohol use. Alternatively, analyses using a prevalent cohort were planned in the event an adequate sample for the primary objective was not available. Descriptive analysis, Kaplan Meier survival and multivariate Cox proportional hazard analysis was conducted using SPSS. Processes and approvals were in place to ensure that patients were protected in an ethical and compliant manner.

As this was a non-randomized study that used secondary data and utilized a non-probability sample of convenience there were inherent threats to both internal and external validity (Szklo & Nieto, 2014, pp. 109-150). Additionally, this study did not

that collected fit for purpose data in a reliable and valid manner ( Szklo & Nieto, 2014. pp. 109-150). Systematic selection bias was a potential issue in this study, as the sample was selected primarily based on availability of laboratory parameters (liver enzyme and platelet counts); there is a potential for differences among this group and the rest of RA patients in the registry. Comparing the study cohort and the registry population assessed this threat of potential bias. Significant differences could be indicative of systematic selection bias (Szklo & Nieto, 2014, pp. 109-150).

There are other limitations with using secondary data for example there maybe missing data for key variables, or key variables may not be available at the pertinent time points (Hofferth, 2005). This was a retrospective study; there is potential risk of misclassification of either the exposure or the outcome. This was a large registry of RA patients across the U.S., who volunteered to participate in the registry. Additionally not all patients at a site were entered into the registry. Thus there was a potential threat to the generalizability of the results (Curtis, et al., 2013).

Using real world data increases the external validity, however, there remain many unmeasured confounders that threaten internal validity and thereby diminishing the inferential power of the study (Frankfort-Nachmias & Nachmias, 2008). This was an exploratory hypothesis-generating study and some of the limitations of observational data can be addressed by adjusting for confounders and assessing interactions along with sub group analysis; these are potential options to consider in order to increase the robustness of the study results (Frankfort-Nachmias & Nachmias, 2008). Thus, results can only be interpreted under the conditions study was conducted, as alternative explanations cannot

be ruled out or cannot be adequately explained through statistical analysis (Szklo &Nieto, 2013, pp. 391-426). The next chapter will present the results of the analysis plan outlined here to determine the incidence, prevalence, and factors associated with NAFLD in the RA population.

# Chapter 4: Results

The purpose of this research study was to determine the presence and factors associated with the development of NAFLD using registry data collected from a cohort of adult RA patients in the U.S. The primary objective of this study was to determine the incidence rate and the factors that predict the development of NAFLD after adjusting for relevant confounders. The contingency plan was to conduct analyses using a prevalent cohort if the sample size was not adequate for the incident cohort analysis. Since the incident cohort sample was sufficiently large the alternate objective to conduct analysis using the prevalent cohort was abandoned. Thus this chapter will focus on the results of the incident analyses. To achieve these objectives an incident cohort was utilized. All patients who met the eligibility criteria without NAFLD (FIB-4 score <1.3) at the index baseline visit were grouped into the incident cohort.

This chapter also includes the results from the descriptive analysis, bivariate analysis, and factors selected for the multivariate analysis. I will summarize the results for each the following research questions.

RQ1 Quantitative: What is the incidence rate of NAFLD among patients with RA from January 2001 - November 2014 in a RA registry in the U.S. using the FIB-4 test?

RQ2 Quantitative: What is the prevalence of NAFLD among patients with RA for one year (e.g. from January 2012-December 2012) in a RA registry in the U.S. determined by using the FIB-4 test?

RQ3: Among patients in the RA incident cohort, what are the baseline differences in clinical and demographic characteristics among those with NAFLD compared to those without?

RQ $H_0$: Among patients in the RA incident cohort, there are no significant baseline differences in the clinical and demographic characteristics among those with NAFLD compared to those without.

RQ3 $H_a$: Among patients in the RA incident cohort, there are significant differences in the baseline clinical and demographic characteristics among those with NAFLD compared to those without.

RQ4 Quantitative: For the RA incident cohort, what are the significant baseline factors (clinical and demographic) that predict the development of NAFLD after adjusting for relevant confounders?

RQ4 $H_0$: For the RA incident cohort, there are no significant baseline factors (clinical and demographic) that predict the development of NAFLD after adjusting for relevant confounders.

RQ4 $H_a$: For the RA incident cohort, there are significant factors (clinical and demographic) that predict the development of NAFLD after adjusting for relevant confounders.

As mentioned above, since the sample size was sufficiently large to answer the primary objective of this research study, the incident analysis, the alternative research questions that relate to the prevalent analyses was dropped and the results will focus on the primary objective only. Chapter 3 outlined *a priori* the statistical analysis plan. In this

chapter, I describe the methods used to select the incident dataset, statistical analysis and also describe any deviations from the plan and the rationale for the modifications.

## Dataset Collection and Preparation

This study used retrospective data from an established RA registry. Recruitment and details about data collection are outlined in Chapter 3. The registry statistician extracted limited, de-identified, patient level data from the registry and only included the pre-specified clinical and demographic data points needed for this study. This included data about demographic and clinical information for the predictor variables; gender, age, race/ethnicity, metabolic syndrome, obesity, diabetes, dyslipidemia, liver enzyme elevation, liver disorders and alcohol use. Factors related to RA were disease duration, disease activity and the RA related medication. All adult patients with a diagnosis of RA of more than 18 years of age in the registry with information necessary to determine the presence of NAFLD (FIB-4 criteria) were included in the study.

The registry statistician extracted the information for the incident cohort and also provided information needed to determine comparability of the NAFLD study cohort and the overall registry population (Table 14). The study period for the incident cohort was from January 2001 to November 2014. Excluded were those for whom the FIB-4 information was not available or those with secondary causes of NAFLD collected in the registry such as Hepatitis C, B, and polycystic ovarian disease, yielding a sample of 17,481 patients for the incident cohort analysis as shown in Figure 20.

The incident cohort included those with an index baseline visit without NAFLD (calculated FIB-4 score <1.30) at or after date of enrollment into the registry and had a

minimum of one or more follow-up visits. An incident case of NAFLD was defined when there was at least two consecutive follow up visits with FIB-4 scores to determine the presence of NAFLD. The dataset for the incident cohort and the data requirements was extracted as planned and described in Chapter 3.

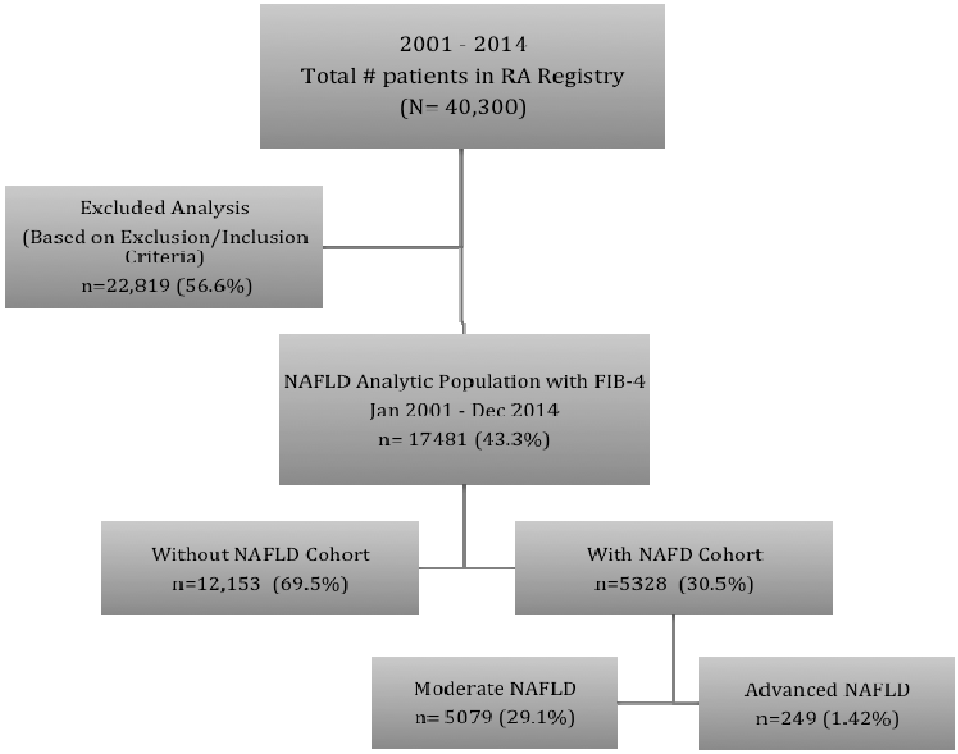



*Figure 20. Flow chart of Study Participants*

**Transforming Data**

The independent predictor variables identified were a combination of demographic and clinical risk factors for NAFLD. Raw data were transformed and operationalized for analysis as previously outlined in Table 8. Frequency tables were then run on each of the variables. Several of the categories had to be further collapsed

due to infrequency of data. Race was collapsed from eight categories into four: Caucasian, African American, Asian and "Other" which included multiracial, Pacific Islander, and unknown. Marital status was collapsed into three groups (single or widowed; married or partnered; divorced or separated). Given the prevalence of obesity in this cohort, this category was collapsed into obese (BMI $\geq$ 25) and non-obese groups. Smoking status was combined from four into two categories, nonsmokers and smokers; the latter included both former and current smokers.

Diabetes included those with a diagnosed history of diabetes or those currently taking diabetic medications. Liver disorders included those with a diagnosis of hepatic disease, or hepatic disease with or without biopsy. Dyslipidemia for the analysis included those with a history of dyslipidemia or using lipid lowering medication. Also included were those with high triglyceride levels, low HDL, high cholesterol, or high LDL. Disease duration categories were classified into clinically meaningful categories of those with early RA with less than 2 years, 2 to 5 years, 5 to 10 Years and those with greater than 10 years of disease (Anderson, Wells, Verhoeven, & Felson, 2000). MTX use included those currently using MTX; conventional diseases modifying antirheumatic drugs (cDMARD) included those on any other RA medications other than MTX. Those on TNFs and non-TNFs were combined into one group of biologic users. Alcohol was collapsed from four categories into two groups; alcohol users (which included those who used alcohol daily, weekly, occasionally, and monthly) and nonusers. Smoking status was combined into two groups; nonsmokers and smokers, the latter included those with

current or past history of smoking. Exercise was divided into two groups: those who exercise and those who do not.

**Descriptive Statistics of the Entire NAFLD Cohort**

A descriptive analysis was conducted to determine comparability of the NAFLD study cohort and the overall registry population (see Table 14). The primary purpose of this descriptive analysis was to evaluate the generalizability of the findings from this study, given that a sample of convenience was used. Specifically, to determine if there were clinically relevant differences between the incident cohort compared to the overall registry population. The overall registry had 40,300 RA patients with almost half of them (17,481) meeting the eligibility criteria to be included in the NAFLD analysis. There was statistically significant difference between those in the NAFLD cohort compared to those remaining in the registry for all the variables except for Hispanic ethnicity and liver disorders. However, the significant *p* values seen in this analysis were driven mainly from the large sample size. Even though there were statistically significant differences the clinical relevance was minimal since the numerical values were similar for most of the variables. For example, the absolute values for many of the factors were similar e.g., three fourth of the cohort were females (74% in overall registry vs. 79% in the NAFLD cohort). However there were some notable absolute differences: the NAFLD cohort was younger (54.2 years vs. 61.1 years); was more likely to have insurance (79.2 % vs. 66.9%); used MTX (70.2% vs. 58.3%); fewer reported CVD history (6.4% vs. 11.6%); and their liver enzyme elevation was lower (8.24% vs. 22.0%).

Table 14

*Overall Registry Patient Compared to NAFLD Study Cohort*

| Variable | Overall Registry Patients (n= 22819) | | NAFLD Cohort (n=17,481) | | *p*-value* |
|---|---|---|---|---|---|
| | n | % | N | % | |
| Gender (Female) | 22436 | 74.4% | 17481 | 78.7% | < .001 |
| Race | 22,819 | | 17,481 | | < .001 |
| Asian | | 1.4% | | 1.6% | |
| African American | | 8.1% | | 6.3% | |
| Other Race [1] | | 5.2% | | 3.5% | |
| Caucasians | | 85.0% | | 88.5% | |
| Ethnicity Hispanic | 1792 | 7.8% | 14199 | 8.17% | 0.363 |
| Marital Status | 22,819 | | 17,481 | | < .001 |
| Single /Widowed | | 22.9% | | 19.6% | |
| Married /Partnered | | 61.0% | | 66.7% | |
| Divorced /Separated | | 12.1% | | 12.3% | |
| Educational Status | 22,819 | | 17,481 | | < .001 |
| Primary | | 4.8% | | 3.7% | |
| High School | | 40.1% | | 36.6% | |
| College/University | | 48.3% | | 55.5% | |
| Insurance Status (Yes) | 19,768 | 66.9 % | 15612 | 79.2 | < .001 |
| MTX Use | 22,819 | 58.3% | 17481 | 70.2% | < .001 |
| cDMARD Use | 22,819 | 32.0% | 17481 | 29.5% | < .001 |
| TNF Use | 22,819 | 32.3% | 17481 | 37.2% | < .001 |
| NonTNF Use | 22,819 | 5.8% | 17481 | 6.7% | < .001 |
| Cardiovascular History | 22,819 | 11.6% | 17481 | 6.4% | < .001 |
| Diabetes | 22,819 | 9.7% | 17481 | 8.1% | < .001 |
| Dyslipidemia | 22,819 | 26.3% | 17481 | 21.8% | < .001 |
| History Cancer | 22,819 | 8.0%) | 17481 | 5.4% | < .001 |
| Liver Disorders | 22,819 | 2.4% | 17481 | 2.2% | 0.174 |
| Liver Enzyme Elevation | 13,832 | 22.0% | 17481 | 8.2% | < .001 |
| Metabolic Syndrome | 22,819 | 22.9% | 17481 | 18.9% | < .001 |
| Alcohol Use (Yes) | 19,087 | 36.3% | 15026 | 39.8% | < .001 |
| Smoker (Yes) | 21,254 | 15.0% | 16228 | 17.4% | < .001 |
| Exercise (Yes) | 21,404 | 68.5% | 16795 | 70.3% | < .001 |
| | n | *M (SD*) | N | *M (SD*) | *p*-value* |
| Age yrs. | 22363 | 61.1 (13.8) | 17481 | 54.2 (12.4) | < .001 |
| Disease Duration yrs. | 22,469 | 9.2 (10.2) | 17242 | 8.2 (8.9) | < .001 |
| CDAI Score | 21,352 | 14.8 (13.2) | 16818 | 13.8 (13.0) | < .001 |
| Body Mass Index | 21,277 | 29.1 (7.2) | 17481 | 29.6% (7.2) | < .001 |

M= mean; SD = Standard Deviations; CDAI= Composite disease activity index; MTX = Methotrexate; cDMARDs = conventional disease modifying antirheumatic drugs; TNF = Tumor Necrotic Factor; 1= multiracial, Pacific Islander, and unknown

**Dataset Preparation**

Upon receipt of data, I conducted a detailed analysis of the dataset to ensure there was sufficient sample size and that the relevant variables were included. Then the NAFLD analytic data set was prepared for descriptive and multivariate analyses. Several analyses for measures of central tendency, distribution, and dispersion of data were assessed. The impact of missing data was evaluated along with assumptions for conducting the Kaplan Meier and Cox proportional analysis. Details and results from these evaluations are provided later in this section.

**Data Preparation for Analysis**

I used the checklist provided by Tabachnick & Fidell (2012, pp. 510-570) as my guide for conducting survival analysis. I evaluated the adequacy of sample size, missing data, normality of distribution, proportionality of hazard assumption and multicollinearity. I also addressed issues with missing data, outliers, and difference between withdrawn and remaining cases, and changes in survival experience over time. The details and results of these evaluations are presented in the following sections.

**Adequacy of Sample Size**

Sample size estimations and considerations are described in detail in Chapter 3. Based on the literature review, taking into consideration the known rates of NAFLD and the effect size of its associated risk factors, with a power of 80% and alpha of 0.05, a sample size of 759 participants would have been sufficient to detect the incidence and prevalence rates and the study would also be adequately powered for the multivariate analysis. The final sample size for this study was 17,481 RA patients with data collected

over a period of 14 years (January 2001 to November 2014), thus sufficiently large to conduct the incident analysis. Additionally, when certain categorical data was collapsed based on the frequency analysis, the analysis was sufficiently powered for the chi-squared test. The assumption was also met of having expected frequencies of greater than five in each of the cells (Field, 2009).

**Assessing Missing Data**

I used missing variable analysis (MVA), a SPSS test to determine if there were identifiable patterns of missing data. The result of this analysis using the Little's MCAR test was not significant ($\chi^2$ (24176) = 1317.39, $p$ = 1.000). Thus it can be assumed that the likelihood of systematically missing values potentially leading to bias was low. In addition, visual outputs and variable summary statistics were generated to determine the number of cases. The impact of missing variables on the outcome variable was assessed as shown in Table 15. The minimum percentage of missing values for a variable to be included in the analysis was set at 1.0%. Among the missing values: ethnicity (Hispanic) had the highest number with 19% with missing values, followed by alcohol use with 14%, and diabetes with 1.4%. These were important variables as they were key predictor variables identified *a priori*. The approach to address these missing values is described in detail in the following section.

Table 15

*Missing Variable Analysis*

| | Missing | | |
|---|---|---|---|
| | N | Percent | Valid N |
| Ethnicity (Hispanic) | 3282 | 18.8% | 14199 |
| Alcohol Use (yes) | 2455 | 14.0% | 15026 |
| Private insurance | 1869 | 10.7% | 15612 |
| Exercise (yes) | 1108 | 6.3% | 16373 |
| Diabetes | 238 | 1.4% | 17243 |
| Marital Status | 223 | 1.3% | 17258 |

**Addressing Missing Data**

With retrospective, secondary data collected over many years, missing data are inevitable and can be a major challenge. There are no firm guidelines for how much missing data can be allowed, allowable tolerance also hinges upon the sample size (Tabachnick & Fidell, 2012, Ch. 4). There are several analytical approaches to address this issue of missing values. All have limitations and none of them are able to produce unbiased estimates with certainty. For this analysis I selected the multiple imputation approach. It is considered one of the more robust analytic approaches to reduce uncertainty (Tabachnick & Fidell, 2012. Ch.4*).* One of the strengths of multiple imputations is that sampling variability is retained. It can be used with data from a single observation and more importantly it accommodates the use of longitudinal data (Tabachnick & Fidell, 2012, Ch. 4*).* Additionally, this approach makes no assumptions about randomly missing variables. It generates a mean estimate for each variable from multiple imputation data sets while taking into account variance within and between these datasets. Thus this approach is able to account for the true uncertainty in the data

(Tabachnick & Fidell, 2012, Ch. 4*)*. Most of the key variables selected apriori did not require imputation except for diabetes and ethnicity. For this analysis using the multiple imputation function available in SPSS, five other imputation datasets were produced in addition to the original data. Given the large sample size and the use of multiple imputations, any substantial impact of missing data was sufficiently mitigated (Tabachnick & Fidell, 2012, pp. 60-116).

**Normality of Distributions**

For continuous variables, the independent-samples t-test was used to determine if there was a statistically significant difference between mean baseline among those with NAFLD versus those without the disease. The factors evaluated were age, BMI, duration of disease and disease activity. Prior to conducting the t-test analysis, I evaluated the normality of distribution and impact of outliers in these two groups in relationship to the dependent variable. I used several analytical approaches including histograms, box-plots, normal Q-Q plots to assess normal distribution and detrended normal Q-Q plot to assess differences between the observed and expected values (data not shown). Age was normally distributed. However, there were a few cases at the very low end and the very high end that deviated from the expected distribution. Similar results were seen for disease duration and disease activity, with outliers at both ends of the distribution. For BMI, the normal distribution as well as expected distribution was deviated, with a large number of cases with very high values. To address this issue of outliers, these variables were transformed into categorical variables for the multivariate analysis. The impact of

these variables was assessed using Mahalanobis regression analysis and presented below (see Table 16).

**Assumptions for Chi-Square**

The chi-square test was used to evaluate if there was a significant association for dichotomous variables. In order to use this test, the sample size should be large and expected frequencies in each cell must be greater than five (Field 2009). For this study both these requirements were met. The sample was sufficiently large (17,481 participants) and the outputs from the analysis showed that none of the cells had less than the expected count of less than five. Strength of relationship or effect size was tested using Cramer's V test (Field, 2009).

**Assessment for Outliers**

Outliers were assessed using Mahalanobis regression analysis. This analysis creates a center point from which the intersection of the means of all the variables and then determines the distance a case is from this center (Tabachnick & Fidell, 2012, pp. 60-116). Outliers were identified by first creating Z score for each covariate's lowest and highest scores. Then Mahalanobis distance was computed using SPSS regression analysis. The impact of the outliers using a multivariate model was assessed and summary statistics for the original as well as for each of the imputed datasets was produced. The outputs showed deviation from normality and were positive for skew and kurtosis (see Table 16). Results for the overall summary Mahalanobis distance analysis showed that only 694 (4%) were identified as outlier cases out of 17481cases after imputation (see Table 17). The issue with multivariate outliers is that cases are discrepant

as a result of combination of outlier scores of one or more variables and not any one score. It is anticipated that the number of possible multivariate outliers was substantially reduced after transformation. There is no clear guidance for allowable missing values, 5% is often an acceptable level (Tabachnick & Fidell, 2012, pp. 60-116). In summary, there were only few outlier cases in this large sample and the data was imputed. Since normality of data is not integral for survival analysis, the inclusion of these outlier cases is not expected to be influential in the multivariate analysis (Tabachnick & Fidell, 2012, pp. 60-116).

Table 16

*Descriptive Mahalanobis Distance*

| | | N | Mean | SD | Skew | SE | Kurtosis | SE |
|---|---|---|---|---|---|---|---|---|
| Original | Mahalanobis Distance | 9381 | 28.99 | 13.91 | 1.50 | .025 | 3.47 | .051 |
| data | Valid N (listwise) | 9381 | | | | | | |
| 1 | Mahalanobis Distance | 16786 | 28.99 | 14.12 | 1.66 | .019 | 4.53 | .038 |
| | Valid N (listwise) | 16786 | | | | | | |
| 2 | Mahalanobis Distance | 16787 | 28.99 | 14.14 | 1.65 | .019 | 4.39 | .038 |
| | Valid N (listwise) | 16787 | | | | | | |
| 3 | Mahalanobis Distance | 16790 | 28.99 | 14.14 | 1.67 | .019 | 4.55 | .038 |
| | Valid N (listwise) | 16790 | | | | | | |
| 4 | Mahalanobis Distance | 16786 | 28.99 | 14.10 | 1.65 | .019 | 4.38 | .038 |
| | Valid N (listwise) | 16786 | | | | | | |
| 5 | Mahalanobis Distance | 16787 | 28.99 | 14.13 | 1.66 | .019 | 4.43 | .038 |
| | Valid N (listwise) | 16787 | | | | | | |

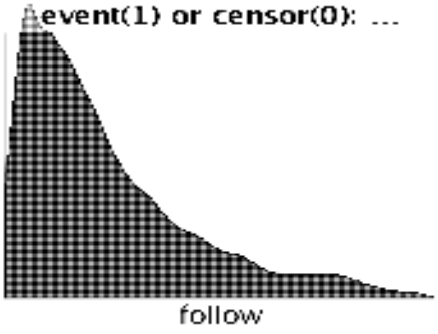
SD= Standard Deviation; SE = Standard Error

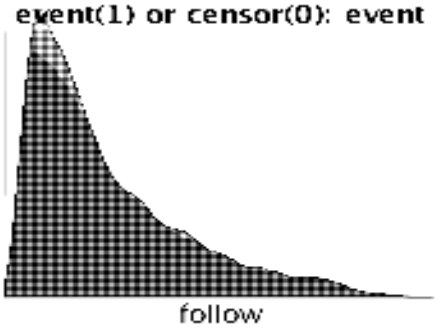
Table 17

*Mahalanobis Distance Summary*

| Imputation Number | | Cases Included | | Excluded | | Total | |
|---|---|---|---|---|---|---|---|
| | | N | Percent | N | Percent | N | Percent |
| Original data | Mahalanobis Distance | 9381 | 53.7% | 8100 | 46.3% | 17481 | 100% |
| 1 | Mahalanobis Distance | 16786 | 96.0% | 695 | 4.0% | 17481 | 100% |
| 2 | Mahalanobis Distance | 16787 | 96.0% | 694 | 4.0% | 17481 | 100% |
| 3 | Mahalanobis Distance | 16790 | 96.0% | 691 | 4.0% | 17481 | 100% |
| 4 | Mahalanobis Distance | 16786 | 96.0% | 695 | 4.0% | 17481 | 100% |
| 5 | Mahalanobis Distance | 16787 | 96.0% | 694 | 4.0% | 17481 | 100% |

For survival analysis, it was assumed that the censored cases and the ones lost during the course of study would not be systematically different from those with an event at the end of the study. Differences would inevitably result in nonrandom loss of cases, violating this assumption would bias the results of the study (Tabachnick & Fidell, 2012, pp. 60-116). This was a retrospective study that used a sample of convenience, selected only patients who met the inclusion criteria. These patients were then followed over time to determine the rate and time to development of NAFLD. Unlike prospective studies where information and reasons for withdrawal would be collected, this information is unknown particularly for the lost cases over time in the registry. Thus in the absence of information, it can only be assumed that the reasons for lost cases would be similar for those censored and those with an event. I conducted an analysis and visually inspected the pattern over time for the event cases and the censored cases. The patterns appear to be similar over time, as shown in Figure 21 for the event cases and Figure 22 for censored cases.




Overall dataset= light blue and cases = grid pattern; X axis = study period in years; Y axis = proportions

*Figure 21*- Event Cases

*Figure 22* - Censored Cases

**Changes in Survival Experience Over Time**

This assumption is important for survival analysis in that it assumes that factors that would affect survival in the beginning would be the same factors affecting survival at the end of the study (Tabachnick & Fidell, 2012, pp. 510-570). Since this was not a prospective study, I was not able to test this assumption. This study used secondary data collected over a period of 14 years and pertinent information to evaluate this assumption is not routinely collected as part of patient care. Thus it can only be assumed that environmental factors and other conditions for survival would be similar over time and these factors would be the same for both the event and censored group. For example, it is assumed that factors such as participants remaining in the registry, treatment of RA, or having laboratory levels measured needed to calculate the FIB-4 score were similar over time.

**Multicollinearity**

Survival analysis is inherently protected against issues with multicollinearity (Tabachnick & Fidell, 2012, pp. 510- 570). However, investigating its impact is

recommended using the FACTOR analysis function; results with greater than 0.90 maybe indicative of multicollinearity. The results of this analysis are presented in Table 18. All the variables had values were less than 0.90, ranging from .22 to the highest value of .76 for metabolic syndrome (See Table 18), indicating no significant multicollinearity.

Table 18

*Factor Analysis Communalities*[a]

| | Initial | Extraction |
|---|---|---|
| Age categories | 1.000 | .528 |
| Gender | 1.000 | .424 |
| Race group | 1.000 | .404 |
| Ethnicity/Hispanic | 1.000 | .420 |
| Marital Status | 1.000 | .410 |
| Education | 1.000 | .441 |
| Insurance Status | 1.000 | .482 |
| Smoking Status | 1.000 | .449 |
| Alcohol Use (yes) | 1.000 | .450 |
| Exercise (yes) | 1.000 | .354 |
| Hypertension | 1.000 | .493 |
| CVD | 1.000 | .277 |
| Dyslipidemia | 1.000 | .494 |
| Cancer History | 1.000 | .467 |
| Diabetes | 1.000 | .325 |
| Liver Disorders History | 1.000 | .549 |
| Liver Enzyme Elevation | 1.000 | .424 |
| BMI categories | 1.000 | .455 |
| Metabolic Syndrome | 1.000 | .768 |
| Disease Duration Categories | 1.000 | .419 |
| CDAI Categories | 1.000 | .354 |
| MTX use | 1.000 | .384 |

Extraction Method: Principal Component Analysis.[a]; a. Imputation Number = 5

**Proportionality of Hazards**

Proportionality of hazards is a major assumption for the Cox proportional hazards model. This assumption assumes that the effect of the given set of covariates is the same over time or that the shape of the survival function is the same for all cases or groups over time (Tabachnick & Fidell, 2012, pp. 510-570). I visually assessed this assumption by generating Kaplan Meier plots for each of the variables similar to the plot shown for age and gender (see Figure 23 and 24). Similarly, survival plots were assessed using the regression analysis where the mean of the covariates was used, the results were significant; $\chi 2$ (21) = 2719.82, $p < .001$. This meant that the relationship between the covariates survival lines was proportional over time and were significantly different, they did not cross each other for the original as well as the imputed data sets (see Table 19, Figure 23 and Figure 24).

Table 19

*Omnibus Tests of Model Coefficients[a] - Mean of Covariates*

| | -2 Log | Overall (score) | | |
|---|---|---|---|---|
| Imputation Number | Likelihood | Chi-square | Df | Sig. |
| Original data | 54714.825 | 1830.40 | 21 | .000 |
| 1 | 90843.581 | 2719.22 | 21 | .000 |
| 2 | 90876.081 | 2717.95 | 21 | .000 |
| 3 | 90841.775 | 2720.68 | 21 | .000 |
| 4 | 90881.167 | 2722.44 | 21 | .000 |
| 5 | 90857.972 | 2719.82 | 21 | .000 |

a. Beginning Block Number 1. Method = Enter; Df= degrees of freedom

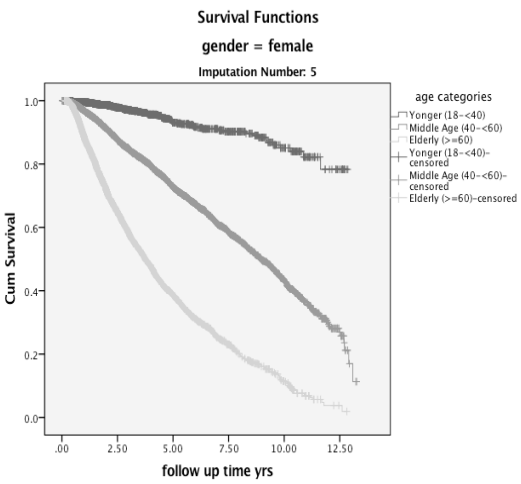



*Figure 23*. Survival Function: Gender and Age

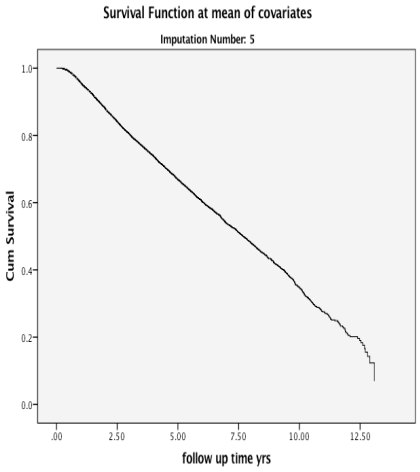



*Figure 24*. Survival Function: Mean of Covariates

I also tested for proportionality of hazards using SPSS COXREG -TIME PROGRAM function. In this analysis, all the covariates were entered into this regression model simultaneously to analyze their effects on survival over time. A non significant result is required to meet the proportionality hazard assumption, meaning that that none of the covariates significantly interacted over time (Tabachnick & Fidell, 2012, pp. 510-570). The results of this analysis was non significant; $p = .709$ (see Table 20).

Table 20

*Test for Proportionality of Hazards- Time with All Covariates*

| | Wald | df | Sig. | Exp (B) | 95.0% CI for Exp (B) | |
|---|---|---|---|---|---|---|
| | | | | | Lower | Upper |
| T_COV_ | .139 | 1 | .709 | 1.000 | 1.000 | 1.000 |

b. Df= degrees of freedom

In summary, using Tabachnick & Fidell's, guidelines for conducting survival analysis each of the issues and assumptions associated with survival analysis was evaluated and addressed. The study was more than adequately powered with a sample size of 17,481 patients with RA with about 14 years of observation time. Missing data

were addressed using the multiple imputation method, an optimal approach that preserved sampling variability of the original data. The large sample size helped minimize the impact of the outliers. Outliers were transformed into categorical variables to further reduce the impact. Impact of outliers was also evaluated by conducting the Mahalanobis distance analysis and it showed that over 96% of cases was included in the analysis and thus not significantly affected by outliers. Multicollinearity was evaluated and all variables were below the accepted threshold of .90. Evaluations of differences between withdrawn and remaining cases, and changes in survival experience over time are important issues for prospective studies designed to evaluate differences between treatments. However, treatment effectiveness was not an objective for this study, and limited information about the survival experience was collected in the registry. Hence it is not known if this assumption was violated and thus the implications are not self-evident. Proportionality hazard assumption was evaluated using Kaplan Meier plots and regression analysis. These analyses showed that none of the covariates or groups significantly interacted with each other over time. Overall, after transforming variables and having adequately met all of the assumptions for survival analysis, this dataset was sufficiently prepared and powered to conduct the ensuing survival analysis.

## Research Question 1

### Results of Incidence Rates and Time to Development of NAFLD

Research question 1 (RQ1) was to determine the incidence rate of NAFLD among patients with RA from 2001 -2014 in the U.S. using the FIB-4 test. Incidence rate was determined using the incident cohort dataset with 17,481 RA patients. Chi-square test

was used to compare frequencies among the categorical variables. The sample was sufficiently large and none of the cells had less than the expected count less than five (Field, 2009). Kaplan-Meier survival analysis was conducted to determine rate and time to development of NAFLD. Breslow (Generalized Wilcoxon) test was used to determine if there were differences in the survival distribution for the categorical variables (Field, 2009). The results of the analysis included the overall cumulative incidence rate during the study period for the cohort, absolute number of incident cases, percent of incident cases, and incidence rate per 1,000 person years (PY) along with 95% confidence intervals.

For this incident cohort of 17,481 patients with RA, data were available for a period of 13.2 years. As shown in Table 21, the overall NAFLD cumulative incidence over this period was 31%, 95%, CI [29.82, 31.18%], with an incidence rate of 95 cases per 1,000 patient years, 95% CI [92.60, 97.45]). The mean overall time to the development of NAFLD was 7.2 years, 95%CI [7.07, 7.29]. Similar results were seen for patients developing mild/moderate NAFLD. Only 249 patients (1.4%), developed advanced NAFLD; however, the mean time to event was longer (12.8 years).

Table 21

*NAFLD Rates: Overall Incidence Rates and Time to Event*

| Variable | N (%) | Incidence Cases n (%) | Incidence Case /1000PY [95% CI] | Time to Event (years) [95% CI] |
|---|---|---|---|---|
| Overall NAFLD | 17481 | 5328 (30.5%) | 95.03 [92.60, 97.45] | 7.18 [7.09,7.29] |
| Mild/Moderate NAFLD | 17481 | 5079 (29.1%) | 90.59 [88.21, 92.96] | 7.35 [7,24,7.47] |
| Advanced NAFLD | 17481 | 249 (1.4%) | 4.44 [3.89, 4.99] | 12.84 [12.78,12.90] |

*p*-value across categories; PY= Patient Years; CI- Confidence Interval; CV = Cramer's V

Table 22 shows the absolute number and percent of overall incident cases, incidence rate (per 1,000 patient years with 95% CI), and time to event for each of the demographic factors. In the overall cohort, baseline age was categorized into three groups; 12% were in the younger group, half of them were middle aged and a third were elderly. In the group of patients that developed NAFLD, most were elderly (56.0%), followed by middle aged (42.1%) and a few were younger (1.7%). Similar results were seen for NAFLD incidence rates, with higher rates for the elderly and middle age group as shown in Table 22. The elderly group developed NAFLD in the shortest mean time of 4.5 years, followed by the middle-aged group with 8.1 years whereas time to incidence of NAFLD was the longest in the younger group (11.6 years). The difference in the mean time to event among the three age categories was statistically significant, $\chi^2$ (2) = 1994.83, $p < .001$. This overall cohort consisted mostly of women (79%). The proportion of patients developing NAFLD and also the incidence rate were three times higher among women then men as shown in Table 22. However, men developed NAFLD in a shorter mean time of 6.4 years compared to 7.4 years for women. There was a statistically significant difference in the time to development of NAFLD by gender, $\chi^2$ (1) = 57.69, $p < .001$.

In the overall cohort most of the patients were Caucasians (89%), with 6.4% African Americans, 1.6% Asians and 3.5% in the "other" race category. The proportion of patients developing NAFLD and also the incidence rate were significantly higher for Caucasians (89.9% and 85.41 cases per 1,000 patient years, 95% CI [83.10, 87.73] respectively) compared to other race groups as shown in Table 22. There was a

statistically significant difference in the mean time to development of NAFLD by race, $\chi^2$ (2) = 12.55, $p < .006$. . Although the absolute mean time to event across the racial/ethnic groups was relatively similar (7 years), the African-American group had a slightly earlier onset of disease in 6.7 years. In the overall cohort most reported not being Hispanic (91%). The proportion of patients developing NAFLD and a higher incidence rate were mostly seen among non Hispanics (93.0% and 88.41 cases per 1,000 patient years, 95% CI [86.06, 90.76] respectively) compared to Hispanics as shown in Table 22. The absolute mean time to event was similar at 7.1 years, 95% CI, [7.07, 7.27] for non Hispanics versus 7.4 years, 95% CI [7.07, 7.88] for Hispanics; however, the difference was statistically significant; $\chi^2$ (1) = 6.76, $p < .009$.

Table 22

*NAFLD Rates: Incidence Rates by Demographic Factors*

| Variable | N (%) | Incidence Cases n=5328 | Incidence Rate Cases/1000 PY [95% CI] | Time to Event (years) [95% CI] | Time $\chi2$ *p*-value* |
|---|---|---|---|---|---|
| Age | | | | | 1994.8 < .001 |
| Younger | 2133 (12.2%) | 92 (1.7 %) | 1.64 [1.31,1.98] | 11.62 [11.36, 11.88] | |
| Middle Aged | 9360 (53.5%) | 2251 (42.1%) | 40.15 [38.52, 41.77] | 8.15 [8.09, 8.31] | |
| Elderly | 988 (34.3%) | 2985 (56.0%) | 53.24 [51.38, 55.10] | 4.56 [4.44, 4.61] | |
| Gender | | | | | 57.69 < .001 |
| Male | 3707 (21.2%) | 1351 (25.4%) | 24.10 [22.83, 25.37] | 6.42 [6.21, 6.64] | |
| Female | 13774 (78.8%) | 3977 (74.6%) | 70.93 [68.81, 73.06] | 7.39 [7.27, 7.52] | |
| Race | | | | | 12.55 < .006 |
| Caucasians | 1546 (88.5%) | 4789 (89.9%) | 85.41 [83.10, 87.73] | 7.18 [7.09, 7.29] | |
| African American | 1116 (6.4%) | 316 (5.9%) | 5.64 [5.02, 6.26] | 6.76 [6.20, 7.25] | |
| Asian | 286 (1.6%) | 68 (1.3 %) | 1.21 [0.92, 1.50] | 7.53 [6.78, 8.29] | |
| Other | 614 (3.5%) | 155 (2.9%) | 2.76 [2.33, 3.20] | 7.71 [7.20, 8.30] | |
| Ethnicity | | | | | 6.76 < .009 |
| Hispanic | 1442 (8.2%) | 371 (7.0%) | 6.62 [5.95, 7.29] | 7.45 [7.07, 7.88] | |
| Not Hispanic | 16039 (91.2%) | 4957 (93.0%) | 88.41 [86.06, 90.76] | 7.16 [7.04, 7.27] | |

* *p*-value across categories; PY= Patient Years; CI- Confidence Interval; CV = Cramer's V

**Incidence Rates and Time to Event by NAFLD Risk Factors**

The pre-identified or known risk factors for NAFLD were baseline obesity, metabolic syndrome, diabetes and dyslipidemia. Included in the analysis were also other potential predictors including liver enzyme elevation, MTX use, liver disorders and alcohol use. Overall rate and time to event for each of these factors are presented in Table 23.

In this cohort 72% of the patients were obese (BMI ≥ 25). The proportion of patients developing NAFLD and also the incidence rate were higher among patients with obesity (72% and 68.38 cases per 1,000 patient years, 95% CI [66.29, 70.47) respectively compared to the non obese group as shown in Table 23. The average time to development of NAFLD was similar for both BMI groups, 7 years; ($\chi^2$ (1) = .061, $p$ = <. 805). In this overall cohort, 18% had metabolic syndrome. Nearly, 20% of patient with metabolic syndrome developed NAFLD, however, the rates for NAFLD were much higher for those with metabolic syndrome, 115.3 per 1000 PY, CI 95% [112.72, 118.00] compared to 45.4 per 1000 PY, CI 95% [43.76, 47.21] among those without metabolic syndrome (see Table 23). There was a statistically significant difference in the time to development of NAFLD for those with metabolic syndrome. This group had an earlier onset of disease with an average of 5.1 years, 95% CI [4.95, 5.43] compared to an average of 7.4 years, 95% CI [7.36, 7.59] for those without (30%); $\chi^2$ (1) =305.309, $p$ < .001.

In this cohort, 6.9% of patients had diabetes. The proportion of patients developing NAFLD and also the incidence rate were significantly higher among the non diabetics (91.7% and 87.14 cases per 1,000 patient years, 95% CI [84.81, 89.48]

respectively) compared those with diabetes as shown in Table 23. However, the mean time to development of NAFLD for those with diabetes was significantly shorter compared to those without diabetes, in 6.1 years, 95% CI [5.77, 6.46] compared to 7.2 years, 95% CI [7.14, 7.37]; $\chi^2$ (1) = 27.78, $p < .001$. Likewise for those with dyslipidemia the mean time to development of NAFLD was significantly sooner, 5.0 years, 95% [CI 4.86, 5.22] compared to 7.5 years, 95% CI [7.47, 7.71] for those without; $\chi 2$ (1) = 351.56, $p < .001$. In this study population only 22% had dyslipidemia. The proportion of patients developing NAFLD and also the incidence rate were about threefold higher among those without a history of dyslipidemia compared to those with dyslipidemia as shown in Table 23.

Only 8% of the cohort had liver enzyme elevation. The proportion of patients developing NAFLD and also the incidence rate were significantly higher for those with normal liver enzyme levels (92.1% and 87.14 cases per 1,000 patient years, 95% CI [85.21, 89.89] respectively) compared to those with liver enzyme elevations as shown in Table 23. There was no difference between these two groups in the mean time to event at 7.1 years ($\chi 2$ (1) $p$= .141). Similar results were seen for those with a history of liver disease at baseline, the difference in mean time to event was not statistically significant with 7.2 years, 95% CI [7.10, 7.32] for those without a history compared to 6.0 years (95% CI, 5.59, 6.49); $\chi 2$ (1) = .972, $p$ = .324. Only 2% reported a history of liver disorders in this cohort. In the group that developed NAFLD most patients did not have a history of liver disorder (97.2%) compared to 2.8% with a history of liver disorder. The overall incidence rate for NAFLD was low for both groups, but slightly higher for those

without a history of liver disease 4.2, per 1000 PY, CI 95% [3.74, 4.82] and 2.6 per 1000 PY, CI 95% [2.26, 3.12] for those with a history of liver disease.

About 40% of the overall cohort reported they were currently or had a history of using alcohol. The proportion of patients developing NAFLD and also the incidence rate were higher for nonusers of alcohol (62.5% and 89.80 cases per 1,000 patient years, 95% CI [87.44, 92.17] respectively) compared to alcohol users as shown in Table 23. There was also a significant difference in the time to development of NAFLD. Those not using alcohol had an earlier onset of NAFLD with a mean time of 6.9 years, (95% CI, 6.79, 7.06) compared to 7.5 years, (95% CI 7.33, 7.69) respectively; $\chi 2\,(1) = 59.989$, $p < .001$.

Table 23

*Incidence Rates and Time to Event by NAFLD Risk Factors*

| Variable | | N (%) | Incidence Case n=5328 | Incidence Rates Cases/ 1000 PY [95% CI] | Time to Event (Years) [95% CI] | $\chi 2$* *p* Value |
|---|---|---|---|---|---|---|
| Obesity | Yes | 12639 (72.3%) | 3834 (72.0%) | 68.38 [66.29, 70.47) | 7.17 [7.04, 7.30] | .061 .805 |
| | No | 4842 (27.7%) | 1494 (28.0%) | 26.65 [25.31, 27.98] | 7.22 [7.02, 7.42] | |
| Metabolic Syndrome | Yes | 2229 (18.3%) | 1078 (20.2%) | 115.36 [112.72, 118.00] | 5.19 [4.95, 5.43] | 305.309 < .001 |
| | No | 14174 (81.7%) | 4250 (79.8%) | 45.48 [43.76, 47.21] | 7.28 [7.36, 7.59] | |
| Diabetes | Yes | 1208 (6.9%) | 442 (8.3%) | 7.88 [7.15, 8.62] | 6.11 [5.77, 6.46] | 27.787 < .001 |
| | No | 16273 (93.1) | 4886 (91.7%) | 87.14 [84.81, 89.48] | 7.25 [7.14, 7.37] | |
| Dyslipidemia | Yes | 3824 (21.9%) | 1378 (25.9%) | 24.58 [23.30, 25.86] | 5.04 [4.87, 5.22] | 351.565 < .001 |
| | No | 13657 (78.1%) | 3950 (74.1%) | 70.45 [68.33, 72.57] | 7.59 [7.47, 7.71] | |
| Liver Enzyme Elevation | Yes | 1440 (8.2%) | 419 (7.9%) | 7.47 [6.76, 8.19] | 7.11 [6.78, 7.48] | 2.169 .141 |
| | No | 16041 (91.8%) | 4909 (92.1%) | 87.14 [85.21, 89.89] | 7.19 [7.08, 7.30] | |
| Liver Disorders | Yes | 391 (2.2%) | 151 (2.8%) | 2.69 [2.26, 3.12] | 6.04 [5.59,6.49] | .972 .324 |
| | No | 17090 (97.8%) | 1517 (97.2%) | 4.28[3.74, 4.82] | 7.21 [7.10, 7.32] | |
| Alcohol use | Yes | 7027 (40.2%) | 1997 (37.5%) | 35.62 [34.08, 37.15] | 7.51 [7.33, 7.69] | 59.989 < .001 |
| | No | 10453 (59.8%) | 3331 (62.5%) | 89.80 [87.44, 92.17] | 6.92 [5.59, 6.49] | |

*p*-value across categories; PY= Patient Years; CI- Confidence Interval; CV = Cramer's V

**Incidence Rates and Time to NAFLD by RA Related Factors**

Included in the incident NAFLD rate analysis were baseline factors related to RA; disease duration, disease activity and use of medication including MTX, conventional synthetic disease modifying antirheumatic drugs (cDMARD), steroids and biologics. Rate and time to event for each of these factors are presented in Table 24.

Most patients had early disease (33%) or greater than 10 years of disease (29.2%). The proportion of patients developing NAFLD and also the incidence rate were the highest among patients with disease duration greater than10 years followed by those with early RA, however, the proportion and rates for the group with 5 to 10 years and 2 to 5 years of disease duration were similar as shown in Table 24. Those with the longest duration of disease (>10 years) developed NAFLD sooner with a mean time of 6.8 years, 95% CI [6.71, 7.07], whereas for the other duration of disease categories the mean time to event ranged from 7.1 years to 7.4 years (see Table 24). These differences in the mean time to development of NAFLD were also statistically significant; $\chi 2\,(3) = 24.16$, $p < .001$ among the groups.

In the cohort at baseline about 18% were in remission, 31% had low disease activity, 28% had moderate disease activity and 22% had high disease activity. As shown in Table 24, the proportion of patients developing NAFLD and also the incidence rate was highest among patients with low disease activity, followed by those with moderate disease activity and then among those with high disease activity. The proportion of patient with NAFLD and incidence rates was the lowest among patients in remission. The mean time to development of NAFLD was statistically significantly different ($\chi 2\,(3) =$

20.28, p < .001, although the absolute mean time to event does not vary greatly across the various disease severity categories (7.0 years to 7.3 years). Time to development of NAFLD was longer for those in remission at 7.3 years, (95% CI, 7.09, 7.61).

Most of the patients in this cohort were currently taking MTX (70.2 %). The proportion of patients developing NAFLD and also the incidence rate was higher among MTX users (72.7% and 69.1 cases per 1000 PY, CI 95% [67.03, 71.23] respectively) compared to nonusers as shown in Table 24. The absolute mean time to event was similar but was statistically different; in 7.0 years, 95% CI [6.96, 7.21] for MTX users versus 7.4 years, 95% CI [7.24, 7.64] for the nonusers; $\chi 2\,(1) = 13.62$, $p < .001$.

About 30% of the overall cohort was on cDMARDs. The proportion of patients developing NAFLD and also the incidence rate was about higher among cDMARDs nonusers (69.2% and 65.78 cases per 1000 PY, CI 95% [63.73, 67.83] respectively) compared to nonusers as shown in Table 24.There was no statistically significant difference in the mean time to development of NAFLD (7.35 years for the users of cDMARDs vs. 7.09 years for nonusers); $\chi 2\,(1) = 27.49$, $p = .596$. About 30% of patients were currently taking steroids. The proportion of patients developing NAFLD and also the incidence rate was higher among steroid nonusers (68.2% and 62.74 cases per 1000 PY, CI 95% [58.66, 66.82] respectively), compared to user as shown in Table 24. Mean time to development of NAFLD for those using steroids was also significant (7.0 years for steroid users vs. 7.1 years for the nonusers); $\chi^2\,(1) = 20.153$, $p < .001$.

The proportion of patients developing NAFLD and also the incidence rate was higher among non biologic users (58.5% and 55.56 cases per 1,000 patient years, 95% CI

[53.66, 57.45 respectively] compared to biologic users as shown in Table 24. There was a statistically significant difference in the mean time to event, biologic nonusers developed NAFLD in 7.05 years, 95% CI, [6.90, 7.19] years versus 7.35 years, 95% CI, [7.18, 7.52] for biologic users; $\chi 2\,(1) = 58.88$, $p < .001$.

Table 24

*NAFLD Incidence Rates by RA Factors*

| Variable | N (%) | Incidence Case n=5328 | Incidence Rate Cases/1000 PY [95% CI] | Time to Event (Years) [95% CI] | $\chi 2$ *p*-value |
|---|---|---|---|---|---|
| RA Disease | | | | | |
| Early RA (< 2yrs) | 5735 (32.8%) | 1556 (29.2%) | 27.75 [26.39, 29.11] | 7.12 [6.91,7.33] | 24.16 (3) < .001 |
| 2 to 5yrs | 3276 (18.7%) | 938 (17.6%) | 16.73 [15.67,17.79] | 7.48 [7.22, 7.73] | |
| 5 to 10yrs | 3340 (19.1%) | 978 (18.4%) | 17.44 [16.36, 18.53] | 7.44 [7.20, 2.69] | |
| > 10yrs | 4990 (28.9%) | 1852 (36.2%) | 33.03 [31.55, 34.51] | 6.89 [6.71, 7.07] | |
| RA Disease Activity | | | | | |
| (CDAI) Remission | 3223 [18.4%] | 865 (16.2%) | 15.43 [14.41, 16.45] | 7.35 [7.09, 7.61] | 20.283 (3) < .001 |
| Low Disease | 5480 [31.4%] | 1678 (31.5) | 29.93 [28.52, 31.34] | 7.12 [7.03, 7.40] | |
| Moderate | 4935 [28.2%] | 1569(29.5%) | 27.98 [26.62, 29.35] | 7.11 [6.09, 7.31] | |
| High | 3835 [21.9%] | 1213 (22.8%) | 21.63 [20.43, 22.84] | 7.01 [6.78, 7.24] | |
| MTX Use | | | | | 13.62 (1) < .000 |
| Yes | 12274 (70.2%) | 3876 (72.7%) | 69.13 [67.03, 71.23] | 7.08 [6.96, 7.21] | |
| No | 5207 (29.8%) | 1452 (27.3%) | 25.90 [24.58, 27.21] | 7.44 [7.24, 7.64] | |
| cDMARD | | | | | .281 (1) .596 |
| Yes | 5183 [29.6%] | 1640 [30.5%] | 29.25 [27.86, 30.64] | 7.35 [7.16, 7.45] | |
| No | 12298 (70.4%) | 3688 (69.2%) | 65.78 [63.73, 67.83] | 7.09 [6.95, 7.22] | |
| Steroids | | | | | 20.153 < .001 |
| Yes | 5199 (29.7%) | 1696 (31.8%) | 30.25 (28.83, 31.67) | 7.01 [6.82, 7.20] | |
| No | 12282 (70.3%) | 3632 (68.2%) | 62.74 [58.66, 66.82] | 7.24 [7.12, 7.38] | |
| RA Medication | | | | | 27.492 < .001 |
| Biologics (Yes) | 7688 (44.0%) | 2213 (41.5%) | 39.47 [37.86, 41.08] | 7.35 [7.18, 7.52] | |
| Biologics (No) | 9793 (56.0) | 3115 (58.5%) | 55.56 [53.66, 57.45] | 7.05 [6.90, 7.19] | |

* *p*-value across categories; PY= Patient Years; CI- Confidence Interval; CV = Cramer's V; cDMARD= Conventional Disease-modifying antirheumatic drugs

**Incidence Rates and Time to NAFLD by Other Factors**

Also of interest were other relevant comorbidities often associated with the development of NAFLD. These factors included assessing NAFLD rates and time to event for CVD, hypertension, smoking, and exercise and are presented in Table 25. Only 6.5 % of this population had a history of CVD. The proportion of patients developing NAFLD and also the incidence rate was significantly higher among those without CVD (90.3% and 85.79 cases per 1,000 patient years, 95% CI [83.47, 88.11] respectively) compared to those with CVD as shown in Table 25. There was a statistically significant difference in the mean time to NAFLD, those with a history of CVD were likely to develop NAFLD significantly sooner, in 4.6 years, 95% CI [4.29, 4.90] compared to 7.3 years, 95% CI, [7.24, 7.47] for those without a history; $\chi 2\,(1) = 183.64$, $p < .001$.

About 37% of the overall cohort reported a history of hypertension. The proportion of patients developing NAFLD and also the incidence rate was marginally higher for those without hypertension (58.5%, 55.63 cases per 1,000 patient years, 95% CI [53.73, 57.53] respectively) compared to those with hypertension as shown in Table 25. However, there was a statistically significant difference in the mean time to event for those with a history of hypertension developed NAFLD in 5.9 years, (95% CI, 5.82, 6.16) compared to those without hypertension with 7.7 years, (95% CI, 7.61, 7.88); $\chi 2\,(1) = 313.26$, $p < .001$.

About 41% of this cohort reported to be a current smoker or had a history of smoking. The proportion of patients developing NAFLD and also the incidence rate was marginally higher for non smokers (59.1% and 55.95 cases per 1,000 patient years, 95%

CI [54.05, 57.85] respectively) compared to smokers as shown in Table 25. There was a statistically significant difference in the mean time to event; smokers had an earlier onset of NAFLD with 6.9 years, (95% CI, 6.73, 7.103) versus to 7.37 years, (95% CI, 7.23, 7.51) for nonsmokers; $\chi 2\ (1) = 22.73$, $p < .001$.

About 70 % of this cohort reported that they exercised once or more during the week. The proportion of patients developing NAFLD and also the incidence rate was higher for patients that reported that they exercised (79.5% and 66.97 cases per 1,000 patient years 95% CI [64.90, 69.04] respectively) compared to the group that did not exercise as shown in Table 25. The mean time to development of NAFLD was not significant with 7.3 years (95% CI, 7.11, 7.49) for the group that did not exercise compared to. 7.1 years for those exercising; $\chi 2\ (1) = 1.07$, $p <= .299$.

Table 25

*Overall NAFLD Incidence by Comorbidities and Other Factors*

| Variable | | N (%) | Incidence Cases n=5328 | Incidence Rate Case/1000 PY [95% CI] | Time to Event (years) [95% CI] | $\chi 2$ *p*-value |
|---|---|---|---|---|---|---|
| CVD | Yes | 1129 (6.5%) | 518 (9.7%) | 9.24 [8.45, 10.03] | 4.60[4.29, 4.90] | 183.64 < .001 |
| | No | 16352(93.5%) | 4810 (90.3%) | 85.79 [83.47, 88.11] | 7. 36 [7.24, 7.7.47] | |
| Hypertension | Yes | 6518 (37.3) | 2209 (41.5%) | 39.40 [37.79, 41.01] | 5.99 [5.82, 6.16] | 313.26 < .001 |
| | No | 10963 (62.7%) | 3119 (58.5%) | 55.63 [53.73, 57.53] | 7.75 [7.61, 7.88] | |
| Smoking Status | Yes | 7208 (41.4%) | 2171 (40.9%) | 38.72 [37.12, 40.32] | 6.91 [6.73, 7,10] | 22.73 < .001 |
| | No | 10193 (58.6%) | 3137 (59.1%) | 55.95 [54.05, 57.85] | 7.37 [7.23, 7.51] | |
| Exercise | Yes | 12171 (69.9%) | 3755 (70.5%) | 66.97 [64.90, 69.04] | 7.13 [7.00, 7,26] | 1.07 .299 |
| | No | 12171 (69.6%) | 1573 (29.5%) | 28.05 [26.69, 29.42] | 7.30 [7.10, 7.9] | |

* *p*-value across categories; PY= Patient Years; CI- Confidence Interval; CV = Cramer's V

## Research Question 2

Research question 3 was to determine the prevalence of NAFLD among patients with RA during the course of one year (e.g. 2012) in the U.S. using the FIB-4 test. As previously mentioned, answering this research question was contingent on not having a sufficiently large sample size to conduct the incident cohort analysis. Since the incident dataset was of sufficient size, data to conduct the prevalence analysis were not included in the dataset provided by the registry for the analysis.

## Research Question 3

RQ3 was the subgroup analysis to determine the differences in baseline characteristics (clinical and demographic) for the incident cohort in those with NAFLD compared to those without. I compared baseline characteristics between the groups, those with NAFLD to those without using the t-test for continuous variables. Chi-square test was used for categorical variables, and effect size was estimated using Cramer's V value. The overall effect size for these variables was small to moderate. Cramer's V results ranged from 6% to 33%.

### Comparison of Baseline Demographic Factors

In the overall study cohort about 31% (n= 17,481) developed NAFLD during the study period (see Table 26). The mean age for the overall cohort with NAFLD was 54 years (SD = 12.45). The NAFLD group was older by 9.8 years; this difference was statistically significant [$t\ (11934) = -44.48$, $p < .001$]. The proportion of patients in the NAFLD group compared to those without NAFLD across the age categories was lower except for the elderly (see Table 26.). The difference between the two subgroups was

statistically significant; $\chi 2$ (2) = 11593.16, $p$ <. 001; the strength of association was moderate (Cramer's V value = .32). In the overall cohort there were more women (79%). As shown in Table 26, there were more men in the NAFLD group compared to the group without NAFLD. The difference between the two subgroups was statistically significant; $\chi^2$ (1) = 474.12, p = <. 001, however, the strength of association was weak with Cramer's $V$ = .06.

The majority of this cohort were Caucasians (89%), with 6% of African Americans, 4% falling into the other race category and less than 2% Asians. The proportion of patients in the NAFLD group compared to those without NAFLD across the race categories was lower except for Caucasians as shown in Table 26. The difference between the two subgroups was statistically significant; $\chi^2$ (3) = 109,23, p = <. 001, however, the strength of association was weak (Cramer's $V$ = .03). Similar results were seen when comparing the two subgroups by ethnicity. The proportion of patients in the NAFLD group compared to those without NAFLD was higher among the non Hispanics than the Hispanic group as shown in Table 26.There was statistically significant difference between the two subgroups; $\chi^2$ (3) = 88.53, p = <. 001, the strength of association was weak (Cramer's $V$ value = .03).

Most of the overall cohort were married (67%), with 20% single or widowed and 13% were divorced or separated. The proportion of patients in the NAFLD group compared to those without NAFLD across the various marital status categories was lower except for the married/partnered group as shown in Table 26. There was statistically significant difference between the two subgroups; $\chi^2$ (2) = 59.82, p = <. 001, the strength

of association was weak (Cramer's *V* value = .02). In the overall cohort about 56% were college/university educated, 37% with high school education and about 4% had primary education. The proportion of patients in the NAFLD group compared to those without NAFLD across the various educational categories was lower except for the group with high school education as shown in Table 26. There was a statistically significant difference between the two groups; $\chi^2$ (3) = 604.76, p = <. 001; the strength of association was weak (Cramer's *V* value = .07). In the overall cohort most had insurance (80%). The proportion of patients in the NAFLD group compared to those without NAFLD was higher among those without insurance as shown in Table 26. There was a statistically significant difference between the two groups; χ2 (3) = 732.59, p = <. 001; however, the strength of association was weak (Cramer's V value = .08).

Table 26

*Comparison of Demographic Factors: Patients with vs. without NAFLD Incidence*

| Baseline Variable | | All Patients N 17,481) | Without NAFLD N=12153 | With NAFLD N=5328 | χ2 [CV] *p*-value* |
|---|---|---|---|---|---|
| Age yrs. *M (SD)* | | 54.28 (12.45) | 51.27 (12.14) | 61.16 (10.23) | 9.89 ** [-10.24, 9.54] |
| | Younger (18 - <40) | 2133 (12.2%) | 2041 (16.8%) | 92 (1.7%) | 1932,19 [.33] |
| | Middle Age (40 -<60) | 9360 (53.5%) | 7109 (58.5%) | 2251(42.2%) | < .001 |
| | Elderly (>=60) | 5988 (34.3%) | 3003 (50.2%) | 2985 (56.6%) | |
| Gender | Male | 3707 (21.2%) | 2356 (19.4%) | 1351 (25.4%) | 474.12 [.67] |
| | Female | 13774 (78.8%) | 9797 (80.6%) | 3977 (74.6%) | < .001 |
| Race | Asian | 286 (1.6%) | 218 (1.8%%) | 68 (1.3%) | 109.23 [.03] |
| | African American | 1116 (6.4%) | 800 (6.6%) | 316 (5.9%) | < .001 |
| | Other[1] | 614 (3.5%) | 459 (3.8%) | 155 (2.9%) | |
| | Caucasians | 15465 (88.5%) | 10676 (69.0%) | 4789 (89.9%) | |
| Ethnicity | Non-Hispanic | 16039 (91.8%) | 11082 (91.2%) | 4957 (93.0%) | 88.53 [.03] |
| | Hispanic | 1442 (8.2%) | 1071(8.8%) | 371 (7.0%) | < .001 |

Table 26 (*table continues)*

*Comparison of Demographic Factors: Patients with vs. without NAFLD Incidence*

| Variable | All Patients (N 17,481) | Without NAFLD N=12153 | With NAFLD (N=5328, | $\chi 2$ [CV] *p*-value* |
|---|---|---|---|---|
| Marital Status | | | | |
| Single/Widowed | 3491 (20.0%) | 2459 (20.3%) | 1032 (19.4%) | 59.20 |
| `Married/Partnered | 11787 (67.4%) | 8111 (66.7%) | 3676 (69.0%) | [.02} |
| | | | | < .001 |
| Divorced/Separated | 2203 (12.6%) | 1583 (13.0%) | 620 (11.6%) | |
| Educational Status | | | | |
| Primary | 653 (3.7%) | 412 (3.4%) | 241 (4.5%) | 604.76 |
| High School | 6406 (36.6%) | 4222 (34.7%) | 2184 (41.0%) | [.07] |
| | | | | < .001 |
| College/University | 9702 (55.5%) | 7059 (58.1%) | 2643 (49.6%) | |
| Insurance Status | | | | 732.59 |
| None | 3632 (20.8%) | 2243 (18.5%) | 1389 (26.1%) | [.08] |
| Yes | 13849 (79.2%) | 9910 (81.5%) | 3939 (73.9%) | < .001 |

**P* value = With Incident NAFLD vs. Without Incident NAFLD; **95% Confidence Interval; 1 - Other includes Mixed Race, Native American, Other, Pacific Islander, Unknown

## Comparison of Baseline RA Factors

In the overall cohort, about 33% had early RA, 19% for those with 2 to 5 years and 5 to 10 years disease duration and 29% for those with > 10 years of disease duration. The proportion of patients in the NAFLD group compared to those without NAFLD across the various categories of disease duration was lower except for the category with greater than10 years of disease as shown in Table 27. There was a statistically significant difference between the two subgroups; $\chi^2$ (3) = 703.34, p = <. 001, but the strength of association was weak (Cramer's *V* value = .08).

In the overall cohort about 18% were in remission, 31% were in low disease activity, 28% moderate disease and 22% with high disease activity. The proportion of patients in the NAFLD group compared to those without NAFLD was higher across the various categories of disease severity except for remission as shown in Table 27. There was a statistically significant difference between the two subgroups; $\chi^2$ (3) = 150.03, p = <. 001, however, the strength of association was weak (Cramer's *V* value = .03).

Most of the overall cohort was currently using MTX (87%). The proportion of patients in the NAFLD group compared to those without NAFLD was slightly higher among the current MTX users (73% vs. 69% respectively) as shown in Table 27. There was a statistically significant difference between the two subgroups; $\chi^2$ (1) = 141.22, p = <. 001, but the strength of association was weak (Cramer's *V* value = .03). In the overall cohort, only 30% were using cDMARDs. The proportion of patients in the NAFLD group compared to those without NAFLD was marginally higher among the cDMARDs users (31% vs. 29%) as shown in Table 27. There was a statistically significant difference between the two subgroups; $\chi^2$ (1) =28.22, p = <. 001; however, the strength of association was very weak (Cramer's *V* value = .01).

In the overall cohort, only 30% were using steroids. The proportion of patients in the NAFLD group compared to those without NAFLD was slightly higher among the steroid users (32% vs. 28%) as shown in Table 27. There was a statistically significant difference between the two subgroups; $\chi^2$ (1) =16.03, p = <. 001; but the strength of association was weak (Cramer's *V* value = .03). In the overall cohort, about 44% were using biologics. The proportion of patients in the NAFLD group compared to those without NAFLD was slightly lower among biologic users (41% vs. 45% respectively) as shown in Table 27. There was a statistically significant difference between the two subgroups; $\chi^2$ (1) = 11.47, *p* = <. 001, but the strength of association was weak (Cramer's *V* value = .03).

Table 27

*Comparison of Baseline RA Factors: Patients with vs. without NAFLD Incide*nce

| Variable | All Patients N =17,481 | Without NAFLD n=12153 | With NAFLD n=5328, | χ2 [CV] *p*-value* |
|---|---|---|---|---|
| Disease Duration yrs. *M (SD*) | 8.29 (8.88) | 7.74 (8.49) | 9.53 (9.78) | -1.79** (2.09- .14) |
| Early RA | 5735 (32.8%) | 4179 (34.4%) | 1556 (29.2%) | 703.34 [.08] |
| 2 to 5 years | 3276 (18.7%) | 2338 (19.2%) | 938 (17.6%) | < .001 |
| 5 to 10 years | 3340 (19.1%) | 2362 (19.4%) | 978 (18.4%) | |
| >10 years | 5109 (29.2%) | 3257 (26.8%) | 1852 (34.8%) | |
| CDAI Score[1] *M (SD*) | 13.94 (12.88) | 13.72 (12.83) | 14.45 (13.00) | -.73 ** (-1.14, -.31) |
| Remission (≤ 2.8) | 3223 (18.4%) | 2358 (19.4%) | 865 (16.2%) | 150.03 [.03] |
| Low Disease (≤ 10) | 5480 (31.3%) | 3802 (31.3%) | 1678 (31.5%) | < .001 |
| Moderate Disease (≤ 22) | 4935 (28.2%) | 3366 (27.7%) | 1569 (29.5%) | |
| High Disease (> 22) | 3835 (21.9%) | 2622 (21.6%) | 1213 (22.8%) | |
| MTX use [2] | | | | |
| None | 5207(29.7%) | 3755 (30.9%) | 1452 (27.3%) | 141.22 |
| Current Use | 12274 (70.2)%) | 8398 (69.1%) | 3876(72.7%) | [.03] |
| | | | | < .001 |
| cDMARD | | | | |
| None | 12298 (70.8%) | 8610 (70.8%) | 3688 (69.2%) | 28.22 |
| Yes | (5183 (29.6%) | 3543 (29.2%) | 1640 (30.8%) | [.016] |
| | | | | < .001 |
| Steroids | | | | |
| No | 12282 (70.3%) | 8650 (71.2%) | 3632 (68.2%) | 16.03 |
| Yes | 5199 (29.7%) | 3503 (28.2%) | 1696 (31.8%) | [.30] |
| | | | | < .001 |
| Biologics Use[4] | | | | |
| No | 9793 (56.0%) | 6678 (54.9%) | 3115 (58.5%) | 11.47 |
| Yes | 7688 (44.0%) | 5475 (45.1%) | 2213 (41.5%) | [.033] |
| | | | | < .001 |

**P* value = With Incident NAFLD vs. Without Incident NAFLD; **95% Confidence Interval;1- CDAI= Clinical Disease Activity Index; 2 -MTX= methotrexate; 3- cDMARD = conventional disease modifying disease-modifying antirheumatic drugs; 4- Biologics – combined TNF = tumor necrosis factor & non TNFs

## Comparison of Baseline Comorbidities and Other Factors

About 37% of this cohort had a diagnosed history of hypertension. The proportion of patients in the NAFLD group compared to those without NAFLD was slightly higher for those with hypertension (41% vs. 35% respectively) as shown in Table 28. There was

a statistically significant difference between the two subgroups; $\chi^2$ (1) =342.69, p = <. 001, however, the strength of association was weak (Cramer's *V* value = .05).

In the overall cohort only 7% had a history of cardiovascular disease (CVD). The proportion of patients in the NAFLD group compared to those without NAFLD was two times higher among those with CVD (10% vs. 5% respectively) as shown in Table 28. There was a statistically significant difference between the two subgroups; $\chi^2$ (1) =810.79, p = <. 001, but the strength of association was weak (Cramer's *V* value = .08).

About 7% of this overall cohort had a history of diabetes. The proportion of patients in the NAFLD group compared to those without NAFLD was slightly higher among those with diabetes (8% vs. 6% respectively) as shown in Table 28. There was statistically a significant difference between the two subgroups for the diabetes; $\chi^2$ (1) =137.80, p = <. 001; the strength of association was very weak (Cramer's *V* value = .03).

In the overall cohort, about 22% of this cohort had a reported history of dyslipidemia. The proportion of patients in the NAFLD group compared to those without NAFLD was marginally higher among those with dyslipidemia (26% vs. 20% respectively) as shown in Table 28. There was a statistically significant difference between the two subgroups; $\chi^2$ (1) =427.96, p = <. 001, however, the strength of association was weak (Cramer's *V* value = .06).

About 6% of the cohort had a history of cancer excluding non melanoma skin cancer (NMSC). The proportion of patients in the NAFLD group compared to those without NAFLD was marginally higher among those with a history of cancer (7% vs. 5% respectively) as shown in Table 28.There was a statistically significant difference

between the two subgroups; $\chi^2$ (1) =272.89, p = <. 001, but the strength of association was weak (Cramer's *V* value = .05).

Only a small proportion of this cohort reported a history of liver disorders (2%). The proportion of patients in the NAFLD group compared to those without NAFLD was slightly higher among those with a history of liver disorders (7% vs. 5% respectively) as shown in Table 28. There was a statistically significant difference between the two subgroups; $\chi^2$ (1) =75.04, p = <. 001, but the strength of association was very weak (Cramer's *V* value = .02).

Overall, there was about 8% of the cohort that had liver enzyme elevation. The proportion of patients in the NAFLD group compared to those without NAFLD was marginally higher among those with normal liver enzyme levels (92.1% vs. 91.6%) as shown in Table 28. There was a statistically significant difference between the two subgroups; $\chi^2$ (1) =8.42, p = .004, however, the strength of association was extremely weak (Cramer's *V* value = .009).

In the overall cohort, about 72% were obese. The mean BMI for the overall cohort was 30 (SD = 7.20) and was similar for those with and without NAFLD. The proportion of patients in the NAFLD group compared to those without NAFLD was also similar among those with and without obesity as shown in Table 28.The difference between the two subgroups was not statistically different; $\chi$2 (1) = 2.68, p - .101, and the strength of association was extremely weak (Cramer's V value = .005).

About 19% of the overall cohort had metabolic syndrome. The proportion of patients in the NAFLD group compared to those without NAFLD was slightly higher

among those with metabolic syndrome (20% vs. 18% respectively) as shown in Table 28. There was a statistically significant difference between the two subgroups; $\chi^2$ (1) =51.84, p = <. 001, however, the strength of association was very weak (Cramer's *V* value = .02).

In the overall about 40% of the cohort reported using alcohol. The proportion of patients in the NAFLD group compared to those without NAFLD was slightly higher among the non alcohol users (62% vs. 59% respectively) as shown in Table 28. There was a statistically significant difference between the two groups for alcohol use: $\chi^2$ (1) = 151.11, p = <. 001, but the strength of association was very weak (Cramer's *V* value = .03).

About 41% of the overall cohort were current smokers or had a history of smoking. The proportion of patients in the NAFLD group compared to those without NAFLD was similar among the smokers and non smokers as shown in Table 28. There was no statistically significant difference between the two subgroups; $\chi^2$ (1) =51.5, p = .023, and the strength of association was very weak (Cramer's *V* value = .01).

Most of this cohort reported exercising (70%). The proportion of patients in the NAFLD group compared to those without NAFLD was marginally higher among those who reported that they exercised (70% vs. 69% respectively) as shown in Table 28. There was a statistically significant difference between the two subgroups; $\chi^2$ (1) =17.15, p = <. 101, but the strength of association was very weak (Cramer's *V* value = .01).

Table 28

*Comparison of Baseline Comorbidities & Other Factors: Patients without and with NAFLD Incidence*

| Variable | | All Patients (N 17,481) | Without NAFLD (n 12153) | With NAFLD (n 5328) | $\chi 2$ [CV] *p*-value* |
|---|---|---|---|---|---|
| Hypertension | No | 10963 (62.7%) | 7844 (64.5%) | 3119 (58.5%) | 342.60 |
| | Yes | 6518 (37.3%) | 4309 (35.5%) | 2209 (41.5%) | [.05] < .001 |
| Cardiovascular Disease | No | 1635 (93.5%) | 11542 (95.0%) | 4810 (90.3%) | 810.79 |
| | Yes | 1129 (6.5%) | 611(5.0%) | 518 (9.7%) | [.088] < .001 |
| Diabetes | No | 16273 (93.1% | 11387 (93.7%) | 4886 (91.7)%) | 137.80 |
| | Yes | 1208 (6.9%) | 766 (6.3%) | 442 (8.3%) | [.03] < .001 |
| Dyslipidemia | No | 13657 (78.1%) | 9707 (79.9)%) | 3950 (74.1%) | 427.96 |
| | Yes | 3824 (21.9%) | 2446 (20.1%) | 1378 (25.9%) | [.06] < .001 |
| History Cancer | No | 16527 (94.5%0 | 11583 (95.3%) | 4944 (92.8%) | 272.89 |
| | Yes | 954 (5.5%) | 570 (4.7%) | 384 (7.2%) | [.05] < .001 |
| Liver Disorders | No | 17090 (97.8%) | 11913 (98.0%) | 5177 (97.2%) | 75.04 |
| | Yes | 391 (2.2%) | 240 (2.0%) | 151 (2.8%) | [.02] < .001 |
| Liver Enzyme Elevation | No | 16041 (91.8%) | 11132 (91.6)%) | 4904 (92.1%) | 8.42 |
| | Yes | 570 (4.7%) | 1021 (8.4%) | 419 (7.9%) | [.009] .004 |
| Obese (BMI) *M (SD*) | | 29.66 (7. 20) | 29.85 (7.43) | 29.22 (6.65) | .62 **[.40, .84] |
| | No | 4842 (27.7%) | 3348(27.5%) | 1494 (28.0%) | 2.68 |
| | Yes | 12639 (72.3%) | 8805(72.5%) | 3834 (72.0%) | [.005] .101 |
| Metabolic Syndrome | No | 14174 (81.1%) | 9924 (81.7%) | 4250 (79.8%) | 51.84 |
| | Yes | 3307 (18.9%) | 2229 (18.3%) | 1078 (20.2%) | [.022] < .001 |
| Alcohol Use | None | 10449 (59.8%) | 7118 (58.6%) | 3331 (62.5%) | 151.11 |
| | Yes | 7032 (40.2%) | 5035 (41.5%) | 1997 (37.5%) | [.03] < .001 |
| Smoking Status | Never | 10193 (58.3%) | 7056 (58.3%) | 3137 (59.1%) | 5.15 |
| | Smoker | 7208 (41.2%) | 5037 (41.7%) | 2171 (40.9%) | [.007] .023 |
| Exercise | None | 5310 (30.4%) | 3737 (30.7%) | 1573 (29.5%) | 17.15 |
| | Yes | 12171 (69.6%) | 8416 (69.3%) | 3755 (70.5%) | [.01] < .001 |

**P* value = With Incident NAFLD vs. Without Incident NAFLD; **95% Confidence Interval; SD = Standard Deviation; BMI = Body Mass Index

## Research Question 4

Research question 4 was to determine the significant baseline factors (clinical and demographic) that predicted the development of NAFLD after adjusting for relevant confounders**.** Prior to conducting the multivariate analysis, I conducted a bivariate analysis to determine the effect of each of the predictor and the outcome variables. The results from the unadjusted analysis were used to inform the multivariate model.

### Unadjusted Analyses of NAFLD Predictors: Baseline Demographic

The unadjusted analyses of baseline demographic predictors for developing NAFLD are presented in Table 29. In this unadjusted analysis there was a significant increased risk for NAFLD with increased age (the middle-aged and elderly) NAFLD risk was lower for women, African American, Caucasians, Hispanics, for those with college/university education, and also for those with insurance.

Table 29

*Unadjusted Analyses of NAFLD Predictors: Baseline Demographic*

| Variable | | Hazard Ratio | [95% CI] | *p*-value |
|---|---|---|---|---|
| Age | Younger (18-<40) | Referent | | |
| | Middle Age (40 -<60) | 5.37 | 4.36, 6.62 | < .001 |
| | Elderly (>=60) | 16.03 | 13.02, 19.73 | < .001 |
| Gender | Male | Referent | | |
| | Female | 0.76 | 0.71, 0.81 | < .001 |
| Race | Asian | Referent | | |
| | African American | 0.78 | 0.61, 0.99 | .050 |
| | Other[1] | 1.10 | 0.98, 1.24 | .080 |
| | Caucasians | 0.79 | 0.67, 0.92 | .004 |
| Ethnicity | Non-Hispanic | Referent | | |
| | Hispanic | 0.88 | 0.79, 0.98 | .021 |
| | Single or Widowed | Referent | | |
| | Married or Partnered | 0.96 | 0.90, 1.03 | .308 |
| | Divorced or Separated | 0.91 | 0.82, 1.01 | .078 |
| Education | Primary | Referent | | |
| | High School | 1.04 | 0.91, 1.19 | 548 |
| | College/University | 0.78 | 0.69, 0.89 | < .001 |
| Insurance Status | None | Referent | | |
| | Yes | 0.62 | 0.58, 0.66 | < .001 |

1 – Other includes Mixed Race, Native American, Other, Pacific Islander, Unknown

**Unadjusted Analyses of NAFLD Predictors: RA Related Factors**

The unadjusted analyses for baseline RA related predictors are presented in Table 30. In the unadjusted analysis, there was a statistically significant decreased risk for NAFLD for those with 2 to 5 years and 5 to 10 years of disease duration, and also for those using biologics. Baseline moderate and high disease severity was associated with increased risk for NAFLD as was using MTX.

Table 30

*Unadjusted Analyses of NAFLD Predictors: Baseline RA Factors*

| Variable | Hazard Ratio | [95% CI] | *P*-value |
|---|---|---|---|
| Disease Duration | | | |
| Early RA | Referent | | |
| 2 to 5 years | 0.89 | 0.82, 0.96 | .006 |
| 5 to 10 years | 0.90 | 0.83, 0.98 | .015 |
| >10 years | 1.06 | 0.99, 1.13 | .072 |
| `CDAI Score | | | |
| Remission (≤ 2.8) | Referent | | |
| Low Disease (≤ 10) | 1.06 | 0.97, 1.15 | .152 |
| Moderate Disease (≤ 22) | 1.12 | 1.03,1.22 | .007 |
| High Disease (> 22) | 1.16 | 1.06, 1.27 | .001 |
| MTX use | | | |
| None | Referent | | |
| Yes | 1.12 | 1.03, 1.21 | .007 |
| cDMARD Use | | | |
| None | Referent | | |
| Yes | .96 | 0.91, 1.02 | .235 |
| Steroids Use | | | |
| No | Referent | | |
| Yes | 1.11 | 1.04, 1.17 | < .001 |
| Biologics Use | | | |
| No | Referent | | |
| Yes | 0.88 | 0.83, 0.93 | < .001 |

1- CDAI= Clinical Disease Activity Index; 2 -MTX= methotrexate; 3- cDMARD = conventional disease modifying disease-modifying antirheumatic drugs (other than MTX)

**Unadjusted Analyses NAFLD Predictors: Comorbidities and Other Factors**

The results of unadjusted analyses by baseline comorbidities and other potential predictors are presented in Table 31. Most of the comorbidities in the model were associated with increased risk for NAFLD except for liver enzyme elevation, obesity, and exercise. The highest increased risk was seen for those with CVD, followed by those with dyslipidemia, metabolic syndrome, and those with hypertension. Other factors associated with increased risk for NAFLD, include liver disorders and smoking. However, alcohol use was associated with decreased risk for NAFLD.

Table 31

*Unadjusted Analyses of NAFLD Predictors: Comorbidities & Other Factors*

| Variable | | Hazard Ratio | 95% CI | *P*-value |
|---|---|---|---|---|
| Hypertension | No | Referent | | |
| | Yes | 1.67 | 1.58, 1.77 | < .001 |
| Cardiovascular Disease | No | Referent | | |
| | Yes | 2.09 | 1.90, 2.29 | < .001 |
| Diabetes | No | Referent | | |
| | Yes | 1.32 | 1.20, 1.45 | < .001 |
| Dyslipidemia | No | Referent | | |
| | Yes | 1.93 | 1.8, 2.05 | < .001 |
| Cancer History | No | Referent | | |
| | Yes | 1.51 | 1.36, 1.67 | < .001 |
| Liver Disorder | No | Referent | | |
| | Yes | 1.19 | 1.01, 1.40 | .032 |
| Liver Enzyme Elevation | No | Referent | | |
| | Yes | 0.96 | 0.87, 1.06 | .479 |
| Obesity (BMI ≥ 25) | No | Referent | | |
| | Yes | 1.06 | .99, 1.04 | .211 |
| Metabolic Syndrome | No | Referent | | |
| | Yes | 01.89. | 1.77, 2.03 | < .001 |
| Alcohol Use | No | Referent | | |
| | Yes | 0.83 | 0.78, 0. 87 | < .001 |
| Smoking Status | No | Referent | | |
| | Yes | 1.16 | 1.10, 1.22 | < .001 |
| Exercise | No | Referent | | |
| | Yes | 1.04 | 0. 98, 1.11 | .127 |

Multivariate analysis was conducted to determine the significant baseline factors that independently predicted the development of NAFLD after adjusting for relevant predictor or explanatory variables. Cox proportional hazards survival analysis was used to identify factors associated with the development of NAFLD (Szklo & Nieto, 2014, pp. 229-305). I ran the unadjusted and adjusted Cox proportional model to determine effect of the confounders on the outcome variable. Incidence (hazard) rates and ratios (HR) are reported with 95% confidence interval and factors that do not include 1 and a p-value < .05 were considered significant. While *p*-values can assess differences, however, it is also important to ascertain the impact or magnitude of the difference to avoid overestimation or underestimation of the associations (Grayson, 1987). I identified factors with significant p-values or those with ratios that changed by 10% or more and included them in the multivariate model as potential confounders (Grayson, 1987; Hernán, Hernández-Díaz, Werler, & Mitchell, 2002). I ran several multivariate models to evaluate the effect of the various predictor variables using the imputed incident data set as well as the original dataset. Multivariate models were analyzed by entering the predictor variables simultaneously into the model. Additionally, I also evaluated the impact on the HR using the original data without imputation.

The process taken to select the variables for the final primary model was as follows. I used the imputed dataset and included all the available demographic, RA related, comorbidities and other relevant variables into the model as described in the bivariate analysis. The outputs were compared to the unadjusted bivariate analysis for each of these variables and then I identified factors that were significant using p value of

0.05 as well those variables that changed by 10% or more. The variables selected a priori were age, gender, race/ ethnicity, obesity, metabolic syndrome, diabetes, dyslipidemia, MTX use, liver enzyme elevation, liver disorders, and alcohol; these were included regardless of significance as they are established clinical risk factors. The variables education, insurance, smoking, exercise, hypertension, cardiovascular, cancer, disease duration, and disease activity were included based on a significant p value or based on change of 10% or more.

I also ran a model where only significant factors and variables selected a priori were included. The results of this analysis were consistently significant for all the variables except in this model, factors such as marital status and exercise were also significant. Included in the multivariate analysis were the original data along with the imputed datasets. The results were similar to the primary model presented in Table 32. Since information about risk factors associated with the development of NAFLD has not been characterized in the RA population, it is important to include as much of the available information as possible to increase robustness of the model to identify predictors of NAFLD. Based on extensive evaluation and consistency of the results for the key predictor variables regardless of the model used, I elected to include all the available variables. They were either selected a priori or were significant. The final model also met the requirements for goodness of fit for model selection and thus enabled the model to predict risk factors associated with NAFLD. The results are presented in Table 32**.**

In the adjusted analyses age a significant independent predictor for NAFLD. HR was 5.16, 95% CI [4.16, 6.40] for the middle aged group ($p < .001$). Similarly there was a significant increased risk for the elderly group with HR of 13.94, 95% CI [11.22, 17.31], $p < .001$. Women were at lower risk for developing NAFLD compared to men, with HR 0.81, 95% CI [0.76, 0.86]; $p < .001$**.** Race and ethnicity (Hispanics) were not significant predictors for the development of NAFLD. In the adjusted analysis, those who were divorced or separated were at a lower risk of developing NAFLD, HR 0.87, 95% CI [0.79, 0.97], p = 0.010. As was those with insurance, with a lower risk for NAFLD (HR 0.89, 95% CI [0.83, 0.95], $p < .001$). Factors such as education, smoking, and use of alcohol were not significant predictors for the development of NAFLD. However, there was an increased risk for NAFLD in the group that exercised (HR 1.09, 95% CI [1.02, 1.16], $p = .009$) in the adjusted analysis.

Those with hypertension, cardiovascular disease and dyslipidemia were at a statistically significant increased risk for NAFLD in the adjusted analysis. Hazard ratios were 1.11, 95% CI [1.04, 1.19], $p < .003$ for hypertension; HR of 1.19, 95% CI [1.08, 1.31], $p < .001$ for cardiovascular disease; and HR 1.31, 95% CI [1.20, 1.42], $p < .001$ for dyslipidemia. Diabetics were at reduced risk for developing NAFLD, HR was 0.87, 95% CI [0.78, 0.98], $p < .020$. The risk for NAFLD was significantly lower for the obese, with a HR= .84, 95% CI [0.79, .90], $p <. 001$. Comorbid conditions such as history of cancer, liver disorders, and liver enzyme elevation groups were not significant predictors for NAFLD after adjusting for confounders.

In the adjusted analysis for RA related factors, all categories of disease duration were associated with lower risk for NAFLD. Those with 2 to 5 years and 5 to 10 years of disease were at lower risk of NAFLD with HR of 0.87, 95%CI [0.80, 0.94], $p$ = .001, and HR of 0.87, 95%CI [0.80, 0.95], $p$ = .001 respectively. The results were similar for those with longer duration of disease greater than10 years; HR 0.88, 95%CI [0.82, 0.95], $p$ = .001. In the adjusted analysis, baseline disease activity was not associated with increased risk for NAFLD. MTX use remained significant predictor in the adjusted analysis with an increased risk for NAFLD, HR 1.08, (95% CI 1.01, 1.15), $p$ = .021. Similar results was seen for cDMARDs use, which was also associated with significant increased risk for NAFLD, HR 1.08, (95% CI 1.01, 1.15), $p$ = .027. The use of steroids and biologics were not associated with increased risk for the development of NAFLD in the adjusted analysis.

Table 32

*Adjusted Analyses of NAFLD Predictors*

| Baseline Factors | Hazard Ratio | 95.0% CI | *p Value* |
|---|---|---|---|
| Age* | | | |
| Younger (18-<40) | Referent | | |
| Middle Age (40 -<60) | 5.16 | 4.16, 6.40 | **< .001** |
| Elderly (>=60) | 13.94 | 11.22, 17.31 | **< .001** |
| Gender (Female) | 0.81 | 0.76,0.86 | **< .001** |
| Race * | | | |
| Asian | Referent | | |
| African American | 1.15 | 0.87, 1.51 | 0.323 |
| Other[1] | 0.94 | 0.70, 1.27 | 0.703 |
| Caucasians | 1.05 | 0.82, 1.35 | 0.677 |
| Ethnicity* (Hispanic) | 1.04 | 0.93, 1.16 | 0.496 |

Table 32
*Adjusted Analyses of NAFLD Predictors* *(table continues)*

| Baseline Factors | Hazard Ratio | 95.0% CI | *p Value* |
|---|---|---|---|
| Marital Status | | | |
| Single or Widowed | Referent | | |
| Married or Partnered | 0.96 | 0.89, 1.03 | 0.279 |
| Divorced or Separated | 0.87 | 0.79, 0.97 | **0.010** |
| Education** | | | |
| Primary | Referent | | |
| High School | 1.00 | 0.87, 1.16 | 0.939 |
| College/University | 0.98 | 0.85, 1.12 | 0.780 |
| Insurance ** | 0.89 | 0.83, 0.95 | **0.001** |
| Smokers** | 0.99 | 0.93, 1.04 | 0.597 |
| Alcohol Use* | 0.97 | 0.91, 1.03 | 0.278 |
| Exercise (Yes) | 1.09 | 1.02, 1.15 | **0.009** |
| Hypertension ** | 1.11 | 1.04, 1.19 | **0.003** |
| Cardiovascular Disease** | 1.19 | 1.08, 1.31 | **0.001** |
| Dyslipidemia * | 1.31 | 1.20, 1.42 | **0.001** |
| Cancer ** | 1.07 | 0.96, 1.20 | 0.195 |
| Diabetes • | 0.87 | 0.78, 0.98 | 0.019 |
| Liver Disorders * | 1.18 | 0.99, 1.39 | 0.061 |
| Liver Enzyme Elevation* | 1.05 | 0.95, 1.17 | 0.331 |
| Obese (BMI >=25) * | 0.84 | 0.79, 0.90 | **< .001** |
| Metabolic Syndrome* | 1.21 | 1.09, 1.35 | **0.001** |
| Disease Duration** | | | |
| Early RA | Referent | | |
| 2 to 5 years | 0.87 | 0.80, 0.94 | **0.001** |
| 5 to 10 years | 0.87 | 0.80, 0.95 | **0.001** |
| >10 years | 0.88 | 0.82, 0.95 | **0.001** |
| Disease Activity (CDAI) ** | | | |
| Remission (≤ 2.8) | Referent | | |
| Low Disease (≤ 10) | 0.98 | 0.90, 1.07 | 0.685 |
| Moderate Disease (≤ 22) | 1.03 | 0.95, 1.12 | 0.484 |
| High Disease (> 22) | 1.08 | 0.98, 1.18 | 0.111 |
| MTX use * | 1.08 | 0.01, 1.16 | **0.021** |
| cDMARD Use** | 1.08 | 1.01, 1.15 | **0.027** |
| Biologics Use** | 1.04 | 0.98, 1.10 | 0.230 |
| Steroid Use ** | 0.98 | 0.92, 1.04 | 0.553 |

BMI = Body Mass Index; CDAI = Clinical Disease Activity Index; MTX= Methotrexates; cDMARD = Conventional disease modifying antirheumatic drugs (excluding MTX). * - Identified apriori; ** met significance (*p* =value or +/_10% difference)

## Summary

In this chapter I described the methods used to select the incident cohort and the steps taking to prepare for analysis. Several variables were transformed, analyses for measures of central tendency, distribution, and dispersion of data were assessed. For the Kaplan Meier and Cox proportional survival analysis, the adequacy of sample size was evaluated, impact of missing data, normality of distribution, proportionality of hazard assumption and multicollinearity were assessed. I also evaluated and addressed issues with missing data, outliers, and difference between withdrawn and remaining cases, and changes in survival experience over time. Missing data were addressed using the multiple imputation method, an optimal approach that preserved sampling variability of the original dataset. The large sample size of 17,481 participants helped minimize the impact of outliers, and was further reduced by transforming them into categorical variables. Since the incident sample size was sufficiently large, the alternative research questions related to the prevalent analysis previously embedded in RQ3 and RQ4 were abandoned. Descriptive analysis, and bivariate analysis were conducted to inform the model for the multivariate analysis.

A descriptive analysis was conducted to determine if the NAFLD cohort was similar to the overall registry, but given the large sample size it was difficult to evaluate using the *p* values. The numerical values were similar for most variables, however, there were some notable absolute differences. The NAFLD cohort was younger, more of this cohort had insurance, and more of them were taking MTX, with fewer reporting CVD

history and liver enzyme elevations. Generally for most of the clinical variables, the NAFLD cohort appears to be mostly similar to the overall registry.

RQ1 was to determine the incidence rate and time to development of NAFLD in this cohort of RA patients. There were about 17,481 patients in the incident cohort with data collected over a period of 13.2 years. The cumulative overall incidence rate over this period was 31%, with a mean overall time to event of 7.1 years, with most developing mild to moderate NAFLD (29%) and only 1.4% had advanced disease.

Research question 2 was to determine the prevalence of NAFLD among patients with RA during the course of one year (e.g. 2012) in the U.S. using the FIB-4 test. As previously mentioned answering this research question was contingent on not having a sufficiently large sample size to conduct the incident cohort analysis. Thus this analysis was not pursued.

RQ3 compared the clinical and demographic variables among those with and without NAFLD. Overall there were statistically significant differences for all of the factors when the two groups of those with and without the disease were compared except for smoking and obesity. The prevalence of demographic factors was lower in the NAFLD group except for the elderly, gender (men). The prevalence of Caucasians, non Hispanics, those married/partnered, those with high school education and those without insurance were higher in the NAFLD group.

The prevalence of RA related factors was lower in the NAFLD group for disease duration except for those with greater than 10 years of disease. Slightly higher prevalence was seen across all disease severity categories with the exception of those in remission.

There was higher prevalence of MTX users, the cDMARDs users, steroid users, and also the users of biologics in the NAFLD group. The prevalence of comorbidities and other factors was higher in the NAFLD group also for hypertension, CVD, diabetes, metabolic syndrome, history of cancer, liver disorders, among those with normal liver enzymes, non users of alcohol and among those who reported that they exercised. The prevalence of obesity and smokers were similar among those with and without NAFLD.

RQ4 identified significant baseline factors (clinical and demographic) that predicted the development of NAFLD after adjusting for relevant confounders. In the bivariate analysis, the following demographic factors were statistically significant for increased risk; age (middle age and elderly), whereas women, race and ethnicity (African Americans, Caucasians, and non-Hispanics), college/university education and having insurance were associated with reduced risk for NAFLD. Among the RA related variables, disease duration (2 to 5 years and 5 to 10 years) was associated with reduced risk. Moderate and high disease activity was associated with increased risk for NAFLD. Use of RA medications including MTX and steroids were associated with increased risk for NAFLD and the use of biologics was associated with lower risk for NAFLD. In the unadjusted analyses of comorbidities such as, hypertension, CVD, diabetes, dyslipidemia, cancer history, history of liver disorders, metabolic syndrome, and smoking were all associated with increased risk. Alcohol use was associated with a reduced risk for NAFLD.

In the multivariate analysis the following variables were statistically significant predictors for increased risk for NAFLD, middle age, elderly, exercise, hypertension,

CVD, dyslipidemia, and metabolic syndrome. Factors associated with statistically significant reduced risk included gender (female), diabetes, obesity, disease duration, being divorced or separated, and having insurance. The relevance and implications of these newly identified predictors for NAFLD in the RA population are elaborated in the next chapter.

In Chapter 5, I provide a summary of findings, interpretation of the results in context of the theoretical framework and current understanding of NAFLD compared to the general population. I discuss the strength and limitations of the study and propose suggestions for future research. I also will review potential impact for social change and recommendations for practice. Finally I conclude this chapter with key messages for the study.

# Chapter 5: Discussion, Conclusions, and Recommendations

The purpose of this quantitative research study was to determine the incidence and factors associated with NAFLD using longitudinal retrospective data collected from a cohort of adult RA patients in the U.S. The primary objective of this study was to first establish the occurrence of NAFLD by using FIB-4, a noninvasive tool in this population. A second goal was to determine if there were significant clinical and demographic factors that independently predict the development of NAFLD using Kaplan Meier and Cox proportional hazard survival analysis.

The finding from this study established for the first time that roughly 31% of patients with RA developed NAFLD (FIB-4 score ≥ 1.3). A large registry dataset with longitudinal data collected over a period of 14 years was used to determine the cumulative incidence rates. About 29% of this cohort had mild to moderate NAFLD and about 1.4% had advanced disease. The overall mean time to event was about 7 years. At baseline, the mean age of this cohort was about 54 years and over 80% of these individuals were Caucasian females. These patients had RA for about 8 years, had moderate disease activity, and over three fourths of them were obese and were currently using MTX. Less than half of this cohort had hypertension, reported using alcohol, or were smokers. About a fourth of these patients had dyslipidemia, metabolic syndrome. Less than 10% had diabetes, cardiovascular disease, and liver enzyme elevation. Very few had a history of liver disorders.

The two subgroups of those with and without NAFLD were compared to determine the differences in baseline clinical and demographic characteristics. There was

a statistically significant difference between the two subgroups of those with and without NAFLD except for obesity and smoking. For the clinical characteristics, prevalence was higher for the known risk factors such as age, gender, and race/ethnicity. In the NAFLD group, the prevalence of RA related factors was higher for those with more than 10 years of disease duration, across all disease severity categories with the exception of those in remission. The prevalence was higher in the NAFLD group for MTX users, cDMARDs users, steroid users, and also for the users of biologics. The prevalence of comorbidities and other factors was higher in the NAFLD group for hypertension, CVD, diabetes, metabolic syndrome, history of cancer, liver disorders, among those with normal liver enzymes, non users of alcohol and among those who reported that they exercised. There were no differences in the prevalence of obesity and smokers among those with and without NAFLD.

Adjusted and unadjusted cox regression analysis was conducted to determine factors associated with the risk of developing NAFLD. In the unadjusted analysis, age (middle age and elderly), baseline moderate and high disease activity, MTX use and steroid use were associated with increased risk for NAFLD. Factors associated with reduced risk for developing NAFLD were gender (women), African Americans, Caucasians, ethnicity (non-Hispanics), disease duration (2 to 5 years and 5 to 10 years), biologic use, alcohol use, college/university education, and having insurance. In the adjusted analysis, age (middle age, elderly), hypertension, cardiovascular disease, dyslipidemia, exercise, metabolic syndrome, MTX use and cDMARDs were significant independent predictors for developing NAFLD. Gender (women), diabetes, obesity,

disease duration, divorced or separated, and having insurance were significant independent predictors for reduced risk for NAFLD

In summary, the results from the bivariate as well as the multivariate results were consistent for most the key variables, except for race and ethnicity, cancer, liver disorder and alcohol use with loss of significance in the adjusted model. Being divorced or separated and having insurance were associated with reduced risk, while diabetes reversed from increased risk to lower risk. The relevance and implications of these newly identified predictors for NAFLD in the RA population will be elaborated in the following section.

## Discussion of Findings

The results of this study agreed with previous NAFLD research in the general population. Finding hypertension, cardiovascular disease, dyslipidemia, metabolic syndrome, and the use of MTX were identified as independent predictors for the development of NAFLD in the RA population. However development of NAFLD was not associated with liver enzyme elevation in this population.

Theories, pathways, and relationships related to the development of NAFLD are still evolving (Erickson, 2009). The conceptual framework for this study was based on the hypothesis that NAFLD and RA would have shared risk factors and the confluence of these factors could contribute to the development of NAFLD. This study further confirmed this framework as metabolic syndrome, dyslipidemia, MTX use, along with hypertension and cardiovascular disease was prevalent in this cohort with RA and all were found to be independent predictors for the development of NAFLD. Interestingly,

the known factors among the general population for increased risk for NAFLD, such as gender, obesity, and diabetes, were associated with decreased risk in the RA population. Exercise was associated with increased risk in the RA population. These findings may be unique to patients with RA, as this population is aggressively treated with anti-inflammatory medications and potentially could alter inflammatory pathways related to obesity, metabolic dysfunction, and lipid metabolism, key precursors to the development of NAFLD (Fabbrini, et.al., 2010).

**Overall NAFLD Incidence Rates**

This is the first study that reports on the incidence rates and time to development of NAFLD for patients with RA using FIB-4. This was a large study of 17,481 patients with RA in the U.S. with data collected over 14 years. During this period, 31% of this cohort developed NAFLD; the rate was 95 cases per 1,000 patient years. The mean time to event was 7 years. Most of the patients that developed NAFLD had mild to moderate NAFLD (29%), while incidence of advance NAFLD was very low (1.4%). The overall incidence rate in the RA population was much higher than the incidence rate reported in the general population of 15% to 20%, and 18 to 31 per 1000 PY (Vernon et al., 2011). The incidence rate was also higher then the only previously reported NAFLD rate for patients with RA with a prevalence of 23% in the U.S. (Bhambhani et al., 2006), however, that study used ultrasound to determine the presence of NAFLD (Chalasani et al., 2012). There are significant variations in the rates of NAFLD depending on the tools used; the rates reported in this study is similar to rates reported when FIB-4 score was used (Eguchi et al., 2012), and also similar to rates several studies that used ultrasound

(Chalasani et al., 2012). Time to development of NAFLD for the RA population was about 7 years and seems to be similar to the general population of 7 to 8.5 years (Zelber-Sagi et al., 2012).

**Risk Factors Associated with Nonalcoholic Fatty Liver Disease**

Overall results of this study showed that age, hypertension, cardiovascular disease, dyslipidemia, metabolic syndrome exercise, MTX use and cDMARDs were significant independent predictors for increased risk for developing NAFLD in persons with RA. Some of these factors (age, hypertension, cardiovascular disease, dyslipidemia, and metabolic syndrome) were similar to the risk factors identified in the NAFLD guidelines for the general population, however, there were other risk factors in the guidelines such as gender, obesity, and diabetes (Chalasani et al., 2012).

Unlike in the general population, race and ethnicity were not significant predictors of NAFLD in the RA population (Chalasani et al., 2012). In the general population, hypertension, presence of cardiovascular disease, and liver enzyme elevation were associated with NAFLD but were not identified as independent risk factors (Lonardo et al., 2015; Chalasani et al., 2012). However in the RA population, all of except for liver enzyme elevation were independent predictors for the development of NAFLD. Unlike the general population, being female, having diabetes, and being obese were independent predictors for reduced risk for NAFLD in the RA population (Chalasani et al., 2012),

Among the demographic variables, age and gender were significant independent predictors for NAFLD in the RA population. Incidence was the highest for the elderly group, with about half developing NAFLD, followed by the middle age group. The

incidence for NAFLD was less common among those less than 40 years of age. There was roughly a 13 fold increased NAFLD risk for the elderly, where the increased risk was five fold for the middle age group. These findings are similar to risk seen in the general population, where the risk for NAFLD increased over time, peaking in the middle and latter ages of life (Lonardo, et al., 2015; Chalasani et al., 2012; Vernon et al., 2011). Being male was also a significant independent predictor for NAFLD in the general population as was for the RA population (Chalasani et al., 2012; Vernon et al., 2011).

In the general population, prevalence and risk for NAFLD differed by race and ethnicity in the U.S., with increased risk for Hispanics, followed by Caucasians, with African Americans consistently having the lowest risk for NAFLD (Stepanova & Younossi, 2012; Vernon et al., 2011, Williams et al., 2011). However in this analysis of RA patients, in the group that developed NAFLD, almost 90% were Caucasians. Race and ethnicity were not significant independent risk factors for NAFLD in the adjusted analysis for patients with RA, though the rates were directionally increased for risk, 15% for African Americans, 5% for Caucasians and 4 % for Hispanics. This discrepancy could be attributed to Caucasians with RA, however, a few studies in the general population also reported increased risk for Caucasians compared to other races (Stepanova & Younossi, 2012; Ong et al., in 2008).

Obesity is a significant risk factor for NAFLD and some studies suggest increased risk even among those with normal BMI. Studies in the U.S. reported overall prevalence rates for NAFLD of more than 65% for the obese (Chalasani et al., 2012; Lazo & Clark, 2008a; Vernon et al., 2011). The results in this study were consistent with these reports,

among the obese about 72 % developed NAFLD, compared to 28% in the non-obese group. Interestingly, in the adjusted analysis obesity was associated with a 16% decreased risk for NAFLD, unlike studies in the general population where the risk was an independent predictor for increased risk (Lazo et al. in 2013). Perhaps this unexpected reversal could be explained by the fact that BMI was used in this study instead of waist circumference, a more sensitive measure for central obesity and a measure recommended by the NAFLD guidelines (Alberti et al., 2009; Crowson, Matteson, Davis, & Gabriel, 2013). This paradoxical finding related to obesity has also been previously reported in the RA literature, where overweight and obesity were associated with reduced relative risk for of all-cause and cardiovascular mortality and this was regardless of age and disease duration (Wolfe & Michaud (2012). As alluded previously, this may be an unique finding related to patients with RA, with chronic inflammation linked to adipose tissue (obesity), some have even reported that there maybe some protective effect with increasing body mass among patients with RA instead of the known harmful effect of obesity (Crowson, et. al, 2013).

In this study about 19% of patients with RA had metabolic syndrome. Among patient with RA metabolic syndrome was identified as an independent NAFLD predictor, with an increased risk of 21%. The proportion of metabolic patients that developed with NAFLD was about 20% and was similar to the findings in the general population with prevalence rates that ranged from 20.5% to 43.% in the U.S. (Smits et al., 2013; Ong et al., 2008). However, unlike the general population, where the risk for NAFLD was associated with the presence of any and all factors related to metabolic syndrome

(Vernon et al., 2011), in this study about 80% of patients without metabolic syndrome developed NAFLD, conceivably another finding unique to patients with RA. This phenomena maybe related to other comorbid conditions prevalent among patients with RA such as insulin resistance, hypertension and increased waist circumference, a measure not available in this study (Ferraz-Amaro, González-Juanatey, López-Mejias, Riancho-Zarrabeitia & González-Gay, 2013).

Similar results were seen for patients with dyslipidemia, more RA patients (74%) without dyslipidemia developed NAFLD. In this study 25 % of patients in the overall cohort developed NAFLD and was within the range of prevalence reported in the U.S. (See Figure 16), Similar to the risk for NAFLD seen in the general population (Chalasani et al., 2012). This study also found dyslipidemia to be an independent predictor for NAFLD with an increased risk of 31%.

Only 7% had diabetes in this study. The NAFLD rate among the diabetics (8.3%) was lower then the prevalence rates reported for the general population in the U.S. of 15.8% to 26.3% (Lazo et al., 2011; Williams et al., 2011). Unlike studies in the general population where diabetes was associated with increased risk for NAFLD (Chalasani et al., 2012), in this study diabetes was an independent predictor for lower risk for NAFLD (16%). It is unknown if this is another unique finding for patients with RA, or perhaps related to underlying insulin resistance, a measure that was not available in this study (Ferraz-Amaro et. al., 2013).

Potential confounders associated with NAFLD were hypertension, cardiovascular disease, liver enzyme elevation, alcohol use, and liver disorders. In the general population

these factors have been associated with NAFLD but are not recognized as independent risk factors for NAFLD by the guidelines (Chalasani et al., 2012). The overall prevalence of these factors was less than 10% for liver enzyme elevation, 37% for hypertension, and 40% for alcohol use in this study. After adjusting for confounders, hypertension and a history of cardiovascular disease were found to be independent predictors for the development of NAFLD for patients with RA. There was an 11% increased risk for NAFLD for those with hypertension and 19% for those with cardiovascular disease.

Only about 8% of this cohort with RA had liver enzyme elevation developed NAFLD. A similar rate was seen in the general population (Chalasani et al., 2012). In the adjusted analysis there was increased risk for NAFLD (5%) for patients with RA but was not statistically significant. More patients not using alcohol developed NAFLD (63%) in the RA population. Unlike the general population where there was an association for increased risk for NAFLD (Chalasani et al., 2012). In the adjusted analysis use of alcohol was not a significant predictor for NAFLD, but had a small risk reduction of 3%. Similar non-significant results were seen for those with a history of liver disorders and history of cancer with an increased risk for NAFLD of 18% and 7% respectively. Additionally, in this study those with education and insurance had a reduced risk for NAFLD. Inexplicably, exercise was associated with increased risk for NAFLD by 9%; this finding is contrary to the NAFLD guidelines, where exercise along with diet was recommended to reduce risk for NAFLD (Chalasani, 2012).

As previously stated, information about risk factors in the general population has been well characterized; however, such information related to RA is not available in the literature. Among those with NAFLD, 35% had disease duration of 10 years or more, 29% had early RA and 18% had disease duration of 2 to 10 years. Inclusion of RA related factors in the model showed that baseline disease duration was an independent predictor for lower risk for NAFLD (12%) and this was regardless of the category of disease duration. Implication of these findings warrants further exploration to understand the impact of disease duration, changes in disease severity, influence of medication and development of comorbidities over time (Ferraz-Amaro et. al., 2013).

Among those with NAFLD, the proportion of patients in low disease activity (32%), moderate activity (30%) and high disease activity (29%) were similar but lower for those in remission (16.2%). It appears that there may be a dose type association with increased baseline disease activity and risk for NAFLD. Baseline disease activity was not a significant independent predictor for the development of NAFLD, although there was an increased risk for NAFLD for those with moderate and high disease activity (3% and 8% respectively), and a reduced risk (2%) for those with low disease activity.

As hypothesized, use of MTX was an independent predictor of development of NAFLD, with an 8% increased risk. Most of this cohort was currently using MTX (70%), and almost three fourths of them developed NAFLD. A similar result was seen with those using cDMARD also with an increased risk of 8%. Other drugs used for RA, including biologics and steroid use were not statistically significant risk factors for NAFLD,

however there were directional changes; steroids were associated with an increased risk of 4% and biologics with a reduced risk of 2%.

In summary, as postulated patients with RA developed NAFLD similar to the rates seen in the general population. A priori it was hypothesized that the following factors would be predictors for the development of NAFLD, these included age, gender, race/ ethnicity, obesity, metabolic syndrome, diabetes, and dyslipidemia. As expected, they were significant independent predictors for increased risk for the development of NAFLD except for obesity and diabetes were associated with reduced risk, and race/ethnicity was not a significant factor. MTX use, liver enzyme elevation, liver disorders, and alcohol use were identified as potential predictors for NAFLD apriori, however, among these factors only current use of MTX was identified as a significant predictor for increased risk for NAFLD. In this population with RA, comorbidities such as hypertension and CVD were found to be independent predictors for increased risk for NAFLD as was disease activity, where as diseased duration was associated with decreased risk.

## Study Limitations

This was a non-randomized study that used secondary data and utilized a non-probability sample of convenience. Threats to internal and external validity were previously outlined in Chapter 1. The data used for this analysis were retrieved from a large U.S. registry of RA patients. It has been previously established that the results from this registry is generalizable to a national U.S. population of patients with RA (Curtis. J., Chen, L., Yun, H., et al, 2013). One of the strengths of this study is its large sample size

and longitudinal data collected over 14 years. However, pertinent to this study is the potential for systematic selection bias, since the cohort was primarily selected based on availability of laboratory parameters (liver enzyme and platelet counts), measures available in the registry. Thus there could be selection bias potentially leading to systematic differences between the study cohort and the rest of the patients in the registry. The impact of this limitation was evaluated by comparing the NAFLD cohort with the overall registry population. There was a statistically significant difference between those in the NAFLD cohort compared to those remaining in the registry for all the variables except for ethnicity and liver disorders. These differences were mainly driven by the large sample size. The overall registry had 40,300 RA patients and almost half of this population was included in the NAFLD cohort. Upon further evaluation, clinically and numerically the NAFLD cohort appeared to be mostly similar to the overall registry for most of the variables, however this may still pose some limitations in terms of generalizability of the results.

Another limitation with this study was related to missing data. It is not known if these values were missing due to loss of follow up or underreporting and thus could potentially lead to bias. This limitation was addressed statistically using the multiple imputations to increase the precision and robustness of the results. There were some other limitations to note related to assumptions associated with survival analysis. For this analysis, I assumed that the censored cases and the ones lost during the course of study were not systematically different from those with an event at the end of the study, as this would inevitably result in nonrandom loss of cases potentially leading to bias. Since this

was a retrospective study that used a sample of convenience such data and reasons for loss were not collected so I can only assume that the reasons for lost cases was similar for those censored and or those with an event. I used the Breslow test to address this issue analytically, as this test weights the differences in survival according to the number at risk at each time point, and showed there was no difference between the cases, thus statistically addressing issues with systematic loss of cases.

Another potential limitation related to survival analysis is the assumption that factors that would affect survival in the beginning would be the same factors affecting survival at the end of the study also. For this study in the absence of data, I assumed that environmental factors and other conditions for survival would be similar over time and these factors would be the same for both the event and censored group. Thus the results of this exploratory hypothesis-generating study using observational data can only be interpreted under the conditions the study was conducted, and thus alternative explanations cannot be completely ruled out (Szklo &Nieto, 2013, pp. 391-426).

## Recommendations for Future Research

The strength of this study was the large sample size and access to data that was collected over a period of 14 years. The findings from this study identified for the first time the incidence and factors associated with the development of NAFLD among patients with RA. Most of the findings were generally consistent with what is known in the general population. The rate of 31% was similar to the rate seen in the U.S. general population, however there were some notable unique differences for the predictors of NAFLD in the RA population. In this study, I did not directly compare the rates between

the RA population and the general population. Such a comparison would be useful from a clinical perspective, particularly to understand if the RA population known to have systemic inflammation would be potentially at higher risk for NAFLD then the general population. In this study among those who developed NAFLD, most developed mild to moderate NAFLD and only a few had advanced disease. Additional long term studies are needed to determine rate and time to progression of advanced disease.

The cohort in this study included mostly Caucasians; further studies are needed to better understand incidence and factors associated with the development of NAFLD in other minority race and ethnic groups. Unlike the general population, in this study patients with diabetes and obesity appeared to have reduced risk for NAFLD. Additional research is needed to better understand if this phenomenon is unique to RA patients with NAFLD having these comorbidities (metabolic syndrome, insulin resistance) or perhaps these patients are affected by the chronic use of immunosuppressants (e.g. anti TNF-alpha) used for the treatment of RA (Ferraz-Amaro et. al., 2013) .

Also, confirmed in this study was that patients treated with MTX are at increased risk for the development of NAFLD; however further studies are needed to identify alternative RA treatments that may reduce the risk for NAFLD. The dataset did not support assessing the effect of time varying variables on the outcome, as only baseline factors were available. It would have been particularly interesting to understand the impact of factors related to RA, such as change in duration and severity of disease, or changes in RA treatment and the impact on NAFLD. These are areas that warrant further exploration. Finally, additional studies using the FIB-4 score are warranted to further

confirm the utility of this tool for the early diagnosis and management of NAFLD.

## Social Change Implications

This study further confirmed existing theories related to NAFLD in another chronic condition, RA. The findings from this study supports previous hypotheses related to the complex relationship between chronic inflammation affecting the liver, factors such as obesity, metabolic dysfunction, altered lipid metabolism and that these factors remain central to the development of NAFLD in the RA population also. Thus this study had some novel findings and adds to the body of knowledge to further the advancement of science but more importantly provides insights for better care of patients with RA.

The findings of this study provide a unique opportunity for social change including the early diagnosis of NAFLD using a novel, less expensive, noninvasive tool. This study used FIB-4, a diagnostic tool that can be used to identify mild/moderate and advanced NAFLD and can easily be incorporated into routine care. Thus FIB-4 is a practical tool for the early diagnosis and management of NAFLD among patients with RA.

The results of this study helps inform future studies as it identified independent predictors for the development of NAFLD among patients with RA, along with providing rates of prevalence of these risk factors. It also sheds light on the time to development of NAFLD for patients with RA. About a third of the RA population developed NAFLD, highlighting the need for policies that support surveillance programs and also those that foster interdisciplinary approaches to identify and manage these interrelated chronic conditions. Predictors and factors associated with the development of NAFLD are useful

information for public health practitioners and clinicians. Such evidence can be used to inform practice, policy, particularly for a newly identified population with NAFLD, patients with RA. The findings of this study and ensuing publications can add substantive evidence to the body of literature and thus can enable increased public awareness of NAFLD among patients with RA.

## Conclusion

The results of this study established for the first time that about 31% of RA patients are at risk for developing NAFLD and the mean time to event was about 7 years. A novel non-invasive tool (FIB-4 score) was used to identify the various stages of NAFLD. Of note was that most developed mild to moderate NAFLD (30%) and only about 1.4% had advanced disease. Overall there were statistically significant differences for all of the clinical and demographic variables when the two subgroups of those with and without NAFLD were compared, except for smoking and obesity where there was no difference. The prevalence of several key comorbidities was higher in the NAFLD group such as hypertension, CVD, diabetes, and metabolic syndrome. Patients with these conditions, nonusers of alcohol and smokers were more likely to develop NAFLD significantly sooner. However there were some unique unexpected findings for RA patients with obesity and diabetes, unlike the general population these factors were associated with reduced risk for NAFLD.

The results from the bivariate as well as the multivariate analysis were consistent for most the key variables, except for race and ethnicity, cancer, liver disorder and alcohol use with loss of significance in the adjusted model. Factors such as being

divorced or separated, and having insurance were associated with reduce risk and diabetes reversed from increased risk to lower risk in the adjusted model. This study using a large dataset with longitudinal data collected over period of 14 years identified for the first time the following variables as independent predictors of increased risk for NAFLD. The risk factors are age (middle age, elderly), exercise, hypertension, cardiovascular disease, diabetes, dyslipidemia, metabolic syndrome and MTX use. Predictors associated with statistically significant reduced risk for NAFLD were gender (female), diabetes, obesity, disease duration, divorced or separated, and having insurance.

Thus this study has identified yet another population at risk for NAFLD those with RA and further confirms its association with other chronic conditions such as dyslipidemia, metabolic syndrome, hypertension and cardiovascular disease. This study furthermore highlights the need for early diagnosis and management of NAFLD, which can be accomplished by using an easily available a noninvasive tool, FIB-4 score. The ubiquitous prevalence of NAFLD continues to be an emerging public health challenge needing concerted attention.

# References

Adams, L., Lymp, J., St. Sauver, J., Sanderson, S., Lindor, K., Feldstein, A., & Angulo, P. (2005). The natural history of nonalcoholic fatty liver disease: A population-based cohort study. *Gastroenterology*, *129*(1), 113-121. doi:10.1053/j.gastro.2005.04.014

Adams, L., Waters, O., Knuiman, M., Elliott, R., & Olynyk, J. (2009). NAFLD as a risk factor for the development of diabetes and the metabolic syndrome: An eleven-year follow-up study. *The American Journal Of Gastroenterology*, *104*(4), 861-867. doi:10.1038/ajg.2009.67

Ahmed, M. (2006). Rheumatoid arthritis induced-fatty liver theory: One reason for global increase in prevalence of diabetes. *Medical Hypotheses*, *66*(4), 862-863. doi:10.1016/j.mehy.2005.11.021

Ahmed, M. H., & Byrne, C. D. (2005). Non-alcoholic steatohepatitis. *Chichester, John Wiley & Sons*, 279–305.

Alamanos, Y., Voulgari, P., & Drosos, A. (2006). Incidence and prevalence of rheumatoid arthritis, based on the 1987 American College of Rheumatology Criteria: A systematic review. *Seminars in Arthritis and Rheumatism*, *36*(3), 182-188. doi:10.1016/j.semarthrit.2006.08.006

Alberti, K., Eckel, R., Grundy, S., Zimmet, P., Cleeman, J., & Donato, K. …Smith. S. (2009). Harmonizing the metabolic syndrome: A joint interim statement of the international diabetes federation task force on epidemiology and prevention; National Heart, Lung, and Blood Institute; American Heart Association; World

Heart Federation; International Atherosclerosis Society; and International Association for the Study of Obesity. *Circulation*, *120*(16), 1640-1645. doi:10.1161/circulationaha.109.192644

Aletaha, D., Funovits, J., Keystone, E., & Smolen, J. (2007). Disease activity early in the course of treatment predicts response to therapy after one year in rheumatoid arthritis patients. *Arthritis & Rheumatism*, *56*(10), 3226-3235. doi:10.1002/art.22943

Aletaha, D., Neogi, T., Silman, A., Funovits, J., Felson, D., & Bingham, C., … Cohen, M. (2010). 2010 Rheumatoid arthritis classification criteria: An American College of Rheumatology/European League Against Rheumatism collaborative initiative. *Arthritis & Rheumatism*, *62*(9), 2569-2581. doi:10.1002/art.27584

Alisi, A., Da Sacco, L., Bruscalupi, G., Piemonte, F., Panera, N., De Vito, R., … Nobili, V. (2011). Mirnome analysis reveals novel molecular determinants in the pathogenesis of diet-induced nonalcoholic fatty liver disease. *Laboratory Investigation*, *91*(2), 283–293. http://doi.org/10.1038/labinvest.2010.166

Amarapurkar, D., Kamani, P., Patel, N., Gupte, P., Kumar, P., Agal, S., … Deshpande, A. (2007). Prevalence of non-alcoholic fatty liver disease: population based study. *Annals of Hepatology*, *6*(3), 161–3.

Anderson, J., Wells, G., Verhoeven, A., & Felson, D. (2000). Factors predicting response to treatment in rheumatoid arthritis: The importance of disease duration. *Arthritis & Rheumatism, 43(1) :22-9*, doi:10643696

Arena, U., Stasi, C., Mannoni, A., Benucci, M., Maddali-Bongi, S., & Cammelli, D., …

Pinzani, M. (2012). Liver stiffness correlates with methotrexate cumulative dose in patients with rheumatoid arthritis. *Digestive and Liver Disease*, *44*(2), 149-153. doi:10.1016/j.dld.2011.08.013

Argo, C., & Caldwell, S. (2009). Epidemiology and natural history of non-alcoholic steatohepatitis. *Clinics in Liver Disease*, *13*(4), 511-531. doi:10.1016/j.cld.2009.07.005

Ascha, M., Hanouneh, I., Lopez, R., Tamimi, T., Feldstein, A., & Zein, N. (2010). The incidence and risk factors of hepatocellular carcinoma in patients with nonalcoholic steatohepatitis. *Hepatology*, *51*(6), 1972-1978. doi:10.1002/hep.23527

Avouac, J., Meune, C., Chenevier-Gobeaux, C., Dieude, P., Borderie, D., & Lefevre, G., … Allanore. T. (2013). Inflammation and disease activity are associated with high circulating cardiac markers in rheumatoid arthritis independently of traditional cardiovascular risk factors. *The Journal of Rheumatology*, *41*(2), 248-255. doi:10.3899/jrheum.130713

Azad, M. (2007). Nonalcoholic Fatty Liver Disease (NAFLD) - A disease of new era. *J. Medicine*, *8*(1). doi:10.3329/jom.v8i1.1375

Babusik, P., Bilal, M., & Duris, I. (2012). Nonalcoholic fatty liver disease of two ethnic groups in Kuwait: Comparison of prevalence and risk factors. *Medical Principles and Practice*, *21*(1), 56-62. doi:10.1159/000331591

Bajaj, S., Luthra, A., Pandey, R., Kondal, D., Bhatt, S., Wasir, J., & Misra, A. (2009). A case-control study on insulin resistance, metabolic co-variants & prediction score

in non-alcoholic fatty liver disease S. *Indian Journal of Medical Research*, *129*, 285-292. Retrieved from http://medind.nic.in/iby/t09/i3/ibyt09i3p285.pdf

Bedogni, G., Miglioli, L., Masutti, F., Castiglione, A., CrocÃ¨, L., Tiribelli, C., & Bellentani, S. (2007). Incidence and natural course of fatty liver in the general population: The Dionysos study. *Hepatology*, *46*(5), 1387-1391. doi:10.1002/hep.21827

Bedogni, G., Miglioli, L., Masutti, F., Tiribelli, C., Marchesini, G., & Bellentani, S. (2005). Prevalence of and risk factors for nonalcoholic fatty liver disease: The Dionysos nutrition and liver study. *Hepatology*, *42*(1), 44-52. doi:10.1002/hep.20734

Bhambhani, N., Gutierrez, J., Cuppari, G., & Disla, E. (2006). Prevalence of non alcoholic fatty liver disease in rheumatoid arthritis. *Arthritis and Rheumatism*, *54*, S194 - S194. Retrieved from https://acr.confex.com/acr/2006/webprogram/Paper5691.html

Bialek, S., Redd, J., Lynch, A., Vogt, T., Lewis, S., Wilson, C., & Bell, B. (2008). Chronic liver disease among two American Indian patient populations in the Southwestern United States, 2000-2003. *Journal of Clinical Gastroenterology*, *42*(7), 849-854. doi:10.1097/mcg.0b013e318054492a

Breslow, N. E. 1970. A generalized Kruskal–Wallis test for comparing K samples subject to unequal patterns of censorship. *Biometrika* 57: 579–594.

Browning, J., Kumar, K., Saboorian, M., & Thiele, D. (2004). Ethnic differences in the prevalence of cryptogenic cirrhosis. *American Journal of Gastroenterology*,

*99*(2), 292-298. doi:10.1111/j.1572-0241.2004.04059.x

Browning, J., Szczepaniak, L., Dobbins, R., Nuremberg, P., Horton, J., Cohen, J., … Hobbs, H. (2004). Prevalence of hepatic steatosis in an urban population in the United States: Impact of ethnicity. *Hepatology*, *40*(6), 1387-1395. doi:10.1002/hep.20466

Caldwell, S., Harris, D., Patrie, J., & Hespenheide, E. (2002). Is NASH under diagnosed among African Americans?. *American Journal of Gastroenterology*, *97*(6), 1496-1500. doi:10.1111/j.1572-0241.2002.05795.x

Centers for Disease Control and Prevention [BMI]. (2014). Obesity and overweight for professionals: Adult. Retrieved November 28, 2014, from http://www.cdc.gov/obesity/adult/defining.html

Centers for Disease Control and Prevention RA]. (2015). Arthritis basics definition Rheumatoid Arthritis. Retrieved November 28, 2014, from http://www.cdc.gov/arthritis/basics/rheumatoid.htm

Chalasani, N., Younossi, Z., Lavine, J., Diehl, A., Brunt, E., & Cusi, K., …Arun, S. (2012). The diagnosis and management of non-alcoholic fatty liver disease: practice guideline by the American Gastroenterological Association, American Association for the Study of Liver Diseases, and American College of Gastroenterology. *Gastroenterology*, *142*(7), 1592-1609. doi:10.1053/j.gastro.2012.04.001

Chen, C., Huang, M., Yang, J., Nien, C., Yang, C., Yeh, Y., & Yueh, S. (2007). Prevalence and etiology of elevated serum alanine aminotransferase level in an

adult population in Taiwan. *Journal Of Gastroenterology And Hepatology*, *22*(9), 1482-1489. doi:10.1111/j.1440-1746.2006.04615.x

Chen, C., Huang, M., Yang, J., Nien, C., Yang, C., Yeh, Y., & Yueh, S. (2006). Prevalence and risk factors of nonalcoholic fatty liver disease in an adult population of Taiwan: Metabolic significance of nonalcoholic fatty liver disease in nonobese adults. *Journal Of Clinical Gastroenterology*, *40*(8), 745-752. doi:10.1097/00004836-200609000-00016

Chen, S., He, F., Zhou, H., Wu, H., Xia, C., & Li, Y. (2011). Relationship between nonalcoholic fatty liver disease and metabolic syndrome. *Journal of Digestive Diseases*, *12*(2), 125-130. doi:10.1111/j.1751-2980.2011.00487.x

Chen, Z., Chen, L., Dai, H., Chen, J., & Fang, L. (2008). Relationship between alanine aminotransferase levels and metabolic syndrome in nonalcoholic fatty liver disease. *J. Zhejiang Univ. Sci. B*, *9*(8), 616-622. doi:10.1631/jzus.b0720016

Chitturi, S., & Farrell, G. (2001). Etiopathogenesis of nonalcoholic steatohepatitis. *Seminars In Liver Disease*, *21*(01), 027-042. doi:10.1055/s-2001-12927

Choy, E., & Sattar, N. (2009). Interpreting lipid levels in the context of high-grade inflammatory states with a focus on rheumatoid arthritis: a challenge to conventional cardiovascular risk actions. *Annals Of The Rheumatic Diseases*, *68*(4), 460-469. doi:10.1136/ard.2008.101964

Chung, C., Oeser, A., Solus, J., Avalos, I., Gebretsadik, T., & Shintani, A. et al. (2008). Prevalence of the metabolic syndrome is increased in rheumatoid arthritis and is associated with coronary atherosclerosis. *Atherosclerosis*, *196*(2), 756-763.

doi:10.1016/j.atherosclerosis.2007.01.004

Chung, G. E., Kim, D., Kim, W., Yim, J. Y., Park, M. J., Kim, Y. J., … Lee, H. (2012). Non-alcoholic fatty liver disease across the spectrum of hypothyroidism. *Journal of Hepatology*, *57*(1), 150–156. doi: 10.1016/j.jhep.2012.02.027

Church, T., Kuk, J., Ross, R., Priest, E., Biltoff, E., & Blair, S. (2006). Association of cardiorespiratory fitness, body mass index, and waist circumference to nonalcoholic fatty liver disease. *Gastroenterology*, *130*(7), 2023-2030. doi:10.1053/j.gastro.2006.03.019

Clark, J., & Diehl, A. (2003). Defining nonalcoholic fatty liver disease: Implications for epidemiologic studies. *Gastroenterology*, *124*(1), 248-250. doi:10.1053/gast.2003.50032

Cohen, J. (1988). *Statistical power analysis for the behavioral sciences*. Hillsdale, N.J.: L. Erlbaum Associates.

Comar, K., & Sterling, R. (2006). Review article: drug therapy for non-alcoholic fatty liver disease. *Aliment Pharmacology Therapy*, *23*(2), 207-215. doi:10.1111/j.1365-2036.2006.02751.x

Corrona.org. (2015). Rheumatoid Arthritis Corrona: Data to empower. Retrieved February 20, 2015, from http://www.corrona.org/observationalregistries/rheumatoid-arthritis/

Crowson, C. S., Matteson, E. L., Davis, J. M., & Gabriel, S. E. (2013). Contribution of obesity to the rise in incidence of rheumatoid arthritis. *Arthritis care & research*, *65*(1), 71-77.

Crowson, C., & Gabriel, S. (2011). Towards improving cardiovascular risk management in patients with rheumatoid arthritis: the need for accurate risk assessment. *Annals Of The Rheumatic Diseases*, *70*(5), 719-721. doi:10.1136/ard.2010.145482

Crowson, C., Myasoedova, E., Davis, J., Matteson, E., Roger, V., & Therneau, T., … Gabriel, D. (2010). Increased prevalence of metabolic syndrome associated with rheumatoid arthritis in patients without clinical cardiovascular disease. *The Journal of Rheumatology*, *38*(1), 29-35. doi:10.3899/jrheum.100346

Curtis, J., Chen, L., Yun, H., Harrold, L., Greenberg, J., Kremer, J. (2013). Generalizability of a U.S. rheumatoid arthritis registry: A comparison of participants' vs. Non-participants' characteristics. [abstract]. *Arthritis & Rheumatism,* 2013;65 Suppl 10 :1048□DOI: 10.1002/art.2013.65.issue-s10

Curtis, J. R., Jain, A., Askling, J., Bridges, S. L., Carmona, L., Dixon, W., … Kremer, J. (2010). A comparison of patient characteristics and outcomes in selected European and US rheumatoid arthritis registries. *Seminars in Arthritis and Rheumatism*, Vol. 40, pp. 2–14. Elsevier. Retrieved from http://www.sciencedirect.com/science/article/pii/S0049017210000491

da Cunha, V., Brenol, C., Brenol, J., Fuchs, S., Arlindo, E., & Melo, I., Xavier, R. (2012). Metabolic syndrome prevalence is increased in rheumatoid arthritis patients and is associated with disease activity. *Scandinavia Journal of Rheumatology*, *41*(3), 186-191. doi:10.3109/03009742.2011.626443

Dassanayake, A., Kasturiratne, A., Rajindrajith, S., Kalubowila, U., Chakrawarthi, S., & De Silva, A. Wickremasinghe. A. (2009). Prevalence and risk factors for non-

alcoholic fatty liver disease among adults in an urban Sri Lankan population. *Journal of Gastroenterology and Hepatology*, *24*(7), 1284-1288. doi:10.1111/j.1440-1746.2009.05831.x

Day, C. P., & James, O. F. W. (1998). Steatohepatitis: A tale of two "hits"? *Gastroenterology*, 114(4), 842–845. http://doi.org/10.1016/S0016-5085(98)70599-2

Del Rincon, I., Williams, K., Stern, M., Freeman, G., & Escalante, A. (2001). High incidence of cardiovascular events in a rheumatoid arthritis cohort not explained by traditional cardiac risk factors. *Arthritis & Rheumatism*, *44*(12), 2737-2745. doi:10.1002/1529-0131(200112)44:12<2737::aid-art460>3.0.co;2-#

Dessein, P., Christian, B., & Solomon, A. (2009). Which are the determinants of dyslipidemia in Rheumatoid Arthritis and does socioeconomic status matter in this context? *The Journal of Rheumatology*, *36*(7), 1357-1361. doi:10.3899/jrheum.090288

Dessein, P., Joffe, B., Botha, A., & Moomal, Z. (2002). The acute phase response does not fully predict the presence of insulin resistance and dyslipidemia in inflammatory arthritis. *The Journal of Rheumatology, 29*(3), 462 - 466. Retrieved http://www.biomedcentral.com/content/pdf/ar428.pdf

Dessein, P., Tobias, M., & Veller, M. (2006). Metabolic syndrome and subclinical atherosclerosis in rheumatoid arthritis. *The Journal of Rheumatology*, *33*(12), 2425 -2432. Retrieved from http://www.jrheum.org/content/33/12/2425.short

Dessein, P., Woodiwiss, A., Joffe, B., & Norton, G. (2007). Aminotransferases are

associated with insulin resistance and atherosclerosis in rheumatoid arthritis. *BMC Cardiovascular Disorders*, *7*(1), 31. doi:10.1186/1471-2261-7-31

Eguchi, Y., Hyogo, H., Ono, M., Mizuta, T., Ono, N., & Fujimoto, K., Saibara. T. (2012). Prevalence and associated metabolic factors of nonalcoholic fatty liver disease in the general population from 2009 to 2010 in Japan: a multicenter large retrospective study. *Journal of Gastroenterology*, *47*(5), 586-595. doi:10.1007/s00535-012-0533-z

Erb, N., Pace, A., Douglas, K., Banks, M., & Kitas, G. (2004). Risk assessment for coronary heart disease in rheumatoid arthritis and osteoarthritis. *Scandinavia Journal Rehabilitations Medicine, 33*(5), 293-299. doi:10.1080/03009740410006899

Erickson, S. (2009). Nonalcoholic fatty liver disease. *Journal of Lipid Research*, *50*, S4126. doi:10.1194/jlr.R800089-JLR200

Fabbrini, E., Sullivan, S., & Klein, S. (2009). Obesity and nonalcoholic fatty liver disease: Biochemical, metabolic, and clinical implications. *Hepatology*, *51*(2), 679-689. doi:10.1002/hep.23280

Fan, J., Li, F., Cai, X., Peng, Y., Ao, Q., & Gao, Y. (2007). The importance of metabolic factors for the increasing prevalence of fatty liver in Shanghai factory workers. *Journal of Gastroenterology and Hepatology*, *22*(5), 663-668. doi:10.1111/j.1440-1746.2007.04892.x

Fan, J., Zhu, J., Li, X., Chen, L., Li, L., & Dai, F., Chen, S. (2005). Prevalence of and risk factors for fatty liver in a general population of Shanghai, China. *Journal of*

*Hepatology*, *43*(3), 508-514. doi:10.1016/j.jhep.2005.02.042

Farrell, G., & Larter, C. (2006). Nonalcoholic fatty liver disease: From steatosis to cirrhosis. *Hepatology*, *43*(S1), S99-S112. doi:10.1002/hep.20973

Ferraz-Amaro, I., González-Juanatey, C., López-Mejias, R., Riancho-Zarrabeitia, L., & González-Gay, M. (2013). Metabolic syndrome in rheumatoid arthritis. *Mediators of Inflammation*, *2013*, 1-11. http://dx.doi.org/10.1155/2013/710928

Field, A. (2009). *Discovering statistics using SPSS*. (3rd ed.). Los Angeles. Thousand Oaks, Calif.: SAGE Publications.

Fischer, G., Bialek, S., Homan, C., Livingston, S., & McMahon, B. (2009). Chronic liver disease among Alaska-Native People, 2003 - 2004. *The American Journal of Gastroenterology*, *104*(2), 363-370. doi:10.1038/ajg.2008.57

Fisher, M., Furer, V., Hochberg, M., Greenberg, J., Kremer, J., & Curtis, J., Solomon, D. (2012). Malignancy validation in a United States registry of rheumatoid arthritis patients. *BMC Musculoskeletal Disorders*, *13*(1), 85. doi:10.1186/1471-2474-13-85

Forthofer, R., Lee, E., Hernandez, M., & Forthofer, R. (2007). *Biostatistics*. Amsterdam: Elsevier Academic Press.

Frankfort-Nachmias, C & Nachmias, D. (2008). *Research methods in the social sciences*. New York: St. Martin's Press.

Frith, J., Day, C., Henderson, E., Burt, A., & Newton, J. (2009). Non-alcoholic fatty liver disease in older people. *Gerontology*, *55*(6), 607-613. doi:10.1159/000235677

Furst, D. E., Chang, H., Greenberg, J. D., Ranganath, V. K., Reed, G., Ozturk, Z. E., Kremer, J. (2009). Prevalence of low hemoglobin levels and associations with other disease parameters in rheumatoid arthritis patients: evidence from the CORRONA registry. Clinical Experiential Rheumatology, 27(4), 560–6. Retrieved from http://www.clinexprheumatol.org/article.asp?a=39

Gabriel, S. (2001). The epidemiology of rheumatoid arthritis. *Rheumatic Disease Clinics of North America, 27*(2), 269 - 281. Retrieved from http://www.sciencedirect.com/science/article/pii/S0889857X05702015

Gabriel, S., & Michaud, K. (2009). Epidemiological studies in incidence, prevalence, mortality, and comorbidity of the rheumatic diseases. *Arthritis Research & Therapy*, *11*(3), 229. doi:10.1186/ar2669

Gehan, E. 1965. A generalized Wilcoxon test for comparing arbitrarily singly censored samples. *Biometrika,* 52: 203–223.

Gerli, R., Sherer, Y., Vaudo, G., Schillaci, G., Gilburd, B., & Giordano, A., … Shoenfeld. Y. (2005). Early atherosclerosis in rheumatoid arthritis: Effects of smoking on thickness of the carotid artery intima media. *Annals of The New York Academy Of Sciences*, *1051*(1), 281-290. doi:10.1196/annals.1361.069

Grayson, D. A. (1987). Confounding confounding. *American Journal of Epidemiology*, *126*(3), 546–553. Retrieved from http://aje.oxfordjournals.org/content/126/3/546

Greenberg, J., Kremer, J., Curtis, J., Hochberg, M., Reed, G., & Tsao, P., … Solomon. D. (2010). Tumor necrosis factor antagonist use and associated risk reduction of cardiovascular events among patients with rheumatoid arthritis. *Annals of The*

*Rheumatic Diseases*, *70*(4), 576-582. doi:10.1136/ard.2010.129916

Grundy, S., Cleeman, J., Daniels, S., Donato, K., Eckel, R., Franklin, B., …Costa, F. (2005). Diagnosis and management of the metabolic syndrome: An American Heart Association/National Heart, Lung, and Blood Institute scientific statement: Executive summary. *Circulation*, *112*(17), e285-e290. doi:10.1161/circulationaha.105.169405

Gupte, P., Amarapurkar, D., Agal, S., Baijal, R., Kulshrestha, P., & Pramanik, S., … Amarapurkar. A. (2004). Non-alcoholic steatohepatitis in type 2 diabetes mellitus. *Journal of Gastroenterology And Hepatology*, *19*(8), 854-858. doi:10.1111/j.1440-1746.2004.03312.x

Hamaguchi, M. (2005). The metabolic syndrome as a predictor of nonalcoholic fatty liver disease. *Annals of Internal Medicine*, *143*(10), 722. doi:10.7326/0003-4819-143-10-200511150-00009

Hamaguchi, M. (2007). Nonalcoholic fatty liver disease is a novel predictor of cardiovascular disease. *WJG*, *13*(10), 1579. doi:10.3748/wjg.v13.i10.1579

Hashimoto, E., Yatsuji, S., Kaneda, H., Yoshioka, Y., Taniai, M., Tokushige, K., Shiratori, K. (2005). The characteristics and natural history of Japanese patients with nonalcoholic fatty liver disease. *Hepatology Research*, *33*(2), 72-76. doi:10.1016/j.hepres.2005.09.007

Hernán, M. A., Hernández-Díaz, S., Werler, M. M., & Mitchell, A. A. (2002). Causal knowledge as a prerequisite for confounding evaluation: An application to birth defects epidemiology. *American Journal of Epidemiology*, *155*(2), 176–184.

http://doi.org/10.1093/aje/155.2.176

Hofferth, S. (2005). Secondary data analysis in family research. *Journal of Marriage and Family*, *67*(4), 891-907. doi:10.1111/j.1741-3737.2005.00182.x

Hu, X., Huang, Y., Bao, Z., Wang, Y., Shi, D., & Liu, F., … Y, X. (2012). Prevalence and factors associated with nonalcoholic fatty liver disease in shanghai work-units. *BMC Gastroenterology*, *12*(1), 123. doi:10.1186/1471-230x-12-123

Hui, A., Wong, V., Chan, H., Liew, C., Chan, J., Chan, F., & Sung, J. (2005). Histological progression of non-alcoholic fatty liver disease in Chinese patients. *Aliment Pharmacology Therapy*, *21*(4), 407-413. doi:10.1111/j.1365-2036.2005.02334.x

Jakobsen, M., Berentzen, T., Sorensen, T., & Overvad, K. (2007). Abdominal obesity and fatty liver. *Epidemiologic Reviews*, *29*(1), 77-87. doi:10.1093/epirev/mxm002

Jimba, S., Nakagami, T., Takahashi, M., Wakamatsu, T., Hirota, Y., Iwamoto, Y., & Wasada, T. (2005). Prevalence of non-alcoholic fatty liver disease and its association with impaired glucose metabolism in Japanese adults. *Diabetic Medicine*, *22*(9), 1141-1145. doi:10.1111/j.1464-5491.2005.01582.x

Kagansky, N., Levy, S., Keter, D., Rimon, E., Taiba, Z., & Fridman, Z., … Malnick, S. (2004). Non-alcoholic fatty liver disease - a common and benign finding in octogenarian patients. *Liver International*, *24*(6), 588-594. doi:10.1111/j.1478-3231.2004.0969.x

Kallwitz, E., Kumar, M., Aggarwal, R., Berger, R., Layden-Almer, J., Gupta, N., & Cotler, S. (2008). Ethnicity and nonalcoholic fatty liver disease in an obesity

clinic: The impact of Triglycerides. *Digestive Disease Science*, *53*(5), 1358-1363. doi:10.1007/s10620-008-0234-x

Karimi, M., Mazloomzadeh, S., Kafan, S., & Amirmoghadami, H. (2011). The frequency of metabolic syndrome in women with rheumatoid arthritis and in controls. *International Journal of Rheumatic Diseases*, *14*(3), 248-254. doi:10.1111/j.1756-185x.2011.01595.x

Karvounaris, S., Sidiropoulos, P., Papadakis, J., Spanakis, E., Bertsias, G., & Kritikos, H., … Boumpas, D. (2006). Metabolic syndrome is common among middle-to-older aged Mediterranean patients with rheumatoid arthritis and correlates with disease activity: a retrospective, cross-sectional, controlled, study. *Annals of Rheumatic Diseases*, *66*(1), 28-33. doi:10.1136/ard.2006.053488

Kelley, D., McKolanis, T., Kuller, L., & Kalhan. C, S. (2003). Fatty liver in type 2 diabetes mellitus: relation to regional adiposity, fatty acids, and insulin resistance. *American Journal of Physiology-Endocrinology and Metabolism*, *285*(4), 906 - 916. Retrieved from http://ajpendo.physiology.org/content/285/4/E906.short

Kichian, K., Mclean, R., Gramlich, L., Bailey, R., & Bain, V. (2003). Nonalcoholic fatty liver disease in patients investigated for elevated liver enzymes. *Canadian Journal of Gastroenterology*, *17*(1), 38-42. Retrieved from http://europepmc.org/abstract/med/12560853

Kim, H., Kim, H., Lee, K., Kim, D., Kim, S., & Ahn, C. et al. (2004). Metabolic significance of nonalcoholic fatty liver disease in nonobese, nondiabetic adults. *Archives of Internal Medicine*, *164*(19), 2169. doi:10.1001/archinte.164.19.2169

Kitade, M., Yoshiji, H., Kojima, H., Ikenaka, Y., Noguchi, R., & Kaji, K., … Takemi, F. (2008). Neovascularization and oxidative stress in the progression of non-alcoholic steatohepatitis. *Molecular Medicine Reports*, doi:10.3892/mmr.1.4.543

Kleiner, D., Brunt, E., Van Natta, M., Behling, C., Contos, M., & Cummings, O., … Unalp-Arida, A. (2005). Design and validation of a histological scoring system for nonalcoholic fatty liver disease. *Hepatology*, *41*(6), 1313-1321. doi:10.1002/hep.20701

Kneeman, J., Misdraji, J., & Corey, K. (2011). Secondary causes of nonalcoholic fatty liver disease. *Therapeutic Advances in Gastroenterology*, *5*(3), 199-207. doi:10.1177/1756283x11430859

Kotronen, A., Yki-Järvinen, H., Männistö,S., Saarikoski, L., Korpi-Hyövälti, E., & Oksa, H., … Tuomilehto, J. (2010). Non-alcoholic and alcoholic fatty liver disease - two diseases of affluence associated with the Metabolic syndrome and type 2 diabetes: the FIN-D2D Survey. *BMC Public Health*, *10*(1), 237. doi:10.1186/1471-2458-10-237

Kwon, Y., Oh, S., Hwang, S., Lee, C., Kwon, H., & Chung, G. (2012). Association of nonalcoholic fatty liver disease with components of metabolic syndrome according to body mass index in Korean adults. *The American Journal of Gastroenterology*, *107*(12), 1852-1858. doi:10.1038/ajg.2012.314

Lonardo, A., Bellentani, S., Argo, C. K., Ballestri, S., Byrne, C. D., Caldwell, S. H., ... & Targher, G. (2015). Epidemiological modifiers of non-alcoholic fatty liver disease: focus on high-risk groups. *Digestive and Liver Disease,* Dec;47(12):997-

1006. doi: 10.1016/j.dld.2015.08.004.

Lazo, M., & Clark, J. (2008). The Epidemiology of nonalcoholic fatty liver disease: A global perspective. *Seminars in Liver Disease*, *28*(04), 339-350. doi:10.1055/s-0028-1091978

Lazo, M., Hernaez, R., Bonekamp, S., Kamel, I., Brancati, F., Guallar, E., & Clark, J. (2011). Non-alcoholic fatty liver disease and mortality among US adults: prospective cohort study. *BMJ*, *343,* (nov182), d6891-d6891. doi:10.1136/bmj.d6891

Lazo, M., Hernaez, R., Eberhardt, M., Bonekamp, S., Kamel, I., & Guallar, E., … Clark, J. (2013). Prevalence of nonalcoholic fatty liver disease in the United States: The Third National Health and Nutrition Examination Survey, 1988-1994. *American Journal of Epidemiology*, *178*(1), 38-45. doi:10.1093/aje/kws448

Lee, J., Kim, K., Lee, S., Yu, E., Lim, Y., & Lee, H., …Shu, D. (2007). Prevalence and risk factors of non-alcoholic fatty liver disease in potential living liver donors in Korea: A review of 589 consecutive liver biopsies in a single center. *Journal of Hepatology*, *47*(2), 239-244. doi:10.1016/j.jhep.2007.02.007

Lee, Y., Lee, H., Lee, J., Shin, Y., & Shim, J. (2010). Association between serum uric acid and non-alcoholic fatty liver disease in Korean adults. *Clinical Chemistry and Laboratory Medicine*, *48*(2). doi:10.1515/cclm.2010.037

Leite, N., Salles, G., Araujo, A., Villela-Nogueira, C., & Cardoso, C. (2009). Prevalence and associated factors of non-alcoholic fatty liver disease in patients with type-2 diabetes mellitus. *Liver International*, *29*(1), 113-119. doi:10.1111/j.1478-

3231.2008.01718.x

Li, H., Wang, Y., Tan, K., Zeng, L., Liu, F., & Zhou, T., … Tang, H. (2009). Prevalence and risk factors of fatty liver disease in Chengdu, Southwest China. *Hepatobiliary Pancreatic Disease International*, *8*(4), 377 - 882. Retrieved http://www.hbpdint.com/EN/article/downloadArticleFile.do?attachType=PDF&id=3050

Lim, J., Mietus-Snyder, M., Valente, A., Schwarz, J., & Lustig, R. (2010). The role of fructose in the pathogenesis of NAFLD and the metabolic syndrome. *Nat Rev Gastroenterology Hepatology*, *7*(5), 251-264. doi:10.1038/nrgastro.2010.41

Lin, Y., Lo, H., & Chen, J. (2005). Sonographic fatty liver, overweight and ischemic heart disease. *World Journal of Gastroenterology*, *11*(3), 4838. Retrieved from https://portalsaudebrasil.com/artigospsb/obes096.pdf

Lin, Y., Chou, S., Huang, P., & Chiou, H. (2011). Risk factors and predictors of non-alcoholic fatty liver disease in Taiwan. *Annals of Hepetology*, 10(2), 125–32. Retrieved from http://www.ncbi.nlm.nih.gov/pubmed/21502673

Lizardi-Cervera, J., Laparra, D., Chavez-Tapia, N., Ostos, M., & Esquivel, M. (2006). Prevalence of NAFLD and metabolic syndrome in asymtomatics subjects. *Revista de Gastroenterologia de Mexico*, *71*(4), 453-9. Retrieved http://www.ncbi.nlm.nih.gov/pubmed/17542278

Loomba, R., & Sanyal, A. (2013). The global NAFLD epidemic. *Nature Review Gastroenterology Hepatology*, *10*(11), 686-690. doi:10.1038/nrgastro.2013.171

Mallet, V., Dhalluin-Vneier, V., Roussin, C., Bourliere, M., Pettinelli, M., & Giry, C., …

Pol, S. (2009). The accuracy of the FIB-4 index for the diagnosis of mild fibrosis in chronic hepatitis B. *Alimentary Pharmacology & Therapeutics*, *29*(4), 409-415. doi:10.1111/j.1365-2036.2008.03895.x

Marchesini, G. (2003). Nonalcoholic fatty liver, steatohepatitis, and the metabolic syndrome. *Hepatology*, *37*(4), 917-923. doi:10.1053/jhep.2003.50161

McPherson, S., Stewart, S., Henderson, E., Burt, A., & Day, C. (2010). Simple non-invasive fibrosis scoring systems can reliably exclude advanced fibrosis in patients with non-alcoholic fatty liver disease. *Gut*, *59*(9), 1265-1269. doi:10.1136/gut.2010.216077

Mohan, V., Farooq, S., Deepa, M., Ravikumar, R., & Pitchumoni, C. (2009). Prevalence of non-alcoholic fatty liver disease in urban south Indians in relation to different grades of glucose intolerance and metabolic syndrome. *Diabetes Research and Clinical Practice*, *84*(1), 84-91. doi:10.1016/j.diabres.2008.11.039

Mohanty, S., Troy, T., Huo, D., O'Brien, B., Jensen, D., & Hart, J. (2009). Influence of ethnicity on histological differences in non-alcoholic fatty liver disease. *Journal of Hepatology*, *50*(4), 797-804. doi:10.1016/j.jhep.2008.11.017

Myasoedova, E., Davis, J., Crowson, C., & Gabriel, S. (2010). Epidemiology of rheumatoid arthritis: Rheumatoid arthritis and mortality. *Current Rheumatology Reports*, *12*(5), 379-385. doi:10.1007/s11926-010-0117-y

National Heart, Lung, and Blood Institute, National Institutes of Health., [NCEP] (2002). *Third report of the National Cholesterol Education Program (NCEP) expert panel on detection, evaluation, and treatment of high blood cholesterol in adults*

*(ATP III), executive summary. Washington, DC: National Heart, Lung, and Blood Institute, National Institutes of Health.* Retrieved October 20,2014, from http://www.nhlbi.nih.gov/guidelines/cholesterol/atp3xsum.pdf

Nascimbeni, F., Pais, R., Bellentani, S., Day, C. P., Ratziu, V., Loria, P., & Lonardo, A. (2013). From NAFLD in clinical practice to answers from guidelines. *Journal of Hepatology*, *59*(4), 859–871. http://doi.org/10.1016/j.jhep.2013.05.044

Nurmohamed, M. (2010). The increased cardiovascular risk in rheumatoid arthritis: when does it start?. *Arthritis Research & Therapy*, *12*(5), 140. doi:10.1186/ar3126

Oh, S., Cho, Y., Kang, M., Yoo, T., Park, J., & Kim, H., … Kim, B. (2006). The association between increased alanine aminotransferase activity and metabolic factors in nonalcoholic fatty liver disease. *Metabolism*, *55*(12), 1604-1609. doi:10.1016/j.metabol.2006.07.021

Omagari, K., Kadokawa, Y., Masuda, J., Egawa, I., Sawa, T., & Hazama, H., … Hayashida, K.(2002). Fatty liver in non-alcoholic non-overweight Japanese adults: Incidence and clinical characteristics. *Journal of Gastroenterology and Hepatology*, *17*(10), 1098-1105. doi:10.1046/j.1440-1746.2002.02846.x

Ong, J., Pitts, A., & Younossi, Z. (2008). Increased overall mortality and liver-related mortality in non-alcoholic fatty liver disease. *Journal of Hepatology*, *49*(4), 608-612. doi:10.1016/j.jhep.2008.06.018

Park, S., Jeon, W., Kim, S., Kim, H., Park, D., & Cho, Y., … Kim, B. (2006). Prevalence and risk factors of non-alcoholic fatty liver disease among Korean adults. *Journal of Gastroenterology and Hepatology*, *21*(1), 138-143. doi:10.1111/j.1440-

1746.2005.04086.x

Preiss, D., & Sattar, N. (2008). Non-alcoholic fatty liver disease: an overview of prevalence, diagnosis, pathogenesis and treatment considerations. *Clinical Science*, *115*(5), 141. doi:10.1042/cs20070402

Puppala, J., Siddapuram, S. P., Akka, J., & Munshi, A. (2013). Genetics of nonalcoholic Fatty liver disease: an overview. *Journal of Genetics and Genomics* 40(1), 15–22. http://doi.org/10.1016/j.jgg.2012.12.001

Ray, K. (2013). NAFLD - the next global epidemic. *Nature Review Gastroenterology Hepatology*, *10*(11), 621-621. doi:10.1038/nrgastro.2013.197

Sahebari, M., Goshayeshi, L., Mirfeizi, Z., Rezaieyazdi, Z., Hatef, M., & Ghayour-Mobarhan, M., … Ferns, G. (2011). Investigation of the association between metabolic syndrome and disease activity in rheumatoid arthritis. *The Scientific World Journal*, *11*, 1195-1205. doi:10.1100/tsw.2011.111

Sakthiswary, R., Chan, G., Koh, E., Leong, K., & Thong, B. (2014). Methotrexate-associated nonalcoholic fatty liver disease with transaminitis in rheumatoid arthritis. *The Scientific World Journal*, *2014*, 1-5. doi:10.1155/2014/823763

Shah, A., Lydecker, A., Murray, K., Tetri, B., Contos, M., Sanyal, A., & NASH Clinical Research. (2009). Use of the Fib-4 Index for non-invasive evaluation of fibrosis in nonalcoholic fatty liver disease. *Clinical Gastroenterology And Hepatology: The Official Clinical Practice Journal Of The American Gastroenterological Association*, *7*(10), 1104. Retrieved form http://www.ncbi.nlm.nih.gov/pmc/articles/PMC3079239/

Shah, A., Lydecker, A., Murray, K., Tetri, B., Contos, M., & Sanyal, A. (2009). Comparison of noninvasive markers of fibrosis in patients with nonalcoholic fatty liver disease. *Clinical Gastroenterology and Hepatology*, *7*(10), 1104-1112. doi:10.1016/j.cgh.2009.05.033

Shen, L., Fan, J., Shao, Y., Zeng, M., Wang, J., & Luo, G., … Chen, S. (2003). Prevalence of nonalcoholic fatty liver among administrative officers in Shanghai: an epidemiological survey. *World Journal of Gastroenterology*, *9*(5), 1. Retrieved from http://www.ncbi.nlm.nih.gov/pmc/articles/PMC3079239/

Shi, X., Wei, Q., He, S., Tao, Y., Sun, J., & Niu, J. (2011). Epidemiology and analysis on risk factors of non-infectious chronic diseases in adults in northeast China. *Journal of Jilin University (Medicine Edition)*, *37*(2), 379 - 384. Retrieved http://en.cnki.com.cn/Article_en/CJFDTOTAL-BQEB201102068.htm

Singh, S., Nayak, S., Swain, M., Malik, R., Agrawal, O., Meher, C., & Rao, M. (2003). Prevalence of nonalcoholic fatty liver disease in coastal eastern India: a preliminary ultrasonographic survey. *Tropical Gastroenterology: Official Journal of the Digestive Diseases Foundation,*, *25*(2), 76 - 79. Retrieved from http://europepmc.org/abstract/MED/15471321

Smith, A., Ayanian, J., Covinsky, K., Landon, B., McCarthy, E., Wee, C., & Steinman, M. (2011). Conducting high-value secondary dataset analysis: an introductory guide and resources. *Journal of General Internal Medicine*, *26*(8), 920-929. doi:10.1007/s11606-010-1621-5

Smits, M., Ioannou, G., Boyko, E., & Utzschneider, K. (2013). Non-alcoholic fatty liver

disease as an independent manifestation of the metabolic syndrome: Results of a US national survey in three ethnic groups. *Journal of Gastroenterology and Hepatology*, *28*(4), 664-670. doi:10.1111/jgh.12106

Solga, S., Clark, J., Alkhuraishi, A., Torbenson, M., Tabesh, A., & Schweitzer, M., … Magnuson, T. (2005). Race and comorbid factors predict nonalcoholic fatty liver disease histopathology in severely obese patients. *Surgery for Obesity and Related Diseases*, *1*(1), 6-11. doi:10.1016/j.soard.2004.12.006

Solomon, D. (2003). Cardiovascular morbidity and mortality in women diagnosed with Rheumatoid Arthritis. *Circulation*, *107*(9), 1303-1307. doi:10.1161/01.cir.0000054612.26458.b2

Soresi, M., Noto, D., Cefalù, A., Martini, S., Vigna, G., & Fonda, M., Averna, M. (2012). Nonalcoholic fatty liver and metabolic syndrome in Italy: results from a multicentric study of the Italian Arteriosclerosis society. *Acta Diabetologica*, *50*(2), 241-249. doi:10.1007/s00592-012-0406-1

SPSS Statistics, Graduate Pack (SPSS Version 22.0) Package for Social Sciences. Armonk, NY: IBM Corp.

Stepanova, M., & Younossi, Z. (2012). Independent association between nonalcoholic fatty liver disease and cardiovascular disease in the U.S. Population. *Clinical Gastroenterology and Hepatology*, *10*(6), 646-650. doi:10.1016/j.cgh.2011.12.039

Suzuki, A., Angulo, P., Lymp, J., St. Sauver, J., Muto, A., Okada, T., & Lindor, K. (2004). Chronological development of elevated aminotransferases in a

nonalcoholic population. *Hepatology*, *41*(1), 64-71. doi:10.1002/hep.20543

Svenson, K., Lundqvist, G., Wide, L., & Hallgren, R. (1987). Impaired glucose handling in active rheumatoid arthritis: Effects of corticosteroids and antirheumatic treatment. *Metabolism*, *36*(10), 944-948. doi: 10.1016/0026-0495(87)90129-6

Szklo, M., & Nieto, F. (2014). *Epidemiology*. Sudbury, Mass.: Jones and Bartlett Publishers.

Tabachnick, B., & Fidell, L. (2012). *Using multivariate statistics* (6th ed.). Harlow, Essex: Pearson Education.

Targher, G., Bertolini, L., Padovani, R., Rodella, S., Tessari, R., & Zenari, L., … Arcaro, G. (2007). Prevalence of nonalcoholic fatty liver disease and its association with cardiovascular disease among type 2 diabetic patients. *Diabetes Care*, *30*(5), 1212-1218. doi:10.2337/dc06-2247

Thoma, C., Day, C., & Trenell, M. (2012). Lifestyle interventions for the treatment of non-alcoholic fatty liver disease in adults: A systematic review. *Journal of Hepatology*, *56*(1), 255-266. doi:10.1016/j.jhep.2011.06.010

Tobón, G., Youinou, P., & Saraux, A. (2010). The environment, geo-epidemiology, and autoimmune disease: Rheumatoid arthritis. *Journal of Autoimmunity*, *35*(1), 10-14. doi:10.1016/j.jaut.2009.12.009

Toms, T., Panoulas, V., John, H., Douglas, K., & Kitas, G. (2009). Methotrexate therapy associates with reduced prevalence of the metabolic syndrome in rheumatoid arthritis patients over the age of 60- more than just an anti-inflammatory effect?: A cross sectional study. *Arthritis Research & Therapy*, *11*(4), R110.

doi:10.1186/ar2765

Torres, D., Williams, C., & Harrison, S. (2012). Features, diagnosis, and treatment of nonalcoholic fatty liver disease. *Clinical Gastroenterology and Hepatology*, *10*(8), 837-858. doi:10.1016/j.cgh.2012.03.011

Tran, T., Changsri, C., Shackleton, C., Poordad, F., Nissen, N., & Colquhoun, S., … Martin, P. (2006). Living donor liver transplantation: Histological abnormalities found on liver biopsies of apparently healthy potential donors. *Journal of Gastroenterology and Hepatology*, *21*(2), 381-383. doi:10.1111/j.1440-1746.2005.03968.x

Tsuneto, A., Hida, A., Sera, N., Imaizumi, M., Ichimaru, S., & Nakashima, E., … Akahoshi. M. (2010). Fatty liver incidence and predictive variables. *Hypertension Research*, *33*(6), 638-643. doi:10.1038/hr.2010.45

Underwood Ground, K. (1982). Liver pathology in aircrew. *Aviation, Space, and Environmental Medicine*. Retrieved from http://psycnet.apa.org/psycinfo/1982-10212-001

Utzschneider, K., & Khan, S. (2006). The role of insulin resistance in nonalcoholic fatty liver disease. *The Journal of Clinical Endocrinology & Metabolism*, *91*(12), 4753 - 4761. Retrieved from http://press.endocrine.org/doi/abs/10.1210/jc.2006-0587

Vallet-Pichard, A., Mallet, V., Nalpas, B., Verkarre, V., Nalpas, A., & Dhalluin-Venier, V., … Pol, S. (2007). FIB-4: An inexpensive and accurate marker of fibrosis in HCV infection comparison with liver biopsy and fibrotest. *Hepatology*, *46*(1), 32-36. doi:10.1002/hep.21669

Vanni, E., Bugianesi, E., Kotronen, A., De Minicis, S., Yki-Jarvinen, H., Svegliati-Baroni, G. (2010). From the metabolic syndrome to NAFLD or vice versa?. *Digestive And Liver Disease*, *42*(5), 320-330. doi:10.1016/j.dld.2010.01.016

Vernon, G., Baranova, A., & Younossi, Z. (2011). Systematic review: the epidemiology and natural history of non-alcoholic fatty liver disease and non-alcoholic steatohepatitis in adults. *Alimentary Pharmacology & Therapeutics*, *34*(3), 274-285. doi:10.1111/j.1365-2036.2011.04724.x

Visser, K., & Heijde, V. (2009). Risk and management of liver toxicity during methotrexate treatment in rheumatoid and psoriatic arthritis: a systematic review of the literature. *Clinical and Experimental Rheumatology*, *27*, 1017-1025. Retrieved from http://www.clinexprheumatol.org/article.asp?a=75

Wagenknecht,L., Scherzinger,A., Stamm, E., Hanley, A., Norris, J., & Chen, Y., … Rotter, J. (2009). Correlates and heritability of nonalcoholic fatty liver disease in a minority cohort. *Obesity*. doi:10.1038/oby.2009.4

Weston, S., Leyden, W., Murphy, R., Bass, N., Bell, B., Manos, M., & Terrault, N. (2005). Racial and ethnic distribution of nonalcoholic fatty liver in persons with newly diagnosed chronic liver disease. *Hepatology*, *41*(2), 372-379. doi:10.1002/hep.20554

Whalley, S., Puvanachandra, P., Desai, A., & Kennedy, H. (2007). Hepatology outpatient service provision in secondary care: a study of liver disease incidence and resource costs. *Clinical Medicine*, *7*(2), 119-124. doi:10.7861/clinmedicine.7-2-119

Williams, C., Stengel, J., Asike, M., Torres, D., Shaw, J., & Contreras, M., … Harrison, S. (2011). Prevalence of nonalcoholic fatty liver disease and nonalcoholic steatohepatitis among a largely middle-aged population utilizing ultrasound and liver biopsy: a prospective study. *Gastroenterology*, *140*(1), 124-131. doi:10.1053/j.gastro.2010.09.038

Wilson, M. (2015). Assessing model adequacy in proportional hazard regression. *Support.sas.com/resources/papers/proceedings13/431-2013*. Retrieved February 9, 2015, from http://support.sas.com/resources/papers/proceedings13/431-2013.pdf

Wolfe, F., & Michaud, K. (2012). Effect of body mass index on mortality and clinical status in rheumatoid arthritis. *Arthritis Care & Research*, *64*(10), 1471-1479.

Wong, V., Chu, W., Wong, G., Chan, R., Chim, A., & Ong, A., … Woo, J. (2011). Prevalence of non-alcoholic fatty liver disease and advanced fibrosis in Hong Kong Chinese: a population study using proton-magnetic resonance spectroscopy and transient elastography. *Gut*, *61*(3), 409-415. doi:10.1136/gutjnl-2011-300342

Xu, C., Yu, C., Ma, H., Xu, L., Miao, M., & Li, Y. (2013). Prevalence and risk factors for the development of nonalcoholic fatty liver disease in a nonobese Chinese population: the Zhejiang Zhenhai study. *The American Journal of Gastroenterology*, *108*(8), 1299-1304. doi: 10.1038/ajg.2013.104

Yan, J., Xie, W., Ou, W., Zhao, H., Wang, S., & Wang, J., … Chen, J. (2013). Epidemiological survey and risk factors analysis of fatty liver disease of adult residents, Beijing, China. *Journal of Gastroenterology and Hepatology*, n/a-n/a.

doi:10.1111/jgh.12290

Zelber-Sagi, S., Lotan, R., Shlomai, A., Webb, M., Harrari, G., & Buch, A., … Oren, R. (2012). Predictors for incidence and remission of NAFLD in the general population during a seven-year prospective follow-up. *Journal of Hepatology*, *56*(5), 1145-1151. doi:10.1016/j.jhep.2011.12.011

Zelber-Sagi, S., Nitzan-Kaluski, D., Goldsmith, R., Webb, M., Blendis, L., Halpern, Z., & Oren, R. (2007). Long term nutritional intake and the risk for non-alcoholic fatty liver disease (NAFLD): A population based study. *Journal of Hepatology*, *47*(5), 711-717. doi:10.1016/j.jhep.2007.06.020

Zhou, Y. (2007). Prevalence of fatty liver disease and its risk factors in the population of South China. *WJG*, *13*(47), 6419. doi:10.3748/wjg.13.6419

Zhou, Y., Li, Y., Nie, Y., Huang, C., & Cao, C. (2012). Natural course of nonalcoholic fatty liver disease in southern China: A prospective cohort study. *Journal of Digestive Diseases*, *13*(3), 153-160. doi:10.1111/j.1751-2980.2011.00571.x.