An Examination of the Relationship of ABO Blood Group and Lifespan in a Hospitalized Population in the Southeastern United States

A dissertation submitted in partial fulfillment of the requirements for the degree of Doctor of Philosophy at Virginia Commonwealth University

by

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List of Abbreviations

ANCOVA = Analysis of Covariance

ANOVA = Analysis of Variance

CDC = Centers for Disease Control and Prevention

CI = Confidence Interval

CV = Covariate

RBC = Red Blood Cells

DHF = Dengue Hemorrhagic Fever

DV = Dependent Variable

DVT = Deep Vein Thrombosis

HIV = Human Immunodeficiency Virus

ICV = Intracranial Volume

IV = Independent Variable

MI = Myocardial Infarction

MS_{effect} = Mean Square Effect

MS_{error} = Mean Square Error

SARS = Severe Acute Respiratory Syndrome

SD = Standard Deviation

SS = Sums of Squares

UNCH = University of North Carolina Hospitals

VCUMC = Virginia Commonwealth University Medical Center

VWD = von Willebrand Disease

VWF = von Willebrand Factor

WFUBMC = Wake Forest University Baptist Medical Center

Abstract

AN EXAMINATION OF THE RELATIONSHIP OF ABO BLOOD GROUP AND LIFESPAN IN A HOSPITALIZED POPULATION IN THE SOUTHEASTERN UNITED STATES

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A dissertation submitted in partial fulfillment of the requirements for the degree of Doctor of Philosophy at Virginia Commonwealth University.

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The clinical significance of ABO blood group is evident and universally accepted with regards to blood transfusion and pregnancy; however, the importance of ABO blood group as it relates to other diseases or disorders and overall mortality is not fully understood by the scientific community. Many studies have suggested associations between blood groups and disease, but consensus has not been reached regarding overall survival or longevity. This epidemiological, retrospective review of ABO blood group and age at the time of death in a hospitalized population in the Southeastern United States is the first multi-site study to examine this relationship.

The study population was 56% male, 63.4% White, 31.0% Black and 2.1% Hispanic. Over half (61.1%) of the population had been transfused with red blood cells within one year of death. Overall, group O (46.6%) was the most prevalent ABO blood group, followed by group

A (36.8%), Group B (12.9%) and group AB (3.7%). The population exhibited differences in the frequencies of ABO blood groups across the races, with the Hispanic population having the highest prevalence of group O (71.2%) and the Black and Asian populations having higher frequencies of group B (22.2% and 23.1% respectively) when compared to the overall population distribution. Lifespan ranged from 0 to 110 with a mean age at death of 58.7 years. While some differences in the mean age at death were noted across ABO blood groups, the main effect of ABO blood group on lifespan did not reach statistical significance when controlling for race, gender and history of red blood cell transfusion. These results contradict other studies that found an association between a particular ABO blood group and lifespan. Future work should consider including cause of death or primary disease as potential confounders and targeting expanded populations over a wider geographic area to increase generalizability and racial diversity.

Chapter One: Introduction

Prior to the discovery of the ABO blood groups, high rates of mortality resulted from using animal and human blood sources for transfusion due to lack of knowledge of the differences in blood composition observed between animals and humans and within the human population (Giangrande, 2000). Since the discovery of the ABO blood groups in 1900 (Landsteiner, 1900), humans and many other primates can be typed for ABO blood group based on the presence or absence of surface antigens on the red blood cells (RBC). The four human ABO groups, A, B, AB, and O, are based on the inheritance of genes on chromosome 9. A person's ABO phenotype does not change as a result of environmental influences during his or her life except in cases of bone marrow transplantation or certain disease states.

Previous research has revealed associations between ABO blood groups and disease (Albert, 1996; Hein, Suadicani, & Gyntelberg, 1998; O'Donnell & Laffan, 2001; Suadicani, Hein, & Gyntelberg, 2007). Some of these associations include malignancy, thrombosis, peptic ulcers, bleeding and infectious diseases (Garratty, 2000). Although ABO blood group is determined by inheritance, natural selection may have influenced the current frequencies of ABO types among populations based on susceptibility to particular diseases or disorders (Aird, Bentall, & Roberts, 1953; Cheng et al., 2005; Glass et al., 1985; Gonzalez Ordonez, Medina Rodriguez, Martin, Alvarez, & Coto, 1999; Hutson, Atmar, Graham, & Estes, 2002; Marinaccio

et al., 1995; Shimazu, Shimaoka, Sugimoto, Taenaka, & Hasegawa, 2000). This project will investigate the relationship between ABO blood groups and length of life. As an introduction, the process of the discovery of the ABO blood group system, the chemical and molecular structure of the antigens, the inheritance of the genes, as well as the geographical and racial/ethnic distribution of the ABO phenotypes will be presented.

Discovery of ABO Blood Groups

Three blood groups (A, B and O) were discovered in 1900 by Landsteiner. He tested blood samples from himself and several colleagues by combining serum specimens with a suspension of RBCs from each person. By observing agglutination in some mixtures but not in others, he was able to classify the blood samples into three groups. Two years later, indications of a fourth group (AB) were found by two of Landsteiner's students (Decastello & Sturli, 1902). Consequently, humans can be divided into four ABO groups according to the reactions with which their RBCs exhibit with normal human sera. These serological reactions depend on the presence or absence of A and B substances on the surface of the RBC, the active parts of which are polysaccharide in nature (Roback, Grossman, Harris, & Hillyer, 2011). In serological terms, the A and B substances are described as antigens or agglutinins. Group A RBCs carry only the A antigen, B cells only the B antigen, AB cells carry both, and O cells carry neither (Table 1).

The antigens of the ABO blood group remain the most well-known and medically important group of red cell antigens. These antigens are defined by carbohydrate epitopes on glycoproteins and glycolipids located on the extracellular surface of the RBC membranes and in other tissues, body fluids and organs (Oriol et al., 1981; Szulman, 1960; Triadou, Audran, Rousset, Zweibaum, & Oriol, 1983; Trickett, Evans, MacIver, Smith, & Slapak, 1983). An ABO antigen is identified by a series of carbohydrates attached to a lipid or protein component on the

Table 1.

Antigens and Antibodies Found in the ABO Blood Groups

Blood Group	Antigens on the surface of RBCs	Antibodies in the plasma or serum
O	neither A nor B	Anti-A; Anti-B; Anti-A,B
A	A	Anti-B
B	B	Anti-A
AB	A, B	none

RBC membrane. These antigens are produced by a series of reactions in which enzymes catalyze the transfer of sugar units. A person's genotype determines the type of enzymes present, and therefore, the type of antigens that are on the RBCs. ABO blood types are inherited through genes on chromosome 9 (Lewis, Kaita, Giblett, & Anderson, 1978). Although they can be detected on the RBCs of five to six-week old embryos, A and B antigens are not fully developed at birth, presumably because the branching oligosaccharide structures develop gradually. By two to four years of age, A and B antigen expression is fully developed and remains fairly constant throughout life. A human serum contains either, both or neither of the corresponding antibodies or hemagglutinins, anti-A and anti-B. Landsteiner concluded from his experiments that human plasma contains A and B antibodies when the corresponding antigen(s) is/are absent from the host red cells (Table 1). ABO antibodies are present in human sera after the first few months of life (Roback, Grossman, Harris, & Hillyer, 2011). Even in 2014, over 100 years after Landsteiner's discovery (1900), human blood is typed by the antigens on the surface of RBCs for certain medical indications.

The serological activity of anti-A and anti-B forms the basis of ABO testing, which uses known sera containing anti-A and anti-B and known A and B cells as reagents to test blood of an

unknown blood group. Anti-A in serum can attach to the A antigen on A or AB cells and cause agglutination. Anti-B behaves similarly with B or AB cells. The ABO blood group system remains most significant for transfusion practice since it is the only system for which the reciprocal (or antithetical) antibodies are consistently and predictably present in the sera of most people who have had no exposure to human red cells (Roback, Grossman, Harris, & Hillyer, 2011).

Structure and Function of ABO Antigens

Establishing the structure of the molecules carrying blood group antigens was built on the base of the serologic knowledge discovered by Landsteiner (1900) and Decastello and Sturli (1902). Though the ABO antigens are also distributed throughout the body in tissues, fluids and organs (Oriol et al., 1981; Szulman, 1960; Triadou et al., 1983; Trickett et al., 1983), they contribute to the architecture of the RBC membrane and have a clear role in adverse events such as hemolytic transfusion reactions, hemolytic disease of the newborn, autoimmune hemolytic anemia, graft rejection and spontaneous abortion. Although the ABO antigens are surface markers on the external surface of the RBC membrane, their function may not be related directly to the RBC. It is possible that we have labeled these antigens as "blood" groups because they are defined serologically by red cells agglutinating with their corresponding antibody and because of the known problems associated with blood transfusion and ABO incompatibility. However, compatibility may not be their sole function. For example, research has shown that many gram negative organisms (such as *E. coli*) have chemical moieties on their membranes resembling the A and B antigens; and this is the stimulus for anti-A and anti-B production by human infants (G. F. Springer, Williamson, & Brandes, 1961). Individuals lacking the A or B antigen make either anti-B or anti-A when exposed to the environmental bacteria that are structurally similar to the A

and B antigen (Garratty, 2005). Therefore, the anti-A and anti-B produced cross-react with red blood cells expressing the corresponding antigen. The biological qualities assigned to these RBC membrane structures are based on observed physiological alteration in RBCs that lack the component, the documentation of similarities in the protein sequence (predicted from the gene's nucleotide sequence) to proteins of known function, and by inference using homologues already identified in other cells.

Molecular structure. The genetics of the ABO blood group system was first described in by Bernstein 1924 (as cited in Hosoi, 2008) as consisting of a set of three allelic genes, A, B and O, at a single genetic locus. In 1976, the chromosomal assignment of the ABO locus was mapped to the region 9q34.2 at the distal end of the long arm of chromosome 9 (Ferguson-Smith, Aitken, Turleau, & de Grouchy, 1976). In 1990, Yamamoto and colleagues described the molecular genetic basis of the ABO blood group system. The genes direct the occurrence and location of A and B antigens; however, the products of the genes are not the antigens themselves, but the enzymes (glycotransferases) that contribute to the production of the A and B antigens (Yamamoto, Clausen, White, Marken, & Hakomori, 1990). The genes that direct A and B antigen development are at three separate loci: ABO, Hh, and Se. Three common alleles (A, B and O) are located at the ABO locus on chromosome 9 at 9q34.1-q34.2 (Zelinski, 1995), and the genes at the other two loci, Hh and Se, are closely linked on chromosome 19 (Larsen, Ernst, Nair, & Lowe, 1990). Hh and Se loci each have two recognizable alleles, one of which has no demonstrable product and is considered an amorph. The active allele at the H locus, H, produces a glycosyltransferase that acts at the cellular level to form the antigen on which A or B is built. The amorph, h, is very rare with a prevalence of 0.004 percent (Das et al., 2001). The Se gene is responsible for the expression of H (and indirectly responsible for the expression of A and B)

on the glycoproteins in epithelial secretions such as saliva. Secretors inherit the Se gene; and their secreted glycoproteins express H, which can be converted to A and/or B antigen if the A and/or B gene is/are also present. The amorph is called se.

The H locus encodes a fucosyltransferase that produces the H antigen on RBCs, which is an essential precursor to the ABO blood group antigens. The *A* gene, encodes a glycotransferase that bonds α-N-acetylgalactosamine to the D-galactose end of the H antigen and produces the A antigen. The *B* gene similarly determines the presence of the B antigen by encoding a glycotransferase that joins α-D-galactose to the D-galactose terminal sugar of the H antigen, creating the B antigen. The *O* gene does not produce a functional protein; and in the heterozygote with an *A* or *B* gene, has little influence on the expression the A or B antigen. Thus, phenotypically, the *O* gene is recessive, and the *A* and *B* genes are codominant (Table 2).

Table 2.

Genotypes of the ABO Blood Groups

Group	Possible Genotypes
O	*OO*
A	*AA, AO*
B	*BB, BO*
AB	*AB*

A and *B* genes differ from one another by seven single-base substitutions, which result in four possible amino acid substitutions (at positions 176, 235, 266 and 268) in the protein sequence of the A and B transferases (Yamamoto & Hakomori, 1990). All variant A and B phenotypes, which have a weaker expression of the A and B phenotypes (subgroups), have been shown to be mutations of the *A* or *B* gene, resulting in less effective transferase production

(Ogasawara et al., 1996). Multiple polymorphisms have been shown in the noncoding regions of genomic sequences of the ABO alleles (Seltsam, Hallensleben, Kollmann, & Blasczyk, 2003), and these sequence variations can affect A and B antigen expression resulting in weak ABO phenotypes (Seltsam et al., 2003). A single base deletion in the O allele shifts the codon reading frame and is responsible for the loss of activity of the A glycotransferase (Yamamoto et al., 1990).

Chemical structure. Because the A and B antigens are derived from a common precursor, the H antigen, the nature of the H antigen is relevant to any discussion of the chemical structure of the ABO blood group antigens. The H antigen is composed of the carbohydrate sequence β-D-galactose, β-D-N-acetylglucosamine, β-D-galactose and 2-linked, α-L-fucose linked to bands 3 and 4.5 proteins (Lowe, 1994). The H antigens are expressed as fucose-containing glycan units, residing on the glycoproteins or glycolipids of RBC membranes or on mucin glycoproteins in secretions (Lowe, 1994). These fucosylated glycans are the direct substrates for the glycosyltransferases that give rise to the epitopes for the A, B and Lewis blood group antigens. Therefore, group O RBCs have the greatest number of H antigenic structures of the ABO blood groups.

The H antigen was first described by Morgan and Watkins (1969). The basic substance from which all the ABO blood group antigens are built is chemically similar to the capsular antigen of pneumococcus type XIV (Mourant, Kopec, & Domaniewska-Sobczak, 1976). In all but a very few persons, an enzyme (the product of the *H* gene), causes a molecule of 1-fucose to be added to the number 2 carbon of the terminal galactose of type 2 oligosaccharide chains, giving rise to the H antigen (Morgan & Watkins, 1969; Morgan, 1970). This is further modified by the *A* and *B* genes of the ABO system, which give rise to a further series of transforming

enzymes, glycosyltransferases (Lowe, 1994). The *O* gene does not encode a functional enzyme. The glucosyltransferases add individual sugars sequentially to sites on oligosaccharides conferring ABO specificity.

Oligosaccharides are chains of sugars that can be attached to either protein (glycoprotein), sphingolipid (glycosphingolipid) or lipid (glycolipid) carrier molecules. When attached to proteins, oligosaccharides are linked either to the amide nitrogen of asparagine via an N-acetylglucosamine or to the hydroxyl oxygen or serine or threonine via an N-acetylgalactosamine (Lowe, 1994). Glycoproteins are associated with the membranes of RBCs and other cells. The body's serous and mucous secretions contain soluble glycoproteins with blood group antigen activity. In glycosphingolipids, structurally similar oligosaccharides are attached via glucose to ceramide residues. Glycosphingolipids form part of the membranes of RBCs, most endothelial cells and some epithelial cells (Oriol et al., 1981; Triadou et al., 1983; Trickett et al., 1983).

A and B glycosyltransferases add specific sugars to oligosaccharide chains that have been converted to H by the fucosyltransferase produced by the *H* gene. The *A* gene encodes alpha1,3-*N*-acetylgalactosaminyltransferase and the *B* gene codes alpha1,3-galactosyltransferase (National Center for Biotechnology Information, 2008). The *O* gene is known as an amorph because any product to which it may give rise has no detectable effect on the H antigen. The *A* gene, however, causes the addition of N-acetylgalactosamine, converting H antigen to A antigen. The *B* gene causes galactose to be added to the H structure, giving rise similarly to the B antigen (Lowe, 1994).

A, B and H antigens are constructed on oligosaccharide chains of four different types (1 to 4) which differ in the linkage of the terminal β-D-galactose to N-acetylglucosamine and in the

characteristics of the carbohydrate chain (Lowe, 1994). The most abundant forms are type 1 and type 2. In type 1 chains, the number 1 carbon of galactose is linked to the number 3 carbon of N-acetylglucosamine; in type 2 chains, the number 4 carbon of N-acetylglucosamine is the acceptor for β-D-galactose. A, B and H antigens on the RBC surface are formed on type 2 chains present in highly branched oligosaccharides attached to integral proteins of the RBC membrane, notably bands 3 and 4.5, and on type 2 oligosaccharides bound to glycolipids (Lowe, 1994).

The transferase produced by the *H* gene adds fucose to the number 2 carbon on the terminal galactose of type 1 and type 2 chains. The *A* and *B* gene transferases can attach their immunodominant sugars to the number 3 sugar of the same galactose only if fucose is already attached, i.e., if the core chain has been converted to H. Attachment of the sugar that defines the A or B antigen diminishes the serological detection of H antigen in a reciprocal manner; the expression of A or B and of H are inversely proportional (Lowe, 1994).

Environmental and Geographic Factors

Antigens of the ABO blood group system have been known since 1900, yet their biological meaning is still largely obscure. The frequencies of ABO blood group phenotypes are not equal and it has been suggested that polymorphic genes provide intraspecies diversity which allows coping with diverse and rapidly evolving pathogens (Marionneau et al., 2001). Based on available knowledge about the genes involved in the blood group antigens' biosynthesis and about their tissue distribution in humans, one could postulate about the selective forces that may maintain or propagate these antigens. For example, some *E.coli* have ABO-like antigens on their cell walls (Andersson, Carlin, Leontein, Lindquist, & Slettengren, 1989; G. F. Springer, Williamson, & Brandes, 1961), and H antigen is chemically similar to the capsular antigen of pneumococcus type XIV (Mourant et al., 1976). These similarities in antigen makeup could

confer some resistance in individuals who manufacture the corresponding antibodies, and increase the susceptibility of people whose blood group matches the antigens (Vogel, Pettenkofer, & Helmbold, 1960). Therefore, the inference is that ABO prevalence is affected by natural selection.

Because environments are dynamic, it may be that a gene or trait that hindered survival and reproduction in the past, might aid in survival and reproduction today. In this way, natural selection and adaptation work to adjust the prevalence of traits so that they are suited for a particular environment. Changes brought about by man, creating environments never before known could influence these tendencies. In order to examine the length of life of those with differing ABO blood groups, it is necessary to consider the distribution of the ABO blood groups and causes for the prevalence of certain blood groups among populations.

ABO Blood Groups and Disease

Beginning with Landsteiner's discovery (1900), our understanding of the ABO blood group system grew, the world of blood transfusion became safer, and scientists were able to study one of the first human characteristics proven to be inherited. The ABO blood group antigens also appear to have been important throughout our evolution because the frequencies of different ABO blood types vary among different populations (Cavalli-Sforza, Menozzi, & Piazza, 1994), suggesting that a particular blood type conferred a selection advantage. Research has shown that specific ABO blood types are thought to be linked with increased or decreased susceptibility to particular diseases (Albert, 1996; Cheng et al., 2005; Hein et al., 1998; Kalayanarooj et al., 2007; Shimazu et al., 2000). For example, individuals of blood group O are at a somewhat higher risk of contracting some infectious diseases, such as cholera (Albert, 1996; Glass et al., 1985; Levine et al., 1979). People of blood group A are at a higher risk for some

malignancies (Aird et al., 1953; Lee et al., 1990). Research suggests that group O is more appealing to mosquitoes, which is a significant factor in contracting malaria. These findings suggest that the ABO blood group antigens could have a physiological role previously unknown.

Although the biological role of ABO antigens is uncertain, it has been suggested that the polymorphic genes of the ABO blood group provide the diversity needed to survive changing environmental conditions and evolving pathogens (Marionneau et al., 2001). There have been many research endeavors regarding the associations of ABO blood groups with specific diseases, but fewer have examined an association with ABO blood groups and overall survival or lifespan. A study of centenarians (persons over 100 years old) in Tokyo demonstrated a higher frequency of group B in centenarians than in controls, suggesting that group B might be associated with exceptional longevity (Shimizu et al., 2004). In contrast, a United States study of patients who died in a tertiary care hospital found that the percentage of group B patients declined with age (Brecher & Hay, 2011), suggesting that group B is not a marker for longevity, but may actually be a marker for earlier death. Further contradiction was found in a Sicilian population in which the authors concluded that the frequencies of ABO blood groups in centenarians and controls did not differ significantly (Vasto et al., 2011). These disparate conclusions concerning a relationship among ABO blood groups and longevity add support to the need for larger, more comprehensive studies to address this question.

A variety of medical research has been concerned with epidemiological studies related to ABO blood groups and associated mortality, diseases and disorders. However, only the hemagglutination problems of incompatible ABO blood type in transfusion and compatibility have been accepted by the scientific community as clinically significant. There remains a lack of consensus about the relationship of ABO blood group with diseases, disorders and lifespan.

This project will look at the significance of ABO blood groups by examining the relationship of ABO blood group, transfusion and length of life in a Southeastern United States population. Findings could provide evidence for a physiologic function of the structure of ABO blood group antigens. The first step will be to clarify whether or not there is a relationship between ABO blood group and lifespan.

Significance

The ABO blood group antigens are of prime importance in transfusion medicine. These antigens are the most immunogenic of all the blood group antigens, and there are some undeniable associations of blood groups with adverse events. Antibodies to blood group antigens can cause hemolytic transfusion reactions, hemolytic disease of the newborn, autoimmune hemolytic anemia, graft rejection, and spontaneous abortion. Despite their known clinical importance in compatibility, the physiological functions of ABO blood group antigens remain unknown. Numerous associations have been made between particular ABO phenotypes and an increased susceptibility to disease (Aird et al., 1953; Albert, 1996; Hein et al., 1998; Lee et al., 1990; O'Donnell & Laffan, 2001; Suadicani et al., 2007). These studies provide increasing evidence that blood groups may play a biological role, though the salience of this issue persists.

The distribution of the four ABO blood groups, A, B, AB, and O, varies in populations throughout the world (Cavalli-Sforza et al., 1994). Prevalence of the blood groups is determined by the frequency of the three alleles at the ABO locus in different populations. Worldwide, group O is the most common, followed by group A, then group B with group AB as the least common (Cavalli-Sforza et al., 1994). Similar frequencies were found in a United States population (Garratty, Glynn, & McEntire, 2004). As part of the Retrovirus Epidemiology Donor Study, Garratty, Glynn and McEntire (2004) collected data on allogeneic and autologous blood

donors over a 10-year period (1991-2000) and reported ABO/Rh(D) phenotype frequencies for the major racial/ethnic groups in the United States. Their study resulted in the largest and most diverse United States sample to date with a sample size of over 3 million. These authors found that the highest percentage of group O was in the Hispanic (56.5%), North American Indian (54.6%) and Black non-Hispanic (50.2%) populations. Among all races/ethnicities, group O was the most prevalent and group AB the least prevalent. The prevalence of group B was highest in the Asian population (25.4%). The ABO phenotype differences between racial/ethnic groups were found to all be statistically significant, ($p < 0.0001$) (Garratty et al., 2004).

One would expect the frequencies of the ABO blood groups to stay the same throughout decades of life in a population, but recent United States data indicates that the percentage of group B declines as age increases (Brecher & Hay, 2011). Although the investigators failed to control for racial or gender differences in life expectancy, these results imply that group B is a marker for earlier death. In contrast to these findings, a Japanese study found group B to be a marker for longevity. This conflict is interesting, especially because the Asian population has a larger percentage of group B than the United States population (Cavalli-Sforza et al., 1994). These are few studies that have investigated an association between ABO blood group and lifespan and they are limited in scope and generalizability.

As of 2014, there exists no large-scale study of ABO blood groups and lifespan in the United States. The proposed project will further examine the relationship of ABO blood group, transfusion and lifespan in the United States and address the limitations of this previous work by surveying a larger sample and by controlling for race and gender. Because research has shown an association between transfusion and adverse outcomes including mortality (Gerber, 2008; Hebert et al., 1999; Marik & Corwin, 2008; Murphy et al., 2007; Reeves & Murphy, 2008;

Taylor et al., 2006), history of transfusion will be included as a potential confounder. A study of the proposed magnitude could provide a footprint for studies in other geographical regions or countries and move towards a definitive answer regarding the relationship between ABO blood group and lifespan.

Although many research endeavors have suggested associations between blood groups and particular diseases or disorders (Aird et al., 1953; Albert, 1996; Hein et al., 1998; O'Donnell & Laffan, 2001; Suadicani et al., 2007), consensus has not been reached. The relationship of ABO blood group with disease or overall mortality is not fully understood by the scientific community. Evidence linking ABO and length of life may imply a functional role, will allow health care professionals to add to the overall understanding of its effect and allow further investigation to focus at the biochemical or molecular level. Some antigens are associated with susceptibility or protection against diseases, but these associations are statistical and the causes are not well understood. While the biological role of blood group antigens is unknown, one is presumed to exist based on the findings of numerous studies (Bennun, Roth, Monferran, & Cumar, 1989; Buchanan & Highley, 1921; Cheng et al., 2005; Glass et al., 1985; Gonzalez Ordonez et al., 1999; Kalayanarooj et al., 2007; Lin, Chang, Wu, & Cheng, 1998). Since previous research indicates those of a particular ABO blood group might be more likely to escape certain illnesses, then it stands to reason that ABO blood group could affect length of life. In recent years advances in molecular genetics and cellular biochemistry have provided an abundance of new information regarding the structure of blood group antigens. This new information and the findings of this study will allow us to further investigate the biochemical mechanisms responsible for a longer lifespan in a particular blood group. Additionally, results will allow us to consider much more thoughtfully how the genetic inheritance of ABO blood

group might relate to environmental factors to improve health or provide protection from illness. Since all humans can be classified by ABO blood group, information regarding its effect has the potential to impact the entire human race.

The purpose of this study is to describe the incidence of ABO blood groups in those who expire at three tertiary care centers. Additionally, the project will examine the relationship of ABO blood group and lifespan. The analysis will control for race, gender and history of transfusion to determine if there is a significant difference in lifespan among persons with differing ABO blood groups.

Aim of Study

The primary aim of this study is to determine if there is a relationship between ABO blood groups and lifespan. Additionally, any relationship will be examined while controlling for race, gender, location and history of RBC transfusion. The specific objectives of this study are to: (1) synthesize patient demographic data, ABO blood group and RBC transfusion history from three tertiary care centers, (2) calculate descriptive statistics for each ABO blood group by race, gender, site and transfusion history, and (3) quantitatively evaluate the effect of ABO blood group on lifespan. To meet these ends, the following preliminary questions must be answered: What is the prevalence of each of the four ABO blood groups in the sample population? What is the prevalence of each of the four ABO blood groups in the sample population by race? What is the prevalence of each of the four ABO blood groups in the sample population by gender? What is the prevalence of each of the four ABO blood groups in the sample population by site? Within each ABO blood group, what is the prevalence of any history of transfusion?

Delimitations and Assumptions

The target population will include all patients who die at the three tertiary care centers during the one-year period, January 1, 2010 through December 31, 2010. Only stillborn infants and those patients without an ABO blood group on file will be excluded from analysis. The three tertiary care centers are Virginia Commonwealth University Medical Center in Richmond, Virginia, Wake Forest University Baptist Medical Center in Winston-Salem, North Carolina, and University of North Carolina Hospitals in Chapel Hill, North Carolina.

This study is based on the following assumptions: (1) the data needed can be collected from a retrospective review of blood bank and electronic clinical records, (2) the data provided by the clinical records is accurate, and (3) by using analysis of covariance techniques, it will be possible to demonstrate that ABO blood group is a marker for lifespan.

Variables

The independent variable is ABO blood group and possible covariates are gender, race, site and history of transfusion. ABO blood group refers to one of the four ABO blood groups (O, A, B, or AB) as determined by serological tests of the antigen on the red blood cell. Gender will be defined and recorded as male (1) or female (2). Race will be defined according to the National Institutes of Health policy on reported race. Therefore, patients' race will be recorded as American Indian or Alaska Native (1), Asian (2), Black or African American (3), Native Hawaiian or Other Pacific Islander (4) and White (5) (National Institutes of Health, 2007). Site refers to the tertiary care center where the sample was collected. History of transfusion refers to transfusion of RBCs any time during the year prior to the patient's death.

Age will serve as the dependent variable and will represent lifespan in this study. Age will be defined as the patient's age at the time of death. It will be recorded as a whole number when the patient's year of birth is subtracted from the year of death.

Summary

The most well-known and clinically important blood group in humans is the ABO blood group. The clinical significance of ABO blood group is evident and universally accepted with regards to blood transfusion and pregnancy. However, the importance of ABO blood group as it relates to other diseases or disorders and overall mortality is not fully understood by the scientific community. Many studies have suggested associations between blood groups and disease (Aird et al., 1953; Cheng et al., 2005; Hutson et al., 2002; Jick et al., 1969; Kalayanarooj et al., 2007; Marinaccio et al., 1995; Shimizu et al., 2004; Suadicani et al., 2007), but consensus has not been reached regarding overall survival or longevity. The proposed project will attempt to answer the question, "Is there a significant relationship between ABO blood group and lifespan?" This will be the first multi-site study in the United States to examine this relationship. An epidemiological approach will be used to arrive at a conclusion using a retrospective study and analysis of covariance techniques.

Organization of the Remaining Chapters

This paper is divided into five chapters. The first has been introductory. Chapter 2 is a review of the literature and includes an overview of the reports of ABO blood group with disease, links of ABO blood group and lifespan, links of transfusion and mortality, and the statistical techniques used for analysis. Chapter 3 is a review of the methodology used for this research project and chapter 4 contains the results and analysis of the research. The final chapter includes a summary of the findings and recommendations for future research.

Chapter Two: Literature Review

This chapter presents a review of the literature relevant to the relationship between ABO blood groups and lifespan. It begins with inheritance and patterns of prevalence of the four ABO blood groups and current knowledge about the evolution of the ABO blood groups. Reports on the associations of blood groups and disease are plentiful and reviewed historically by medical condition. Additionally, published links between ABO blood group and lifespan as well as that of transfusion and outcomes are examined. Lifespan is described in terms of life expectancy in the United States and the variables that may affect life expectancy.

ABO Blood Group Patterns of Prevalence

Antigens of the ABO blood group system were identified in the early 20th century, yet their biological meaning is still largely obscure. The frequencies of blood group phenotypes are obtained by testing the red cells of a large sample of randomly selected people of the same race or geographic location and observing the proportion of positive and negative reactions with a known antibody. In a given blood group system, the sum of phenotype frequencies should equal 100 percent. It has been suggested that highly polymorphic genes provide intraspecies diversity that allows coping with diverse and rapidly evolving pathogens (Marionneau et al., 2001). Based on the available knowledge about the genes involved in their biosynthesis and about their tissue distribution in humans, one could postulate about the selective forces that may maintain or propagate these oligosaccharide antigens.

Transient polymorphism, a polymorphism in which one allele is in the process of displacing another, is maintained only during the time that a gene is spreading through a population (Ford, 1945). Because environments are not stable, genes or traits that hindered survival in past, could aid in survival today. The prevalence of traits better suited for a particular environment increase due to natural selection and adaptation. As environmental conditions change, different phenotypes may be subject to Darwinian natural selection and have varying survival. Therefore, we could expect that the phenomenon of transient polymorphism could be observable when natural selection works to reduce or eliminate one phenotype or trait that is less fit in a particular environment. In order to examine the life expectancy of ABO blood groups in this context, it is necessary to consider the distribution of the ABO blood groups among populations.

Evolution is a change in allele frequency over time. In addition to natural selection, mutations and genetic drift may be sources of change in allele frequency. Mutation results when there is a change in the DNA sequence of a particular gene. The most common mutations are single nucleotide changes which are thought to occur in a given gene with a regular frequency. A new mutation that is transmitted to the next generation results in a change in allele frequency, simply because the new allele did not exist in the previous generation. In large populations, genetic drift usually acts to counter the accumulation of mutated alleles, because high frequency alleles have a better chance of being contributed to the next generation, while low frequency alleles have a better chance of being lost. When dramatic examples of genetic drift occur, alleles that were at low frequencies in the parent population can be raised to high frequencies in the new population. An example of this is a population bottleneck in which a population is reduced to a few individuals and the alleles of the survivors rise to higher frequencies in the populations of

their descendants. The northern elephant seal experienced this phenomenon when humans hunted them in the 1890s and reduced their population size to as few as 20 by the end of the 19th century. Their population has since rebounded to over 30,000, but their genes still carry the marks of this bottleneck and they have much less genetic variation than the original population. The evolutionary changes that resulted in the diversity of certain ABO blood groups among populations are unclear. It is possible to make inferences about the evolution of ABO blood group genes by examining nucleotide sequences of the alleles (Saitou & Yamamoto, 1997). However, it is not known for certain the exact event or events that gave rise to our current distribution or that the distribution has ever been any different than it is today. Further, in order to understand how natural selection pertains to ABO allele frequencies, it is necessary to understand the biological function of the ABO antigens. Others have suggested that the frequencies of the various alleles reflect the effect of post or ongoing epidemics and of genetic drift (Marionneau et al., 2001) . For example, blood group antigens might interact with pathogens that use cell surface carbohydrates as primary receptors and individuals devoid of the antigens to which the pathogen binds may be protected from the disease. This is exemplified with uropathogenic strains of *Escherichia coli* which bind glycolipids of non-secretors (Stapleton, Nudelman, Clausen, Hakomori, & Stamm, 1992). Because they lack the FUT2 gene, the R45 strain binds to the galactosylgloboside in the genitourinary tract of non-secretors. However, in secretors, the FUT2 enzyme adds a fucose to the galactosylgloboside that masks this *E.coli* receptor. Thus, it stands to reason that the polymorphic genes of the ABO blood group may provide some of the diversity needed to cope with the environment, specifically, various and rapidly evolving pathogens (Marionneau et al., 2001).

Observations related to escape from pathogens have given rise to a theory suggesting that the current diversity of ABO alleles is due to natural selection. Because there are so many different pathogens that can make use of the A, B and H antigens to infect cells (Albert, 1996; Bennun, Roth, Monferran, & Cumar, 1989; Glass et al., 1985; Kalayanarooj et al., 2007; Lindesmith et al., 2003; Marionneau et al., 2002), natural selection favors populations comprised of individuals with many different ABO phenotypes. If all pathogens used only the H antigen to infect cells, then there would be selective pressure for everyone in the population to have only A or B antigens; however, since there are pathogens that use H, A or B for infection, the population can survive by having some individuals with no H, some with no A and some with no B antigens. This form of selection called balancing selection, results in relatively high allele frequencies for multiple alleles and can counter the action of genetic drift in eliminating low frequency alleles.

United States phenotypic distribution. Three publications report on the distribution of blood groups in the United States population. The earliest works by Buckwalter and colleagues (1958) and Mourant and colleagues (1976), both studied populations of a single race. The population investigated by Buckwalter and colleagues (1958) included North American Black persons (n = 6722) while the population investigated by Mourant and colleagues, although larger in size, included only North American White persons (n = 120,281). The most recent study conducted by Garratty, Glynn and McEntire (2004) resulted in the largest and most diverse United States sample to date with a sample size of over 3 million. These investigators collected data on allogeneic and autologous donors over a 10 year period (1991-2000) and race/ethnicity was self-reported. Among all races/ethnicities, group O was the most prevalent and group AB the least prevalent. The highest percentage of group O was in the Hispanic (56.5%), North

American Indian (54.6%) and Black non-Hispanic (50.2%) populations. A summary of the results are provide in Table 3. The prevalence of group B in the Asian population (25.4%) is highest among the racial/ethnic groups. The ABO phenotype differences between racial/ethnic groups all were found to be statistically significant (chi-square, $p<0.0001$) (Garratty, Glynn, & McEntire, 2004).

Table 3.

ABO Blood Groups by Race/Ethnicity in the United States

	Blood Group Phenotype (%)			
Race/ethnicity	O	A	B	AB
White non-Hispanic	45.2	39.7	10.9	4.1
Hispanic	56.5	31.1	9.9	2.5
Black non-Hispanic	50.2	25.8	19.7	4.3
Asian	39.8	27.8	25.4	7.1
North American Indian	54.6	35.0	7.9	2.5
All	46.6	37.1	12.2	4.1

Note. Adapted from "ABO and Rh(D) phenotype frequencies of different racial/ethnic groups in the United States," by G. Garratty, S.A. Glynn, and R. McEntire, 2004, *Transfusion*, 44.

Buckwalter and colleagues' (1958) early study of North American Black persons found greater differences and reported higher percentage of group O and group B persons and a lesser percentage of group A when compared to the results of Mourant and colleagues and Garratty, Glynn and McEntire. Although Mourant and colleagues' study was limited to only White persons, the frequencies of ABO blood groups were similar to the overall frequencies found in the more diverse population studied by Garratty, Glynn and McEntire.

Regardless of ethnicity, all three studies indicate O as the most prevalent ABO blood group, followed by A, B and then AB (Table 4). The two studies that included a Black population show higher frequencies of group O and group B individuals and a lesser frequency of group A individuals as compared to the White population (Buckwalter et al., 1958; Garratty et al., 2004). Little reliable data is available on other ethnic groups in the United States. Garratty and colleagues (2004) were able to add newer and more accurate data on the other ethnic groups in the current United States population; however, their data represent the racial and ethnic categories designated by United States donors. Therefore, the ABO frequencies observed may not directly reflect the ABO frequencies of populations in the racial/ethnic group's country of origin, where less racial admixtures occur.

Table 4.

Comparison of ABO Blood Group Prevalence in United States by Race/Ethnicity and by Author

			Phenotype (%)			
Author, Date	Sample Size	Racial Makeup	O	A	B	AB
Garratty et al., 2004	2,215,623	White	45.2	39.8	10.9	4.1
	236,050	Black non-Hispanic	50.2	25.8	19.7	4.3
	3,086,215	All	46.6	37.1	12.2	4.1
Mourant, et al, 1976	120,281	White	45.8	39.7	10.8	3.7
Buckwalter et al, 1958	6,722	Black	49.1	26.5	20.1	4.3

Looking at other primate species, substantial frequencies of A, B and O alleles are reported as well. With the exception of B alleles in humans and gorillas, the frequency of ABO alleles in other primate species differ greatly from human ABO alleles (Corvelo, Schneider, & Harada, 2002). The ABO alleles in our species appear to be millions of years old due to the

relatively large number of nucleotide differences among the three alleles for ABO (Saitou & Yamamoto, 1997). Though considered to result from a mutation, it could be that the frequency of O alleles has always been high and that it has not risen over the course of human history.

Global phenotypic distribution. The distribution of the four ABO blood types varies in populations throughout the world. The most comprehensive data concerning blood groups in different populations is found in *Blood Groups and Diseases* by Mourant, Kopéc, and Domaniewska-Sobczak (1976). Phenotypic distribution is determined by the frequency of the three alleles of the ABO gene in different populations. Worldwide, group O was reported to be the most common, followed by group A, group B and group AB.

Persons of group O are the most common around the world, including the United States and Western Europe. Among indigenous populations of Central and South America, the frequency of group O is extremely high, approaching 100 percent. It is also high among Australian aborigines and can range from 80-100 percent in Native American populations. Group A is common in Central and Eastern Europe. In countries such as Austria, Denmark, Norway and Switzerland, about 45-50 percent of the population are group A in comparison to about 40 percent of Poles and Ukrainians. The highest frequencies of group A are found in small, more isolated populations. For example, about 80 percent of the Blackfoot Indians of Montana are group A. Group B is relatively common in Chinese and Indians, being present in up to 25 percent of the population. It is less common in European countries and Americans of European origin, being found in about 10 percent of these populations. Group B ranges up to 30 percent in some Asian populations, particularly in the Himalayas. Group AB is the rarest of blood groups. It is most common in Japan, regions of China and in Korea, present in about 10 percent of these populations (Mourant, Kopec, & Domaniewska-Sobczak, 1976).

Causes of global differences in ABO prevalence. With genetic drift, random effects most likely account for the 100 percent frequency of group O in some Native South American groups and the 50 percent frequency of group A in Australian and Native American populations. The relative small differences in ABO frequencies between neighboring groups may be due to gene flow between populations. The inference is that ABO prevalence is affected by natural selection. For example, some infectious disease organisms have ABO-like antigens on their cell walls, conferring some resistance in individuals who manufacture the appropriate antibodies, and increasing the susceptibility of people whose blood group matches the antigens (Vogel, 1970). Specifically, the Himalayan region has peak frequencies of group B (Mourant et al., 1976). This area has a history of epidemics of smallpox. Vogel, Pettenkofer and Helmbold (1960) suggest that the smallpox virus possesses material similar to the group A antigen, and this may be responsible, in part, for resistance of smallpox infection. Pettenkofer and colleagues (1962) asserted that in small pox infection, persons with blood groups B or O, in whom anti-A normally occurs, have an immunological advantage over persons of blood groups A and AB, who lack this antibody. In support of their work the authors report that of 103 Germans with severe post-vaccination reactions, a significantly higher proportion belonged to groups A and AB. Therefore, the inference is that the smallpox epidemics selected against individuals of groups A and AB, resulting in unusually high frequencies of group B.

It is also possible that there are antenatal processes having a selective effect on the ABO blood group frequencies. ABO incompatibility between the mother and fetus would select for fetuses with matching or compatible blood groups, thus favoring group O. Indeed the high frequency of group O in isolated populations in and around Europe, on islands, mountain ranges, and deserts, to which travel has been slower and more difficult may be result of ABO differences

between mother and fetus (Mourant et al., 1976; Mourant, Kopec, & Domaniewska-Sobczak, 1978). This clearly has not happened in most populations, and evidence of other processes must be sought and alternate explanations must be considered. The high frequency of group O may represent the blood group composition of one or more early populations of the region who were driven by later immigrants into these relatively inaccessible areas. If some common epidemics do favor persons of group A or groups A and B, then it may be that in areas of easier communications and transmissions of infections, the processes favoring group O are partly balanced by these others, leading to higher equilibrium frequencies of A and B in the more continental areas.

Associations of ABO Blood Groups and Disease

From 1917 to 1940 there were already more than 300 reports in the literature showing a statistical relationship of certain ABO blood groups with certain medical conditions or characteristics such as criminality or personality traits. Geneticist, E.B. Ford (1945), said, "It is reasonable to conclude, from what we know of polymorphism, that individuals belonging to the different blood groups are not equally viable…A valuable line of enquiry which does not yet seem to have been pursued in any detail would be to study the blood group distributions in patients suffering from a wide variety of diseases. It is possible that in some conditions, infectious or otherwise, they would depart from their normal frequencies, indicating that persons of a particular blood group are unduly susceptible to the disease in question." (p. 85).

Clinical Significance of ABO Blood Groups

A variety of medical literature has been concerned with epidemiological studies related to ABO blood groups and associated mortality, diseases and disorders. However, only a small number of issues have been accepted by the scientific community as clinically significant. These

include the hemagglutination problems of incompatible ABO blood type in transfusion and pregnancy. In fact, the chemical structures were labeled as RBC antigens due to the problems they caused in transfusing blood (Garratty, 2000). Most transfusions provide safe and effective temporary replacement of blood components. However, severe hemolytic reactions can occur when transfused RBCs interact with preformed antibodies in the recipient. The interaction of antibody with antigen can initiate a sequence of neuroendocrine responses, complement activation, coagulation effects, and cytokine effects that result in clinical manifestations of an acute hemolytic transfusion reaction (Roback, Grossman, Harris, & Hillyer, 2011). Most severe acute hemolytic transfusion reactions results from transfusion of ABO-incompatible RBCs (Sazama, 1990).

Levine (1943) demonstrated that hemolytic disease of the fetus and newborn (HDFN) was caused by immunization of the mother by paternally derived fetal antigens she lacked. Maternal IgG antibodies cross the placenta, sensitize fetal RBC antigens and can cause bilirubinemia, anemia and heart failure. Because IgG is the dominant class of Anti-A,B in group O persons, infants of group O mothers are at a higher risk of ABO hemolytic disease of the newborn. Although small quantities of IgG antibodies are present, IgM is the predominant immunoglobulin class of anti-A and anti-B in group B and group A persons, respectively (Rawson & Abelson, 1960a; Rawson & Abelson, 1960b). Because IgM does not cross the placenta, infants of group A and group B mothers are at a lesser risk of ABO hemolytic disease of the newborn (Kochwa, Rosenfield, Tallal, & Wasserman, 1961).

ABO blood group and disease. Differences in ABO blood groups are determined by antigens in the outer carbohydrate coating (glycocalyx) of erythrocytes (Lowe, 1994). Because these antigens are present in most tissues as well (Oriol et al., 1981; Szulman, 1960; Triadou,

Audran, Rousset, Zweibaum, & Oriol, 1983; Trickett, Evans, MacIver, Smith, & Slapak, 1983), differences in the glycocalyx expressed by cells might elicit differing responses in biomedical phenomena in other diseases or disorders apart from the hemagglutination events already discussed. Although many studies have examined the relationship of ABO blood group with disease, there remains a lack of consensus regarding statistical and clinical associations. Studies on this association date back as far as 1927, and the most noteworthy are reviewed here.

Prokop and Uhlenbruck (1969) published a review of early literature of associations of blood groups and disease. Although most of the research examines associations of ABO with disease, early papers describe associations with hangovers, teeth or personality characteristics. One report indicated that hangover was worse in individuals of group A and that group B individuals defecate the most (Warnowsky, 1927). Additionally, in 1930 an investigator reported that group O persons have the best teeth (Suk, 1930). Further there are several papers and books published relating ABO blood groups to personality characteristics. For example, in his book, *Character, Blood Groups and Constitution*, Schafer (1941) found that military personnel who were group O had less satisfactory strength of character and personality, and that group B individuals were more impulsive. Bohmer (1927) and others have suggested an association with group A and criminality (as cited in Garratty, 1996; Palmieri, 1929). These early reports lacked adequate sample sizes and the rigor of modern statistical methods, but demonstrate the numerous speculations that have been made regarding associations with ABO. Reports of such associations with characteristics persist today. The book entitled "You are Your Blood Type – The Biochemical Key to Unlocking the Secrets of Your Personality" was published in 1988 (Nomi & Besher, 1988). This book details how Japan's largest companies use blood types in marketing and for evaluating job applicants. In 1998, one of the *New York*

Times best sellers was a book titled, "Eat Right 4 Your Type" (D'Adamo, 1997). The same author wrote a subsequent book that describes special diets for each ABO blood group (D'Adamo, 1999). The author hypothesizes that the original blood group was O and that these people were hunter-gatherers and meat eaters. In contrast, later generations who were group A became farmers and ate more grain and vegetables, and drank milk; groups B and AB were said to be the last to emerge because of racial intermingling (D'Adamo, 1997). However, contemporary molecular studies show that the *O* gene appears to be a mutation of the *A* gene, and thus group O appeared later than the other ABO groups (Saitou & Yamamoto, 1997).

Although there are thousands of publications on the associations of blood groups with disease, many are based merely on statistical associations without a basis for biological or physiological association (such as associations with hangovers and personality characteristics). Additionally, most of the early studies are controversial because they were small studies, had inadequate controls or had been analyzed incorrectly. However, there exists a general pattern that has emerged from the large body of statistical data on malignancy, coagulation and infection (Table 5) (Garratty, 2000). Many of these early statistical associations now have some associated scientific findings (such as membrane chemistry, tumor immunology and microbial receptors for infectious disease) that support a scientific rationale for the statistical associations (Garratty, 1994).

The scientific literature provides a variety of data regarding the relationship of ABO phenotype with diseases and disorders, as well as survival (Albert, 1996; Hein, Suadicani, & Gyntelberg, 1998; O'Donnell & Laffan, 2001; Suadicani, Hein, & Gyntelberg, 2007). Reports of positive correlations exist for a particular ABO blood group with infectious disease, deep vein thrombosis (DVT), heart disease, pulmonary function, malignancy, obesity, survival, and even

Table 5.

Statistical Relationship of ABO Blood Groups with Disease

Disease	A > O	O > A	B/AB > A/O
Malignancy	X		
Thrombosis	X		
Peptic Ulcers		X	
Bleeding		X	
E. coli/Salmonella infections			X

Note. Adapted from "Blood Groups and Disease: A Historical Perspective," by G. Garratty, 2000, *Transfusion Medicine Reviews*, 14.

behavioral traits (Table 6). The following summary is organized by disease and emphasizes any association found between ABO blood group and disease or mortality.

Infectious disease. Studies of the relationship of ABO blood group and infectious disease are plentiful in the literature and include *Helicobacter pylori*, *Vibrio cholerae, Escherichia coli*, Norwalk virus, Severe Acute Respiratory Syndrome and Dengue Fever (Aird, Bentall, Mehigan, & Roberts, 1954; Alkout, Blackwell, & Weir, 2000; Boren, Falk, Roth, Larson, & Normark, 1993; Cheng et al., 2005; Clark et al., 1956; Guillon et al., 2008; Hein et al., 1998; Hutson, Atmar, Graham, & Estes, 2002; Kalayanarooj et al., 2007; M. M. Levine et al., 1979; Marionneau, Airaud, Bovin, Le Pendu, & Ruvoen-Clouet, 2005; Mentis et al., 1991; Sharara, Abdul-Baki, ElHajj, Kreidieh, & Kfoury Baz, 2006). The A, B, H and other related carbohydrate antigens are highly expressed on gut cells in the gastric and duodenal regions (Glynn, Holborow, & Johnson, 1957; Green et al., 1988) and have been associated with susceptibility to pathogens, and in particular, gut pathogens. Evidence has accumulated for

Table 6.

ABO Blood Group Associations by Disease/Disorder

Disease/Disorder	Author, Year of Publication	Association with ABO Group
Helicobacter pylori	Aird et al., 1954	O
	Clark et al., 1956	O
	Rotter, 1983	O
	Mentis et al., 1991	O
	Davidson & Triadafilopoulos, 1992	O
	Boren et al., 1993	O
	Lin et al., 1998	O
	Hein et al., 1998	O
Vibrio cholerae	Levine et al., 1979	O
	Glass et al., 1985	O
	Albert, 1996	O
Escherichia coli	Armstrong et al., 1999	B (protective effect)
	Shimazu et al., 2000	B (protective effect)
Norwalk Virus	Hutson et al., 2002	B (protective effect)
	Lindesmith et al., 2003	B (protective effect)
	Marionneau et al, 2005	A
Severe Acute Respiratory Syndrome (SARS)	Cheng et al., 2005	O (protective effect)
	Guillon et al., 2008	O (protective effect)
Dengue Fever & Dengue Hemorrhagic Fever (DHF)	Kalayanarooj et al., 2007	AB (more severe case of the disease)
Deep Vein Thrombosis (DVT)	Jick et al., 1978	A, B, AB
	Koster et al., 1995	A, B, AB
	Robert et al., 2000	A, B, AB
Heart Disease	Medalie et al., 1971	A, B, AB
	Garrison et al., 1976	Non-O
	Rosenberg et al., 1983	A
	Whincup et al., 1990	A
	Meade et al., 1994	AB
	Green et al., 1995	A, B, AB
	Suadicani et al., 2000	O
	O'Donnell & Laffan, 2001	O
	Ketch et al., 2008	Non-O
	Anvari et al., 2009	O
Pulmonary Function	Menkes et al., 1984	A

Table 6. Continued

Disease/Disorder	Author, Year of Publication	Association with ABO Group
Malignancy	Aird et al., 1953	A
	Lee et al., 1990	A
	Marinaccio et al., 1995	A
	Suadicani et al., 2007	O
Obesity	Suadicani et al., 2007	O
Intelligence Quotient (IQ)	Gibson et al., 1973	A_2 and O
Suicide	Lester, 1987 & 2004	O (protective effect)
Temperament	Neumann et al., 1991	O (type A behavior)
Bipolar Disorder	Shapiro et al., 1977	O
	Zonda & Lester, 2002	O
Survival/Mortality	Shimizu et al., 2004	B (protective effect)
	Hong et al., 2006	B (protective effect)
	Brecher & Hay, 2011	B
	Vasto et al., 2011	No Association

associations between ABO blood group and gastric ulcer and carcinoma (Boren et al., 1993; Langman & Doll, 1965; Merikas, Christakopoulos, & Petropoulos, 1966).

In 1954, Aird reported that group O individuals were more likely than group A, B or AB individuals to have peptic (gastric and duodenal) ulcers. Taking the ABO group and secretor status into account yielded a relative increase of 35 percent in group O secretors among those with peptic ulcer when compared to a control group. Langman and Doll (1965) and Merikas and colleagues (1966) also investigated this association and supported the association of group O with ulcers and bleeding ulcers. Using blood donors as the control group, the original study was performed on only hospital patients, thus creating a potential bias if only patients with bleeding ulcers were admitted. Within this context, it has also been shown that among the cases of duodenal ulcers, the group O patients are more likely to bleed (Langman & Doll, 1965). Thus,

the bleeding tendency that brought the patient to the hospital may have accounted for the excess of group O reported by Aird and colleagues (1954). In 1993, Boren and colleagues reported that Lewis blood group antigen, Le^b (which has close associations with the ABO system), is the receptor for *Helicobacter pylori*. *H. pylori* is associated with gastric adenocarcinoma and is thought to be a major cause of gastric ulcers (Boren et al., 1993).

Additionally, there is evidence that suggests that the mosquito, *Anopheles gambiae*, recognizes ABO blood group variation and is selective in its feeding, with a preference for blood group O (Wood, Harrison, Dore, & Weiner, 1972). The basis for this recognition and selection is not obvious, although ABH blood group substances do occur on skin cells and have been reported in sweat of secretors (Roback, Grossman, Harris, & Hillyer, 2011; Saito, 1994). This finding could be of great importance in infectivity rates, the development of acquired immunity and natural selection for ABO blood groups in malarious regions.

Individuals possess antibodies directed toward the A or B antigen absent from their own RBCs. One hypothesis for the development of these antibodies is based on the fact that the configurations that confer A and B specificities also exist in other biological entities, notably bacterial cell walls. Springer and colleagues (1961) showed that many gram negative organisms, such as *Escherichia coli*, have chemical moieties on their membranes resembling A and B blood group antigens. A separate, later report indicates that this is the stimulus for anti-A and anti-B production by human infants (Springer & Horton, 1969). Bacteria are widespread in the environment and their presence in intestinal flora, dust, food and other widely distributed agents ensures a constant exposure of all persons to A-like and B-like antigens (Roback, Grossman, Harris, & Hillyer, 2011). Immunocompetent persons react to the environmental antigens by producing antibodies to those that are absent from their own systems. This environmental

explanation for the emergence of anti-A and anti-B remains a hypothesis that has not been proven, but there is evidence that anti-B recognizes and kills *E. coli* in vitro (Check, O'Neill, O'Neill, & Fuscaldo, 1972; Muschel & Osawa, 1959). These studies illustrate an increase in the bactericidal reaction when *E. coli* is mixed with anti-B and lend support to the hypothesis of ABO antibody development. However, it is not known whether ABO antibodies can destroy bacteria in vivo and, therefore, serve a functional role or offer a protective effect. Although this remains uncertain, others have suggested that the differences seen in the prevalence of ABO blood groups in different parts of the world may be attributable to epidemics that have occurred in the past (Mourant et al., 1976; Mourant et al., 1978).

Deep vein thrombosis. Deep vein thrombosis (DVT) refers to a clot that forms mainly in the larger veins of the leg. This clot can interfere with blood flow, and it may break off and travel through the bloodstream, or embolize. The embolus can lodge in the brain, lungs, heart or other area, severely damaging that organ (*Vascular Disease Foundation,* 2007). Thrombophlebitis occurs when a blood clot causes inflammation in one or more veins. The affected vein may be near the surface of the skin, or deep within a muscle (*Vascular Disease Foundation,* 2007). ABO blood group distribution has been associated with thrombotic disease with an excess of the non-O (in other words, A, B, and AB) blood group in patients with venous thrombosis (Jick et al., 1969). In 1978, Jick and colleagues examined the association between thrombophlebitis and ABO phenotype in 86 cases with a discharge diagnosis of thrombophlebitis of the lower extremity and 344 matched control patients. The authors found that the association between non-O blood type and thrombophlebitis of the lower extremity was strongest among the younger patients and women (Jick, Dinan, Herman, & Rothman, 1978). A later case-control study added support to Jick and colleagues' findings and demonstrated that non-O blood group

increased the risk of deep vein thrombosis (Koster, Blann, Briet, Vandenbroucke, & Rosendaal, 1995). Similar findings were noted in a 2000 study of subjects with an identified thrombosis risk factor such as the factor V Leiden mutation (Robert et al., 2000).

Heart disease and coagulation. The literature shows a tendency for group O individuals to bleed and non-O groups to thrombose (Jick et al., 1978; Koster et al., 1995; Robert et al., 2000). One report describes group A individuals as having a higher average level of factor VIII in their plasma than group O individuals (Preston & Barr, 1964). Preston and colleagues (1964) suggested that the different levels of factor VIII in the plasma, even within the normal range, may determine whether clinically detectable bleeding may occur from an ulcer or a blocked or narrowed blood vessel. Another investigator found a higher frequency of group O in the atherosclerotic patients with increased mural atheroma when compared to the frequency of group O in the occlusion group (Kingsbury, 1971). This investigator attributed these findings to a tendency of the group O patients to hemorrhage (rather than thrombose) into the arterial walls, thus sustaining more tissue damage.

Several other works have confirmed the association of group A with a higher factor VIII level (Garratty, 1994; Souto et al., 2000); and there also have been associations with other coagulation factors, such as von Willebrand Factor (VWF) (Garratty, 1994; Gonzalez Ordonez, Medina Rodriguez, Martin, Alvarez, & Coto, 1999). VWF plays an essential role in hemostasis by participating in platelet adhesion and aggregation and stabilizing Factor VIII. Deficiency or abnormalities of VWF leads to von Willebrand disease (VWD) (U.S. National Library of Medicine & National Institutes of Health, 2007a). Additionally, the level of VWF appears to correlate with ABO blood group (Gill, Endres-Brooks, Bauer, Marks, & Montgomery, 1987; McCallum, Peake, Newcombe, & Bloom, 1983; Mohanty et al., 1984; Orstavik et al., 1985;

Stormorken & Erikssen, 1977). Group O individuals have been reported to have lower plasma concentrations of VWF, than persons with other blood types (Green, Jarrett, Ruth, Folsom, & Liu, 1995; O'Donnell & Laffan, 2001). However, these studies have demonstrated a relationship between ABO blood group and levels of factor VIII and VWF only by association and not by linkage studies. Also unclear from these reports is whether the ABO locus exerts a functional effect directly on these plasma factors or whether the ABO locus is in linkage disequilibrium with another locus that controls these factors.

Souto and colleagues (2000) applied statistical methods combining linkage and association tests in a pedigree-based sample using ABO genotypes. This allowed for differentiating AO from AA and BO from BB. The results demonstrated significant linkage between the ABO locus and VWF antigen ($p=0.00075$). In addition, factor VIII coagulant activity and activated partial thromboplastin time showed suggestive linkage with the ABO locus ($p=0.10$ and $p=0.13$). All three phenotypes (A, B and AB) showed significant differences between OO and non-OO genotypes. In addition, VWF antigen exhibited significant differences between O heterozygotes and non-OO homozygotes. This study is unique because it used a combined linkage and association test, which indicated that the ABO locus itself has a functional effect on these phenotypes. Since elevated VWF carries increased risk for ischemic heart disease, these studies support the suggestion that cardiovascular events might be less frequent in group O individuals.

Because an association with the Le(a-b-) phenotype and increased risk of coronary heart disease for older males had been reported (Hein, Sorensen, Suadicani, & Gyntelberg, 1992), Green and colleagues (1995) investigated the relationship between Lewis phenotype and cardiovascular risk factors in 1714 participants in their Coronary Artery Risk Development in

Young Adults Study. Although no significant differences were observed among Lewis phenotypes, in White men with blood groups A, B or AB and the Le(a-b-) phenotype, significantly higher levels of factor VIII and VWF factor were observed than those with other Lewis phenotypes. The results indicated a significant interaction between ABO blood group and Lewis phenotype in terms of relationship to factor VIII. A similar trend was observed in Black men with blood type A, B or AB and the Le(a-b-) phenotype for factor VII/VWF and in women with blood type A, B or AB and the Le(a-b-) phenotype for factor VIII. The authors concluded that the data suggest the Le(a-b-) phenotype and blood groups A, B and AB (by virtue of their association with raised levels of factor VIII and VWF), may be risk markers for future cardiovascular disease (Green et al., 1995).

An increased risk of heart disease has been found for non-O individuals (Garrison et al., 1976). The Framingham Heart Study cohort was followed for the occurrence of cardiovascular events for a period of 10 years. A significant association was found between ABO blood group and intermittent pain caused by too little blood flow (claudication), with blood group O showing the lowest rates. Slight (although non-significant) excesses for certain other heart disease events were also found in non-O individuals. Serum cholesterol showed marginally significant but consistent elevations in non-O subjects, but the increased risk in non-O individuals was found to occur independently of the known intermittent claudication risk factors. Because the observed relationship between blood group and intermittent claudication occurred independently of the usual atherosclerotic risk factors, the authors concluded that ABO blood group, should be considered in the pathogenesis of intermittent claudication, possible through an effect on clotting (Garrison et al., 1976).

Jick and colleagues (1978) used a case-control design to study the role of estrogens and other factors in myocardial infarction (MI) in young women. The study included 83 women less than 46 years old with acute MI and 154 controls. In this early study, non-O blood group was determined to be positively associated with MI. A subsequent case-control study aimed to evaluate the risk factors for first nonfatal MI in women younger than 50 years old, and included 255 women with MI and 802 controls (Rosenberg et al., 1983). Results indicated that blood group A was a factor significantly associated with MI in women of this age group.

Suadicani and colleagues (2000) tested the hypothesis that an uneven socioeconomic distribution of ABO phenotypes could contribute to an explanation for the association of socioeconomic status with the risk of ischemic heart disease. This prospective study controlled for age and other relevant confounders, such as smoking, physical activity, wine consumption, and serum lipids. The study participants were enrolled in the Copenhagen Male Study (CMS) and were 2993 men aged 53-74 without overt ischemic heart disease. The outcome measure was the incidence of ischemic heart disease in an 8-year follow-up. The results indicated that ABO phenotype was not a confounder for the association of socioeconomic status with the risk of ischemic heart disease. However, ABO blood group was a strong risk or effect modifier for the association of socioeconomic status with the risk of ischemic heart disease in group O men. The authors concluded that their findings helped to clarify the roles of socioeconomic status and ABO blood group as cardiovascular disease risk factors (Suadicani, Hein, & Gyntelberg, 2000).

Most recently Anvari and colleagues (2009) examined over 10,000 Iranian patients awaiting coronary artery bypass graft surgery in an effort to determine if ABO blood group is related to ischemic heart disease. Using Chi Square analysis, this prospective study reported a statistical difference between gender and ABO ($p = 0.074$ for group O and $p = 0.031$ for group

A), with the number of group A male patients differing from the number of group A female patients. However, males were overrepresented in the sample population (71.9%). More importantly, the authors also reported that group B was less susceptible to hyperlipidemia compared to other groups ($p = 0.024$) and suggested that group B is a protective factor for cardiovascular disease risk. An additional conclusion was the prevalence of coronary artery disease in group O as markedly higher than in all other ABO blood groups. The authors found diabetes, hypertension, and cerebrovascular disease significantly more common in the group O patients compared to all other ABO blood groups (Anvari et al., 2009). Although the sample appears to be from a single ethnic group, it is not clear if the authors controlled for gender or age.

The results of Anvari and colleagues (2009) differ from other United States (Ellison et al., 1999; Ketch et al., 2008; Rosenberg et al., 1983) and European (Meade et al., 1994; Medalie et al., 1971; Whincup, Cook, Phillips, & Shaper, 1990) studies which found more cardiovascular events in non-group O patients. The results of Ketch and colleagues (2008) point to higher thrombosis in the non-group O patients despite less extensive atherosclerosis. However, when they examined outcomes at one year, the groups were similar. Additionally, the research by Ketch and colleagues is limited due to its retrospective nature and smaller sample size (1198). Neither of these studies measured VWF or Factor VIII levels which may have prognostic value independent of ABO group (Jager et al., 1999) or accounted for environmental factors (such as diet) that could be a stimulus for cardiovascular disease.

Pulmonary function. Menkes and colleagues (1984) performed an epidemiologic study of obstructive airway disease using cross-sectional and longitudinal data to investigate relationships among risk factors, pulmonary function and mortality. In the 2539 non-patient adults, 11 potential risk factors, including ABO group, were found to be significantly associated

with airway obstruction. When combinations of risk factors were examined, cross-sectional data indicated that some of these factors were important risk factors in cigarette smokers but less evident in never smokers. ABO blood group, familial component, coffee drinking and diet soda intake were related to marked differences in lung function in cigarette smokers, but had little impact on never smokers. Consistently greater declines of lung function were noted in males, older subjects, smokers, Whites, and individuals carrying the type A blood group allele (Menkes, Cohen, Beaty, Newill, & Khoury, 1984).

Malignancy. Studies of the association between ABO blood group and malignancy have included stomach cancer, gynecological tumors, and lung cancer. Many early studies lacked large sample sizes and adequate controls. The first reliable report was published by Aird and colleagues (1953), who showed that 20 percent more group A individuals had cancer of the stomach than did group O individuals. This was a large study, and its results have been confirmed in subsequent reports (Vogel & Krugr, 1968; Vogel, 1970).

Lee and colleagues (1990) carried out descriptive and analytical studies to examine the epidemiological characteristics and multiple risk factors of stomach cancer in Taiwan. The authors found that group A was associated with the development of stomach cancer with an odds ratio of 1.61, $p<0.01$ (Lee et al., 1990). Marinaccio and colleagues (1995) conducted a study to examine gynecological tumors and ABO phenotype. This report was a retrospective analysis of 968 women affected by gynecological tumors conducted to assess the existence of a difference in survival between patients with different blood groups. The authors reported a better 5-year and 10-year survival associated with group O when compared with group A. Specifically, endometrial and ovarian cancer occurred more frequently in group A women than those in the

other blood groups and in the same tumors, group A was associated with a poorer prognosis (Marinaccio et al., 1995).

The Copenhagen Male Study supported the concept that compared with other ABO phenotypes, males of phenotype O may exhibit a stronger systemic inflammatory response following occupational exposure to particulate air pollution (Suadicani, Hein, & Gyntelberg, 2002). The increasing recognition of inflammation as a contributing factor to the development of cancer motivated Suadicani and colleagues to hypothesize that the role of inflammation-related risk factors for lung cancer may depend upon ABO blood group. Consequently, Suadicani and colleagues (2007) examined the relationship of lung cancer mortality, risk of lifestyle and occupational factors previously linked to inflammation and ABO phenotype. The authors concluded that the predictive role of inflammation-related risk factors for lung cancer mortality was significantly stronger among males of phenotype O than A (Suadicani et al., 2007).

A review of the literature indicates that associations of ABO groups with malignancy, overall group A predominates over group O (Aird, Bentall, & Roberts, 1953; Lee et al., 1990; Marinaccio et al., 1995) . Altered expression of the ABH antigens have been found on all types of carcinomas and are often associated with unfavorable prognosis (S. Hakomori, 1996; Le Pendu et al., 2001). As cells (in tissue) become malignant, they tend to lose normal antigens and acquire new antigens. These "new" antigens may be precursors of antigens normally present or antigens that should not be present on a genetic basis (Garratty, 2000). Oh-Huti (1949) was the first to describe a loss of ABH antigens from malignant cells, and his report was confirmed by Masamune and colleagues (1953), Kay and Wallace (1961), Nairn and colleagues (1962) and Davidsohn (1972). It has been proven that ABO antigens diminish on malignant cells as the

malignancy progresses and the loss of A, B, and H antigens is proportional to the metastatic potential of the tumor (Garratty, 1994).

Obesity. The definition of obesity varies; but in general, it is a chronic condition defined by an excess amount body fat. Obesity is not just a cosmetic consideration, but also increases the risk of insulin resistance, type 2 diabetes, heart attack and stroke (U.S. National Library of Medicine & National Institutes of Health, 2007b). Suadicani and colleagues (2002) identified a relationship between occupational airborne pollutants, ABO phenotypes, and risk of ischemic heart disease when they found that long-term exposure to air pollutants was associated with a significantly increased risk of heart disease among men with phenotype O, and not among men with other ABO phenotypes. Long-term occupational exposure (> 5 years of exposure) to various airborne pollutants such as soldering fumes, welding fumes and plastic fumes was associated with a significantly increased lifetime prevalence of myocardial infarction. Odds ratios (95% confidence limits) for these factors were 3.0 (1.6–5.8), p = 0.002, 2.1 (1.05–4.2), p = 0.05, and 8.3 (2.6–27.0), p = 0.003. In this report, the authors suggested that the biological pathway could be a stronger systemic inflammatory response in men with blood group O. Several inflammatory mediators likely to increase the risk of ischemic heart disease have been linked also to obesity, suggesting that long-term exposure to airborne pollutants might play a role in the etiology of obesity. As a result, in 2005, Suadicani and colleagues tested the hypothesis that long-term occupational exposure to airborne pollutants would be more strongly associated with obesity in men with phenotype O than in men with other ABO phenotypes. The subjects were participants of the Copenhagen Male Study. The authors found no differences in the prevalence of obesity between men with the O phenotype and men with other phenotypes. However, only among men with the O phenotype was long-term occupational exposure to

various respirable airborne pollutants associated with an increased prevalence of obesity. Even after adjustment for lifestyle factors, age and social class, the authors found a relationship of airborne exposures with obesity in men with phenotype O (Suadicani, Hein, & Gyntelberg, 2005).

The authors suggest these observations are biologically plausible since air pollution is a heterogeneous, complex mixture of gases, liquids, and particulate matter. Systemic inflammatory responses have been described by Yudkin and colleagues (2000), who suggested that interleukin-6 (IL-6) may be the link connecting coronary heart disease and obesity (Suadicani et al., 2005). Additionally, there is evidence that individuals of blood type O have an increased inflammatory response when exposed to *H. pylori*, with a significantly increased release of IL-6, one of the inflammatory parameters also previously linked with risk of ischemic heart disease (Alkout et al., 2000) and obesity (Das, 2001; Yudkin, Kumari, Humphries, & Mohamed-Ali, 2000). Researchers offer that this finding of a strong relationship between long-term exposure to airborne pollutants, ABO phenotypes, and risk of obesity could lead to new possibilities for clarifying mechanisms underlying the global obesity epidemic (Suadicani et al., 2005).

Behavioral traits. In addition to diseases, the relationship between behavioral traits or personality characteristics such as intelligence quotient (IQ), suicide, temperament, and bipolar disorder with ABO blood groups has been examined in various studies. In a 1973 letter to *Nature*, Gibson and colleagues described an association they found between IQ and ABO phenotypes. Their data showed a significant difference in mean age-adjusted IQ between some of the ABO phenotypes. In particular, the A_2 group had the highest mean IQ and the A_2 and O phenotypes each had significantly higher mean IQs than the A_1 phenotypes (Gibson, Harrison,

Clarke, & Hiorns, 1973). Although some publications have no scientific basis (e.g., a book entitled, "You are Your Blood Type – The Biochemical Key to Unlocking the Secrets of Your Personality" (Nomi & Besher, 1988)), other associations have stronger biological foundations and some can be linked to overall health and mortality.

In 1977, Shapiro and colleagues studied 66 manic-depressive patients and identified them as either bipolar or unipolar. Within this group, they found that a significantly higher percentage of bipolar patients (than unipolar patients) were group O (Shapiro, Rafaelsen, Ryder, Svejgaard, & Sorensen, 1977). A 2002 study of type I bipolar patients in Budapest confirmed these results (Zonda & Lester, 2002).

Lester (1987) has explored the association between the distribution of blood types in nations and their suicide and homicide rates. He found that in 17 industrialized nations, the percentage of people with group O blood was negatively associated with their suicide rates but was not associated with their homicide rates (Lester, 1987). In 2004, Lester looked at a larger sample of nations. He found that the greater the proportion of people with group O blood in a nation, the lower the suicide rate and the higher the homicide rate (Lester, 2004). The proportions with group A and AB blood were associated with higher suicide and lower homicide rates (Lester, 2004). Even when the author controlled for gross domestic product per capita, it did not change these patterns.

There have been some correlations found between physical dysfunctional states and blood groups. Since some of these disorders have possible psychosomatic components, such as duodenal ulcer or MI, Neumann and colleagues (1991) wanted to focus on the relationship between blood groups and various indices of behavior patterns in young patients who had an initial MI. Examples of the behavior patterns used were type A behavior scores and anger

ratings. Results indicated that patients with the group O phenotype scored higher than patients with the group A phenotype on type A behavior scales. Group B patients responded on several scales between those with group A and group O (Neumann, Chi, Arbogast, Kostrzewa, & Harvill, 1991). Authors suggested that these results might be used in future research to predict MI.

Summary of ABO blood group associations with disease. The statistical associations of ABO blood group with disease that are of most interest are those with malignancy, peptic ulcers, coagulation and infection (Table 5). Many of the early statistical associations now have some associated scientific findings that suggest a rationale for the associations (Garratty, 1994). Table 6 provides a more detailed historical perspective of the published works in each of the broad disease or disorder categories covered in this manuscript. The diseases or disorders in the table had a positive association with the ABO indicated in the table, unless otherwise noted that the ABO was found to have a "protective effect" for the particular disease or disorder.

ABO blood groups and survival. While the biological role of ABO antigens is uncertain, it has been postulated that the polymorphic genes of the ABO blood group provide the diversity needed to survive changing environmental conditions and evolving pathogens (Marionneau et al., 2001). Although there have been many research endeavors regarding the associations of ABO blood groups with specific diseases, fewer have examined an association with ABO blood groups and overall survival or lifespan. What follows is a review of the few studies that have examined ABO blood group and lifespan from a macro-perspective.

A 2006 study addressed the effectiveness of liver transplant, as measured by 1-year survival (Hong et al., 2006). The authors performed a retrospective review of 1164 liver transplant recipients in Canada, and ABO blood group was included as an independent variable.

The results indicated that the patient's ABO blood group had a significant effect on recipient survival. Specifically, group B patients had a 12 percent higher survival probability; and group AB patients had a 7 percent lower survival probability compared to group O (Hong et al., 2006). The authors offer no possible explanations for these findings and merely state that this phenomenon is not clearly understood.

Another study investigated the association between blood groups and life expectancy (Shimizu et al., 2004). The authors compared frequencies of ABO blood groups in 269 centenarians (persons over 100 years old) living in Tokyo and those in 7153 regionally matched controls. Differences between centenarians and controls and between observed and expected frequencies were investigated by Chi Square tests. Group B was observed more frequently in centenarians than in controls ($\chi^2 = 8.41$, $p = 0.04$). This suggests that group B might be associated with exceptional longevity. This conclusion is limited since the predominance of blood group B among centenarians was not statistically significant. Additionally, the authors tabulated diagnoses of hypertension (n=78), cardiovascular disease (n=51), apoplexy (n=37), diabetes (n=9), femoral fracture (n=66), malignancy (n=24), and chronic lung disorder (n=29) in the centenarians. One-third of the centenarians were free of these diseases, but this did not correlate with the group B centenarians. Seventy-seven percent of the group B centenarians had one or more of these diagnoses. The authors concluded that this finding indicates that group B persons are more likely to survive serious disease rather than escape it. The authors did not report disease frequency for the control group. Because plasma concentrations of VWF (a cardiac risk factor) are lower in group O (O'Donnell & Laffan, 2001), one would expect a predominance of group O; but the frequency of group O in centenarians was lower (Shimizu et al., 2004). Shimizu and colleagues (2004) do not offer a biological explanation for these

findings except to state that group B contributes to longevity by some biochemical mechanism that allows this group to better survive disease.

To assess the validity of Shimizu and colleagues' (2004) observations, Brecher and Hay (2011) collected data on the ABO blood groups of patients who died in a United States tertiary care hospital over a 1-year period. If group B was a marker for a longer lifespan, it would be expected that the percentage of group B patients would rise with age at the time of death and those of other blood groups would decline. A total of 772 patients were included in the study and were presented as ABO proportion stratified by age (Brecher & Hay, 2011). The researchers found that the percentage of group B patients declined with age, and this result was statistically significant ($p<0.01$; $R^2 = 0.68$). None of the other blood groups showed a statistically significant increase or decrease when plotted against decade of death (Brecher & Hay, 2011). Additionally, when survival curves for the ABO blood groups were compared, group B exhibited decreased survival compared to the other groups (Figure 1). Inspection of the survival curves shows that most of the difference occurs at the younger ages. Although the aim of this study was to investigate a relationship of ABO blood group with longevity, when examining the frequencies of death stratified by decade, it is important to note that the sample revealed a disproportionate number of deaths in the first decade of life. It is not possible from the data here to theorize a cause for this, but it is important to note since diseases processes in the young may be different than older patients. Overall, these results suggest that group B is not a marker for longevity, but may actually be a marker for earlier death. Although these results are pertinent to the United States population, and provide interesting information, the authors did not control for race or gender, and did not collect information on disease comorbidities.

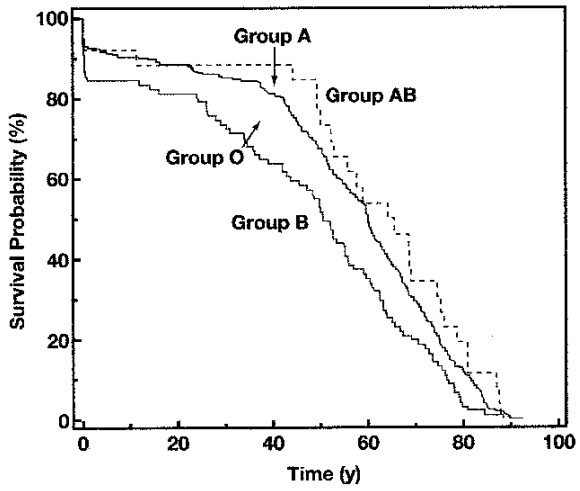

Figure 1. ABO Blood Group Survival Comparison.

Survival curves showing the decreased survival of people with group B blood compared with people with groups O, A, and AB over time ($P < .01$). There was no statistical difference in groups A, O, and AB ($P = 0.47$). From "ABO Blood Type and Longevity" by M.E. Brecher and S.N. Hay, 2011, *American Journal of Clinical Pathology*, 135, p. 97. Copyright 2011-2014 American Society for Clinical Pathology, Copyright 2011-2014 American Journal of Clinical Pathology. Reprinted with permission.

Using the same 2004 data set as the Brecher and Hay (2011) study, a pilot analysis was performed using Analysis of Covariance (ANCOVA) and controlling for race, gender and transfusion history. This sample (n=771) resulted in overall ABO frequencies similar to the general population (Table 7) and a bimodal distribution of age at the time of death (Figure 2). The sample consisted of 404 (52.4%) males and 367 (47.6%) females and the majority of the population had been transfused (76.1%). The racial composition was White (62.6%), Black (31.4%), Hispanic (3.0%), Asian (0.6%), Native American (0.8%) and unknown (1.6%). The estimated marginal mean age at the time of death was lowest for the group B patients at 48.0 years and highest for group AB at 62.5 years (Figure 3). Although this could be explained by a higher prevalence of group B Black persons, the analysis found a statistically significant difference, F(3, 763), p=0.018, in the age at the time of death among the ABO blood groups when controlling for race, gender and transfusion history.

Table 7.

ABO Blood Group Frequencies in 2004 Pilot Data

Phenotype	Frequency	Percentage(%)
O	389	50.5
A	264	34.2
B	92	11.9
AB	26	3.4

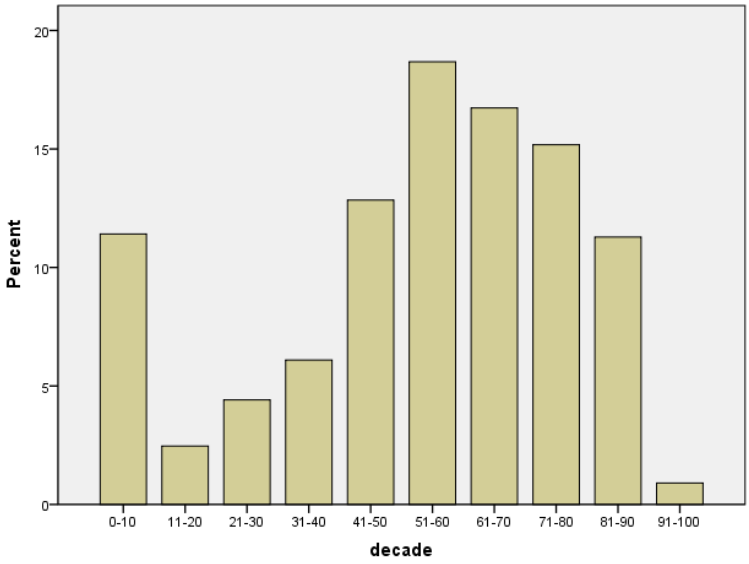

Figure 2. Distribution of Age at the Time of Death, Stratified by Decade of Death.

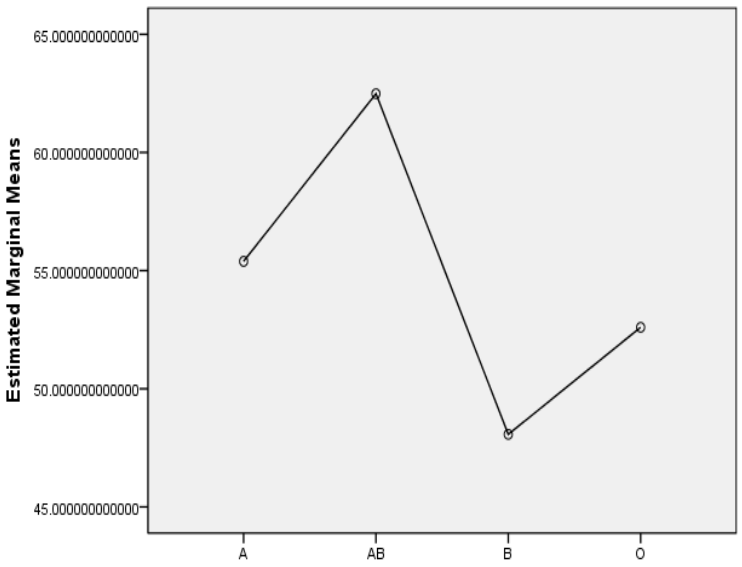

Figure 3. Estimated Marginal Means of Age at Death for each ABO Blood Group

Most recently a Sicilian population of 38 centenarians and 59 healthy controls were studied to examine a relationship between ABO and longevity (Vasto et al., 2011). This group of centenarians (age range 100-107) had no cardiac risk factors or other age-related diseases. The control group (age range 45-65) was recruited from blood donors and judged to be healthy on the basis of clinical history and blood tests (complete blood cell count, erythrocyte sedimentation rate, glucose, urea nitrogen, creatinine, electrolytes, C-reactive protein, liver function tests, iron, proteins, cholesterol and triglycerides). Samples were genotyped to determine ABO blood group and Chi Square analysis was used to determine the statistical significance of differences in ABO of centenarians and controls. Although statistical values were not reported, the authors concluded that the frequencies of ABO blood groups in the centenarians and controls did not differ significantly. The inferences are limited due to the small sample size and regional population, but this study provides an additional disparate conclusion concerning a relationship among ABO blood groups and longevity and more support for larger studies to address this question.

The scientific literature provides a variety of data regarding the relationship of ABO phenotype with diseases and disorders, as well as overall length of life and survival. Reports of positive correlations exist for: infectious disease, heart disease, pulmonary function, cancer, obesity, length of life, survival, and even behavioral traits. It stands to reason that if a particular blood group provides a protective mechanism against serious illness, then that blood group should exhibit a longer lifespan. While the overwhelming majority of reports indicate an association with a particular ABO phenotype, it is important to note the potential for positive outcome bias. It is possible that studies indicating no association with ABO phenotype are less likely to be submitted for publication or to be accepted for publication.

Transfusion and Mortality

Since 1981 investigators have provided evidence that transfusion presents risks beyond infectious disease and incompatibility (Kamper-Jorgensen et al., 2008; Kleinman, Marshall, AuBuchon, & Patton, 2004; Tynell, Norda, Shanwell, & Bjorkman, 2001; Tynell, Norda, Montgomery, & Bjorkman, 2005; Vamvakas & Taswell, 1994; Vamvakas & Moore, 1997; Wallis, Wells, Matthews, & Chapman, 2004; Whyte, 1988). These findings along with the fact that up to 71 % of hospitalized patients are transfused before they die (Hay, Scanga, & Brecher, 2006), make transfusion history an important factor to consider when examining lifespan or mortality in any group. Blood transfusions (including all blood components such as red cells, platelets, and plasma) more than doubled from 1997 to 2007 (Agency for Healthcare Research and Quality, 2009) and approximately 12 million units of blood are transfused to 3.5 million patients each year in the United States (Goodnough, Soegiarso, Birkmeyer, & Welch, 1993). The practice of transfusion is in transition. While the risks of contracting life threatening infections, such as Human Immunodeficiency Virus (HIV), from blood transfusion are well

understood, some believe the danger posed by the transfusion itself is more serious (Robinson et al., 2005). There is a substantial body of evidence demonstrating strong associations between transfusion and adverse outcomes in a range of clinical settings, including cardiac surgery and acute coronary syndrome (ACS), where transfusion is administered for reasons other than life threatening bleeding (Gerber, 2008; Marik & Corwin, 2008; Murphy et al., 2007; Reeves & Murphy, 2008; Taylor et al., 2006). Some evidence suggests that anemia is not tolerated as well in critically ill patients (Carson et al., 1996; Hebert et al., 1997), and therefore, prior standard practice has called for red cell transfusions at a lower hemoglobin threshold to improve oxygen delivery in an effort to avoid the harmful effects of oxygen debt. However, optimal transfusion practice in this patient group is being questioned due to reports of adverse outcomes including postoperative pneumonia, sepsis, immunosuppression, myocardial infarction, heart failure, stroke, renal failure and mortality. Some adverse reactions such as bacterial contamination leading to sepsis and incompatibility between the donor and recipient leading to renal failure are well understood; however, for other adverse outcomes, the etiology is less clear. For example, heart attack and stroke have been reported (Murphy et al., 2007), but a causal relationship has not been established. The most notable study to date, a randomized controlled trial of liberal versus conservative red blood cell use (Hebert et al., 1999), demonstrated that, for patients who were less acutely ill, mortality was lower in the group receiving fewer transfusions and, for high risk patients, mortality was similar in the liberal and conservative transfusion groups. The investigators did not postulate explanations for their findings except to mention that critically ill patients may be at increased risk for the immunosuppressive (Bordin, Heddle, & Blajchman, 1994; van de Watering et al., 1998) and microcirculatory (Baker, Wilmoth, & Sutton, 1986; Langenfeld, Livingston, & Machiedo, 1991) complications of red cell transfusion. Future

endeavors should include randomized trials to investigate if transfusion causes adverse outcomes, and secondly, to identify patient groups in which the benefit of transfusion outweigh these risks.

Survival after transfusion. A summary of the literature addressing post-transfusion survival lends support to including transfusion as an important variable when examining mortality or lifespan (Table 8). Even though not all of the studies are generalizable to the United States population or free of bias or historical concerns, the results are consistent in finding increased mortality among transfused patients. Three population-based analyses of post-transfusion survival in the United States have been published. The first followed a cohort of 802 patients transfused in 1981 in one Midwestern United States county (Vamvakas & Taswell, 1994), and the second followed surgical patients who were transfused perioperatively in 1986 through 1988 in the same county (Vamvakas & Moore, 1997). The third study is an observational study that used data extracted from a managed care administrative claims database and is the largest United States study to date addressing post-transfusion survival with a sample of 6779 patients (Kleinman et al., 2004).

Kleinman and colleagues (2004) reported an overall annual mortality of 31 percent in year one after transfusion, 14 percent in year two, and 10 percent in each of years three through five. Compared to earlier work (Vamvakas & Taswell, 1994), these researchers report a seven percent decreased survival at year one and a 14 percent decreased cumulative survival at year five (Kleinman et al., 2004). The authors attribute this to the greater percentage of their population over age 65 and report that transfusion mortality was much higher in recipients older than age 65. The earlier two studies lack generalizability to a current United States population due to their timeframe and the changes in transfusion practice over time, lack of representation

Table 8.

Comparison of Different Studies of Survival After Transfusion

Author	Year of Transfusion	N	Location	Time after transfusion (%) 1 year	2 years	5 years	7 years
Vamvakas and Taswell (1994)	1981	802	US	76	70	60	48
Kamper-Jorgensen, et al. (2008)	1983-2002	1,118,261	Denmark and Sweden	74	NR[a]	53	NR
Whyte (1988)	1984	367	New Zealand	79	75	NR	NR
Vamvakas and Moore (1997)	1986-1988	1540	US	83	78	67	NR
Tynell, et al. (2001)	1993	1734	Sweden	66	58	NR	NR
Wallis, et al. (2004)	1994	2899	Northern England	68	59	47	NR
Kleinman, et al. (2004)	1995	6779	US	69	60	46	NR
Tynell, et al. (2005)	1993	932	Sweden	68	59	45	39
Tynell, et al. (2005)	2000	990	Sweden	74	NR	NR	NR

[a] NR = Not reported

from other geographic areas in the United States, and the inclusion of only surgical patients in the second study. Because all of the study participants studied by Kleinman and colleagues (2004) were enrolled in managed care, it is possible that this population was healthier than the populations of the earlier studies. The populations of each of these studies are problematic because survival rates of transfused patients would be expected to differ between patient groups, and none of the United States studies to date have sampled the general population.

A fourth United States study examined Medicare beneficiaries with a new Myelodysplastic Syndrome (MDS) diagnosis in 2003 (Goldberg et al., 2010). Although the

primary aim of this study was to determine incidence of complications of MDS among Medicare beneficiaries, the findings regarding survival post-transfusion were striking. A retrospective review of United States Medicare claims was performed to determine the incidence, clinical and economic consequences of MDS, focusing on those beneficiaries greater than 65 years old. The symptoms of MDS reflect peripheral cytopenias and for most patients with MDS, supportive care with blood transfusions is a requirement of treatment. Compared with the MDS patients who were not transfused, the MDS patients who received blood transfusions had a greater prevalence of comorbidities such as cardiac events (82.4% in transfused, 67.1% in nontransfused, $p < 0.001$), diabetes mellitus (44.4%, 37.1%, $p = 0.10$), dyspnea (62.9%, 40.4%, $p < 0.001$), and infectious diseases (81.0%, 55.7%, $p < 0.001$). Additionally, the authors noted that although acute myeloid leukemia developed within 3 years in 9.6% of patients, there was a much higher transformation among those who had received transfusions (24.6%, $p < 0.001$). The 3-year adjusted survival for MDS patients was 60%, significantly lower than for the general Medicare population (84.7%; hazard ratio, 3.08; $p <0.001$), and nontransfused patients had a higher 3-year-age-adjusted survival rate than transfused patients (69.0% versus 40.9%). After adjusting for age, transfusion was associated with an increased risk of death among patients with MDS (hazard ratio, 2.41; 95% CI, 1.84-3.15; $p < 0.001$). The authors noted increased rates of comorbidities, transformation to acute leukemia and a decreased overall survival rate for transfused MDS patients when compared to nontransfused MDS patients. Some of this effect could be related to an increased need for transfusion among patients with more advanced disease; however transfusion dependency identifies patients with MDS at additional risk of shortened survival.

Post-transfusion mortality in the United States appears to be similar to European findings and suggests that data collected during similar timeframes in different developed countries may be generalizable to other geographical locations. Kamper-Jorgensen and colleagues (2008) examined short and long-term mortality among transfusion recipients versus the general population using blood bank databases and administrative data in Denmark and Sweden. This descriptive study of over one million transfusion recipients provided absolute and relative survival after transfusion and concluded that survival was poorer in men versus women, and in all but the 0 to 19 age group, increased age at first transfusion was associated with worse survival. Absolute mortality was high in the period shortly after transfusion. Standardized mortality ratios were 17.6 times higher three months post-transfusion. Even 17 years after transfusion, the data show a 30 percent increase in mortality. Although Kamper-Jorgensen and colleagues (2008) use mortality data from the general population for comparison, matched groups with similar baseline characteristics who are not transfused are needed to draw stronger conclusions regarding causation.

Conclusions concerning the relationship between blood transfusion and an increased risk of death cannot be drawn from the post-transfusion survival studies conducted to date. Thus far, studies have been descriptive, not etiologic. However, as suggested by Kamper-Jorgensen (2008), the poor survival reported after blood transfusion reflects the fact that transfusions are given to patients who already are at increased risk of dying from trauma, surgeries or serious illness.

Survival rates of transfused patients are expected to differ between patient groups and data on survival need to be related to the case mix. Transfusion criteria have changed over time due to concerns about the blood product supply, the safety of blood products after the emergence

of HIV, or the demand for more aggressive treatment in elderly patients. Because transfusion practice could be more aggressive or more conservative depending on timeframe or patient group, caution should be exercised when making direct comparisons of absolute survival between studies. Differences in survival could also be explained by the use of different transfusion triggers, population specific characteristics, variation in transfusion policy or differences in standards of medical care between countries, between hospitals, or over time. The strength of these associations across a range of clinical settings suggests that confounding and bias, the chief limitations of all observational studies, are unlikely to account for all of these observations.

Cardiovascular disease, transfusion and survival. It is recognized that patients with cardiovascular disease may be adversely affected by anemia (Jan & Chien, 1977). A retrospective study of 1958 patients who refused blood transfusion reported an increase in the risk of death (Carson et al., 1996) with decreasing preoperative hemoglobin concentrations in patients with cardiovascular disease compared to other patients (Carson et al., 1996). Although transfusing blood to anemic patients with ischemic heart disease may theoretically increase oxygen delivery and improve outcomes, there is no definitive evidence to support transfusion. In fact, studies of patients following cardiovascular surgery and patients in circulatory shock report no increase in tissue oxygenation with blood transfusion (Casutt et al., 1999; Dietrich, Conrad, Hebert, Levy, & Romero, 1990; Fortune et al., 1987). Despite consistent findings from physiological and observational studies regarding the risks of anemia in patients with ischemic heart disease, there remains limited evidence that blood transfusions increase survival in this population. Conflicting results have been reported in studies attempting to discern whether blood transfusions improve outcomes in patients with ischemic heart disease (Hebert et al., 1999;

Rao et al., 2004; Wu, Rathore, Wang, Radford, & Krumholz, 2001). Small randomized trials with samples ranging from 25 to 99 patients report no difference in outcomes for groups randomized to restrictive or conservative transfusion strategies (Bush, Pevec, & Holcroft, 1997; Fortune et al., 1987; Johnson et al., 1992).

In the largest randomized trial to date, Hebert and colleagues (1999) examined whether a restrictive transfusion strategy versus a liberal transfusion strategy produced equivalent results in critically ill patients. This study of 838 patients compared rates of all-cause mortality at 30 days and severity of organ dysfunction. Thirty day mortality was similar in both groups, but the rates were lower with the restrictive strategy among patients who were less acutely ill and patients who were less than 55 years old. Rates were not lower among patients with clinically significant cardiac disease. The mortality rate during the hospital stay was significantly lower in the restrictive strategy group. Hebert and colleagues (1999) concluded that the restrictive strategy is at least as effective as and possibly superior to a liberal transfusion strategy in critically ill patients, with the possible exception of patients with acute myocardial infarction (MI) and unstable angina.

In contrast, Wu and colleagues (2001) retrospectively studied 78,974 Medicare beneficiaries who were hospitalized with a primary diagnosis of acute MI and categorized patients according to their admission hematocrit level. Only 3680 patients in this sample received a blood transfusion. The authors used Chi square analysis to evaluate the association between blood transfusion and 30-day mortality among hematocrit groups and reported that patients with lower hematocrit values had increased 30-day mortality. Patients were categorized based on their hematocrit at admission. The investigators found that blood transfusion was associated with decreased 30-day mortality among the patients with hematocrit values of

between 5 and 33 percent and with lower short-term mortality among elderly patients with acute MI if hematocrit was less than or equal to 30 percent at admission. Patients who received a transfusion despite having a hematocrit greater than 36 percent had a higher risk of death within 30 days than patients with similar hematocrit that were not transfused. When the patients that died within two days of admission were removed from the data, then transfusion was associated with lower mortality among patients with hematocrit less than or equal to 30 percent. This study was among the first to demonstrate that red blood cell transfusion may be beneficial in patients with acute MI. However, the results are limited by biases. This study suffered from a low rate of transfusion (4.7%). Additionally, the group of patients that were transfused differed significantly from the group that was not transfused with regard to clinical presentation and coexisting factors, and the analysis was not based on the hematocrit value associated with the transfusion, rather the hematocrit at admission. Wu and colleagues (2001) attempted to account for survivor bias in a secondary analysis by excluding patients who died within two days of admission. This eliminated the association between transfusion and improved mortality in patients with hematocrits between 30 and 33 percent.

Following the results of Wu and colleagues (2001), Rao and colleagues (2004) attempted to overcome some of these limitations. Rao and colleagues (2004) used detailed prospectively collected data, focused on a patient population that required more aggressive intervention and greater exposure to blood products and used more robust statistical techniques to better adjust for the influence of baseline characteristics and time. The authors reported that transfusions were not associated with improved survival when nadir hematocrit values were in the range of 20 percent to 25 percent and were clearly associated with worsened outcomes when values were greater than 30 percent. Rao and colleagues (2004) conclude that transfusion in the setting of

ACS is associated with higher mortality even after adjustment for other predictive factors and timing of events. Limitations of this study include a smaller sample (2400 transfused patients) that would not allow for detection of an overall mortality difference as large as 1 percent, the potential for unmeasured confounders that could account for increased mortality with transfusion, and the use of patients enrolled in a clinical trial as they could differ from the general population.

Although their patient populations differ in age and comorbidities, both Wu and colleagues and Rao and colleagues (2004) demonstrated that patients who receive red blood cells at a higher hematocrit level appear to be harmed by transfusion. At hematocrit levels less than 30 percent it is possible that the interpretation given by the authors represents aspects of the true effects, especially since there are many differences between studies. Wu and colleagues (2001) derived observations from Medicare claims data from a wide population of elderly patients who had acute MI, whereas Rao and colleagues (2004) only included younger individuals who required aggressive interventional management. It is plausible that a higher transfusion threshold would benefit elderly patients because of the greater degree of diffuse vascular disease, the presence of additional comorbid illnesses, or the inability to augment cardiac output as a means of compensation for anemia. Younger patients may derive less benefit from transfusions because of widespread use of aggressive revascularization procedures, statins, anti-platelet agents and other therapies.

Most recently, Weightman and colleagues (2009) conducted a prospective observational study of patients who underwent cardiac surgery. The association between length of survival, blood products transfused and risk factors for long-term survival at entry to the study were determined by Cox proportional hazards regression. The authors reported no association

between transfusion and long-term survival. The sample size (1062) was small compared to other observational studies and the participants included low-risk cardiac surgery patients. The authors also included anemia as a risk factor in their analysis. Preoperative anemia is a predisposing factor for blood transfusion, and it could be argued that any association between anemia and adverse outcome is actually the result of blood transfusion. In addition, the investigators stratified the data into four groups based on the number of units transfused. It is possible that stratification diluted the conclusions and lead to a Type II error. The group without transfusion was disproportionately large compared to the other three groups, despite an overall transfusion rate in the study of 57 percent. The authors acknowledge that the study is inadequately powered. Another difference in this study is the inclusion of plasma, platelets and cryoprecipitate in equal weight to red cell transfusions. This confounds the analysis and makes this study different than previous studies. It is unlikely that the impact of acellular blood components would be identical to that of red cells.

Red cell transfusions are standard practice for many patient groups including critical care, but there are contradictory views on the risks of anemia and the benefits of transfusion. The body of evidence indicates various adverse effects including pneumonia, sepsis and mortality in patients who receive blood transfusion (Gerber, 2008; Marik & Corwin, 2008; Murphy et al., 2007; Reeves & Murphy, 2008; Taylor et al., 2006). Although an association with transfusion and overall survival is suggested (Kamper-Jorgensen et al., 2008; Kleinman et al., 2004) , contemporary data from a United States population and evidence from randomized trials are lacking. Many investigators have focused their research question on the survival of patients after transfusion in the context of ACS (Hebert et al., 1999; Rao et al., 2004; Weightman, Gibbs, Sheminant, Newman, & Grey, 2009; Wu et al., 2001). The lack of randomized trials and the

apparent contradictory results from the observational studies leave this topic up to debate. Based on current studies, it is evident that transfusions in the presence of ACS are rarely beneficial when the hematocrit level exceeds 30 percent (hemoglobin > 10.0 g/dL) in the absence of acute blood loss. It is reasonable to conclude that the benefits of transfusion exceed the risks when hemoglobin concentrations fall below 7.0 g/dL. Between 8.0 and 10.0 g/dL, there remains no consensus. Randomized controlled trials in different populations of patients with ischemic heart disease are needed. Information on survival of transfusion recipients is essential if risk-benefit and cost-effectiveness calculations are to be made.

Life Expectancy in the United States

Because the primary outcome of this research endeavor is length of life or lifespan, it is important to consider the life expectancy of the pertinent population and how it may differ when other risk factors (such as gender or race) are included. Life expectancy is a hypothetical measure that uses current age-specific death rates to predict the future survival of a cohort. This prediction is necessary as calculating actual life expectancy would be unreasonable due to the requirement for following a particular cohort until the death of the last survivor. As a measure, life expectancy is often used to gauge the overall health of a population. Life expectancy is calculated as the average number of years of life remaining for persons who have attained a certain age. As a summary measure of mortality, life expectancy represents the average number of years of life that could be expected if current death rates were to remain constant (Jekel, Katz, Elmore, & Wild, 2007). Death rates at or above a given age, independent of mortality at younger ages, are used to predict life expectancy. Changes in life expectancy can be used to help explain trends in mortality (Caselli, Vallin, & Wunsch, 2006).

The National Center for Health Statistics provides data on life expectancy in the United States (Table 9). The United States Life Tables provide data by gender and by race for Whites and Blacks, but does not further differentiate by race. From 1900 though 2004, life expectancy at birth increased 29 years for men (to age 75) and 32 years for women (to age 80). Life expectancy at age 65 also increased during this period rising from 12 to 17 years for men and from 12 to 20 years for women. In contrast to life expectancy at birth, which increased sharply early in the 20th century, life expectancy at age 65 improved primarily after mid-century. Decreased death rates are explained by improved access to health care, advances in medicine, healthier lifestyles, and better health before age 65. In 2004, life expectancy at birth showed a disparity among race with 76 years for White males compared with 70 years for Black males and 81 years for White females compared with 76 years for Black females. However, life expectancy at birth increased more for the Black than for the White population between 1990 and 2004. During this period, the gap in life expectancy between White males and Black males narrowed from 8 years to 6 years. During the same period, the gap in life expectancy between White females and Black females decreased from 6 years to 5 years. The gap in life expectancy between White and Black people at age 65 is narrower than at birth. Between 1990 and 2004, the difference in life expectancy at age 65 between White males and Black males remained stable at 2 years. In 2004, life expectancy at age 65 was 17 years for White males and 15 years for Black males. The difference in life expectancy between White and Black females has also been stable in recent years; in 2004, at age 65, White females and Black females could expect to live an additional 20 and 19 years, respectively (National Center on Health Statistics, 2007).

Socioeconomic status. Also related to lifespan and virtually all health outcomes in most countries is socioeconomic status. People with more education or income live longer and

Table 9.

United States Life Expectancy (in years) by Race and Gender

	Male	Female	Total
White	75.7	80.8	78.3
Black	69.5	76.3	73.1
All Races	75.2	80.4	77.8

Note. From "*Health, United States, 2007 with Chartbook on Trends on the Health of American,"* The National Center for Health Statistics, 2007, Hyattsville, MD.

experience fewer adverse health events. Racial or ethnic disparities do not simply reflect differences in income and the likelihood of poor or fair health are seen within each income group (Robert Wood Johnson Foundation, 2008). Blacks, American Indians, Hispanic Americans, Pacific Islanders and some Asian-American groups are disproportionately represented among the more socioeconomically disadvantaged groups in the United States. This reflects a long history of racial inequality in which race or ethnic origin was legally used to exclude individuals from employment, educational opportunities and property ownership. Although most explicit uses of race to demean or exclude people from participation in society have been outlawed, racial residential segregation persists.

A report from the Robert Wood Johnson Foundation (2008) describes the scope of health disparities in the United States, specifically how the poor and middle class are less healthy and how factors in our society contribute to health disparities. In the United States, where education and income are the most frequently used measures of socioeconomic status, Americans who are poor and those who have not graduated from high school experience considerably worse health on average than more affluent or educated Americans. Health differences across income and education groups are seen in a range of health conditions from the beginning of life to old age.

Individuals with lower family incomes are more likely to have a chronic disease that limits their activity. Poor adults are more than three times as likely as affluent adults to report activity limitation due to chronic illness. Lower income also means higher rates of type 2 diabetes. Poor adults are twice as likely as affluent adults to have diabetes, which is a major cause of severe illness, disability and premature death. Similar patterns are seen for coronary heart disease. The rate of coronary heart disease, the leading cause of death in the United States, is nearly 50 percent higher among poor adults than among the most affluent adults (Robert Wood Johnson Foundation, 2008).

In one study, researchers at the United States Centers for Disease Control and Prevention (CDC) estimated that 38 percent of the two-fold excess mortality among Black adults compared with Whites in the United States was related to differences in income (Otten, Teutsch, Williamson, & Marks, 1990). The role of genetic differences in health disparities has been debated, but scientific sources have concluded that race is primarily a social, not biological, construct (American Association of Anthropology, 1998; American Association of Physical Anthropologists, 1996).

It has long been recognized that those of lower socioeconomic status have higher mortality in the United States (Kitagawa & Haser, 1973). Differential socioeconomic status is assumed to be the cause of large differences in healthy life expectancy for racial groups in the United States (Crimmins, Saito, & Ingegneri, 1989; Hayward & Heron, 1999; Sullivan, 1971; Williams & Collins, 1995). At older ages, the reported pattern of differences in healthy life expectancy by race varies somewhat (Branch et al., 1991; Guralnik, Land, Blazer, Fillenbaum, & Branch, 1993; Manton & Stallard, 1991). In a national sample of age 70 and over, African American and White total life expectancy was quite similar, while White healthy life expectancy

exceeded African American healthy life expectancy (Crimmins, Hayward, & Saito, 1996). However, in a North Carolina sample, African American life expectancy above age 75 has been found to exceed White values (Guralnik et al., 1993; Land, Guralnik, & Blazer, 1994). Crimmins and Saito's (2001) report examined differences in healthy life expectancy, which summarizes the combined effects of different levels of mortality and morbidity on the overall length of healthy life. It provides a summary indicator of the total health impact of differences in socioeconomic wellbeing. This report examined healthy (disability free) life expectancy by gender and education for Whites and African Americans in the United States at three dates (1970, 1980 and 1990). The authors reported large racial and educational differences in healthy life expectancy at each date (Crimmins & Saito, 2001). They reported that the differences by education in healthy life expectancy are even larger than differences in total life expectancy. Arthur and colleagues (2008) sought to determine whether race was associated with risk of in-hospital death after injury. They found that relative to injured White patients, Black and Asian patients had a higher risk of death (1.5% vs. 2.1%). Other racial/ethnic groups showed no significant mortality difference from White patients. The authors concluded that Black and Asian patients have a higher risk of death after injury than White patients (Arthur, Hedges, Newgard, Diggs, & Mullins, 2008).

Summary

The particular problem addressed by this project is whether individuals belonging to different ABO blood groups differ in their length of life or lifespan. The question is not a new one. As long ago as 1921, investigators examined associations of ABO blood groups with peptic ulcer, pernicious anemia and other malignant diseases (Buchanan & Highley, 1921). Interest decreased for the next 30 years and then Aird and colleagues (1953) published a paper showing

that there was a striking relationship between group A and carcinoma of the stomach. Since this time, a great deal of literature has accumulated.

There are some undeniable associations of blood groups with disease. Blood group antibodies can cause hemolytic transfusion reactions, hemolytic disease of the newborn and autoimmune hemolytic anemia, graft rejection and spontaneous abortion. What is more open to debate is whether the many statistical associations with other diseases have clinical significance, and whether blood group antigens have a biologic role. Much progress has been made since the early reports of statistical associations of ABO blood groups and various diseases. Some of the seemingly coincidental associations now have a scientific basis. As Garratty (2000) suggests, even though blood groups were discovered in the process of trying to successfully transfuse blood, "common sense should dictate that blood group antigens and antibodies are not there only to give us problems in this unnatural process."

ABO blood group antigens, first identified on RBCs, are now known to be important as receptors and ligands for microbes and immunologically important proteins. Increasing evidence indicates that some blood group antigens may play a biologic role, but that role may not be directly related to the RBC (Garratty, 2000). Immunohematologists labeled these chemical structures as "blood group" antigens because they caused problems in transfusing blood. However, as Garratty (2000) proposes, it should be remembered that the anti-A and anti-B are naturally occurring antibodies probably because they are antibacterial antibodies that cross-react with RBCs. Contemporary findings in the fields of membrane chemistry and molecular biology add some rationale to some of the earlier statistical associations found. However, it remains unclear if blood group antigens play a biological role or if there is an association with ABO blood group and lifespan.

It is clear from the abundance of published works on the association between ABO phenotype and a myriad of human conditions that it is significant and ubiquitous. Researchers have come to conflicting conclusions on relationships and it is evident that further studies must be conducted. Since ABO blood group is a characteristic of all people, this study has the potential to provide information about the human race, including correlations of ABO blood groups with diseases and disorders. Although environmental characteristics may influence length of life and proliferation of a particular blood group, the fact that ABO blood group is entirely a genetic component makes this topic of great importance and globally relevant.

The geographic inheritance patterns of ABO blood groups and how ABO blood group relates to environmental factors to improve health or escape illness has been questioned almost since the ABO blood system was first discovered. This project will answer the question, is there a relationship between ABO blood group and lifespan in a hospitalized Southeastern United States population? It is relevant because existing evidence regarding a relationship among ABO blood groups and lifespan is slight and studies are contradictory. This non-experimental, retrospective study will have a target population of all patients who died at three tertiary care centers over a one-year period. A statistical model will be employed to determine length of life across ABO blood groups and ANCOVA will be used to test for differences in the mean lifespan of patients. This investigation of the overall association of ABO blood group and lifespan from a macro-perspective in a large population and controlling for covariates such as race and gender is a first step towards determining if blood group antigens have a functional role. A broad scale study of this magnitude has yet to be performed in the United States. Consequently, the results of this study combined with the advances in molecular genetics and cellular biochemistry will

allow us to further investigate biochemical mechanisms responsible for longer life spans for persons of a particular blood group.

Chapter Three: Methods

The details of the research design are provided in this chapter and include: identification of the methodology, variables, population and sample, data collection procedures and analytical procedures. Because previous research efforts suggest that certain ABO blood groups are linked to protection from various diseases or disorders (Aird, Bentall, & Roberts, 1953; Brecher & Hay, 2011; Gonzalez Ordonez, Medina Rodriguez, Martin, Alvarez, & Coto, 1999; Kingsbury, 1971; Shimizu et al., 2004; Suadicani, Hein, & Gyntelberg, 2007), it stands to reason that individuals of a particular group will demonstrate a longer lifespan. A particular ABO blood group may contribute to length of life via biomedical mechanisms favorable for surviving or eluding serious disease. A vast number of research endeavors point to an association of ABO blood groups with specific diseases, but the biological role of ABO antigens remains uncertain. Fewer studies have examined an association with ABO blood groups and lifespan or longevity, (Brecher & Hay, 2011; Hong et al., 2006; Shimizu et al., 2004; Vasto et al., 2011) and the results are inconsistent. The proposed project is the largest to date to examine the relationship between ABO blood group and lifespan from a macro-perspective and includes covariates not previously studied.

Unquestionable associations of disease with blood group include hemolytic transfusion reactions, hemolytic disease of the newborn, autoimmune hemolytic anemia and graft rejection (Roback, Grossman, Harris, & Hillyer, 2011). Statistical associations have been reported with ABO blood group and malignancy, thrombosis, peptic ulcers, bleeding and infectious disease

(Aird et al., 1953; Aird, Bentall, Mehigan, & Roberts, 1954; Hein, Suadicani, & Gyntelberg, 1998; Robert et al., 2000; Shimazu, Shimaoka, Sugimoto, Taenaka, & Hasegawa, 2000; Suadicani et al., 2007). The biological role of ABO antigens remains uncertain. It has been proposed that the polymorphic genes of the ABO blood group provide the diversity needed to survive changing environmental conditions and evolving pathogens (Marionneau et al., 2001). Although the statistical associations with ABO blood group and disease are prevalent in the literature, fewer studies have examined an association with ABO blood group and overall survival or longevity. Shimizu and colleagues (2004) compared frequencies of ABO blood groups in centenarians in their Tokyo population, and Brecher and Hay (2011) collected data on the ABO blood groups of a population of patients who died in a United States tertiary care center. While Shimizu and colleagues found group B more frequent in the centenarian population, the prevalence of group B declined with age in the United States population. Thus, the findings of Brecher and Hay suggest that group B is not a marker for longevity. Both of these studies conflict with a report from a Sicilian population that found no difference in ABO blood groups with regard to longevity (Vasto et al., 2011). Because the existing evidence is scarce and the results are contradictory, this project further examined the relationship between ABO blood group and lifespan in a Southeastern United States population. By assessing a larger population and controlling for more variables, this study provided more robust data for analysis and a foundation for future studies to investigate this question at the molecular level or from an aspect of glyconomics (the study of sugar-modifications to proteins that affect the structure and function of blood group antigens).

Research Design

The proposed project answered the question, "Among patients who die in a hospital, is there a significant relationship between ABO blood group and lifespan?" An epidemiological approach was used to arrive at a conclusion using a retrospective study and analysis of covariance techniques. The sample population included all patients who expired at three tertiary care centers over a one-year period. If a significant relationship exists between ABO blood group and the lifespan of patients, as determined by age at death, in a tertiary care center setting, this study design would allow for this determination. ANCOVA was used to determine if a significant difference in mean age at death exists among the ABO blood groups of this population. The study was based on the following assumptions: (1) the data needed could be collected from a retrospective review of electronic blood bank and administrative records, (2) the data provided by the clinical records was accurate, and (3) by using ANCOVA techniques, it would be possible to illustrate that ABO blood group is a marker for lifespan.

The design used for this study was a non-experimental, retrospective study with no control group. In this non-experimental design, a control group was not necessary since the ANCOVA analysis would statistically equate the groups on given variables. Within the study population, ANCOVA was used to compare the ABO blood groups: A, B, O and AB, which represented the independent variable (IV). Lifespan (measured as age at the time of death) was the dependent variable (DV) and the statistical analysis adjusted the means of the DV such that all study subjects were equal with respect to covariates (CV). Differences among subjects on CVs were removed so that the only differences that remained were related to the effects of the ABO blood group variable.

This project employed a retrospective review of electronic clinical records for a one-year period from three tertiary care centers: Wake Forest University Baptist Medical Center (WFUBMC), Virginia Commonwealth University Medical Center (VCUMC) and the University of North Carolina Hospitals (UNCH). The project was an observational epidemiologic study that required no intervention. Due to the nature of the study, IVs could not be manipulated; and therefore, the non-experimental design was necessary. The design prevented the investigator from assigning patients to conditions or using randomization. The investigator provided descriptive statistics and attempted to establish comparability among the different ABO blood groups and among the different sites of collection.

Variables. ABO blood group and age served as IV and DV, respectively. Age represented the age at the time of death and was recorded in years as a whole number. Gender, race, transfusion history and site of data collection served as CVs. Gender was defined and recorded as male (1) or female (2). Race was defined according to the existing options in the hospital information systems and was recorded as: White (1), Black (2), Native American (3), Hispanic (4), Asian (5), Other (6) or Unknown (7). The "Other" category included patients who identified themselves as a racial group not listed as an option. The "Unknown" category included patients who did not have a race indicated in their medical record. Transfusion history was recorded as a dichotomous variable (yes/no) and was defined as exposure to RBCs during the year prior to the patient's death at the facility where the patient died. It was expected that the patient populations from the three sites (WFUBMC, UNCH, or VCUMC) would be similar; however, site of collection was included as a CV to rule out a proxy geographic effect or differences in hospital practice.

Research questions. Initially, the prevalence of the ABO blood groups were determined by calculating the percentage of each blood group in the sample population. If a relationship between ABO blood group and lifespan exists, the prevalence of ABO blood groups in the sample population may differ when compared to the known proportions of ABO blood groups in the United States population (Garratty, Glynn, & McEntire, 2004). Secondly, mean lifespan (as measured by mean age at the time of death) was calculated for each ABO blood group and compared to the other ABO blood groups. The sample was also stratified by race, gender and site in order to observe descriptive statistics by ABO blood group. An ANOVA (Analysis of Variance) was performed to examine differences in the age of death among the ABO blood groups, and ANCOVA analysis was used to determine if CV effects existed and if age at death was related to ABO blood group when the sample was adjusted for CVs. The total analysis answered the question, "Does lifespan, as defined by age at death, differ with ABO blood group in a hospital population when controlling for race, gender, transfusion history or site?" The statistical analysis will test the null hypothesis that age at the time of death does not differ with ABO blood group after adjusting for race, gender, transfusion history and site.

Description of sites. VCUMC (Richmond, Virginia), UNCH (Chapel Hill, North Carolina), and WFUBMC (Winston-Salem, North Carolina) are all academic medical centers in the Southeast United States and each consists of an integrated network of hospitals, clinics and health sciences schools. While one facility or a smaller geographic region could have been chosen to minimize confounding variables, this would limit the generalizability of the results. Tertiary care centers were chosen in order to provide the largest sample sizes and most diverse patient populations that reflected the population of the region. Each of the three centers is a

teaching facility, and all are categorized as large hospitals (US Department of Health and Human Services, 2008) based on bed sizes of 708 (UNCH), 779 (VCUMC) and 872 (WFUBMC).

All three facilities serve as regional referral centers in Virginia or North Carolina and care for patients in almost every county of their state. Each center is an integrated health care system that operates acute care, rehabilitation and long-term care beds, outpatient services, and community health and information centers. The centers have outreach activities throughout the region, including satellite clinics, health fairs and consulting services. They provide a continuum of care that includes primary care centers, outpatient rehabilitation, dialysis centers, home health care and long-term nursing centers.

Population and sample. The target population included all patients who expired at the three tertiary care centers during a one-year period (January 1, 2010 through December 31, 2010). Only stillborn infants and those patients with missing data were excluded from analysis. All variables were screened for missing values and their distributions were evaluated. The sample was inclusive of all patients who died within the project timeframe; thus, producing a non-random, convenience sample. This seemed appropriate since the goal was to examine the lifespan of all patients with respect to a particular ABO blood group.

Sample size and power. Previous research indicated that 85% of patients who expire in a tertiary care center have blood types in their medical record (Brecher & Hay, 2011). Therefore, it was anticipated that 15% of deceased patients would be excluded from the sample because of missing data. Using 2004 data from the facility with the smallest bed size (UNCH), this sampling method resulted in 772 patients (Brecher & Hay, 2011). Therefore, the estimated sample size for this project, using three facilities of about equal size and offering very similar services was (772 patients x 3 centers) 2,316 patients.

For comparative questions, sample size requirements are determined based on the effect size desired at a usual power of 0.80. If power is set at 0.80, then each ABO blood group needed 60 patients in order to detect a medium effect size (0.50) (Cohen, 1988). Known population statistics for ABO blood groups (Garratty et al., 2004) were used to predict the number of patients of each blood group in the expected sample of 2,316 patients. Blood groups B and AB are the rarest of the blood groups, making up 11% and 4%, respectively, of the United States population (Garratty et al., 2004). Using these statistics and the study's total sample size, an estimate of the number of B and AB patients in the sample was 255 and 93, respectively. With an anticipated sample of 93 in the rarest blood group, this design was predicted to meet the sample requirements.

Data collection procedures. The data used for this project were extracted from the hospital and blood bank information systems at three tertiary care centers: VCUMC, WFUBMC and UNCH. Data were collated and synthesized in a comprehensive data base by the principal investigator. The collection process and project timeline are summarized in Figure 4 and Table 10.

The following project liaisons were established at each of the three tertiary care centers to facilitate access to the data required: Dr. Yara Park (UNCH), Dr. Emmanuel Fadeyi (WFUBMC), and Dr. Susan Roseff (VCUMC). These project liaisons formed a support team, which worked with the principle investigator to prepare Institutional Review Board (IRB) proposals and manage the data acquisition keeping this consistent among all centers. Additionally, project liaisons helped to expedite access to any other required personnel at each facility. Once IRB approval was received, hospital records staff supplied a list of all patients who expired at the three institutions from January 1, 2010 through December 31, 2010. The

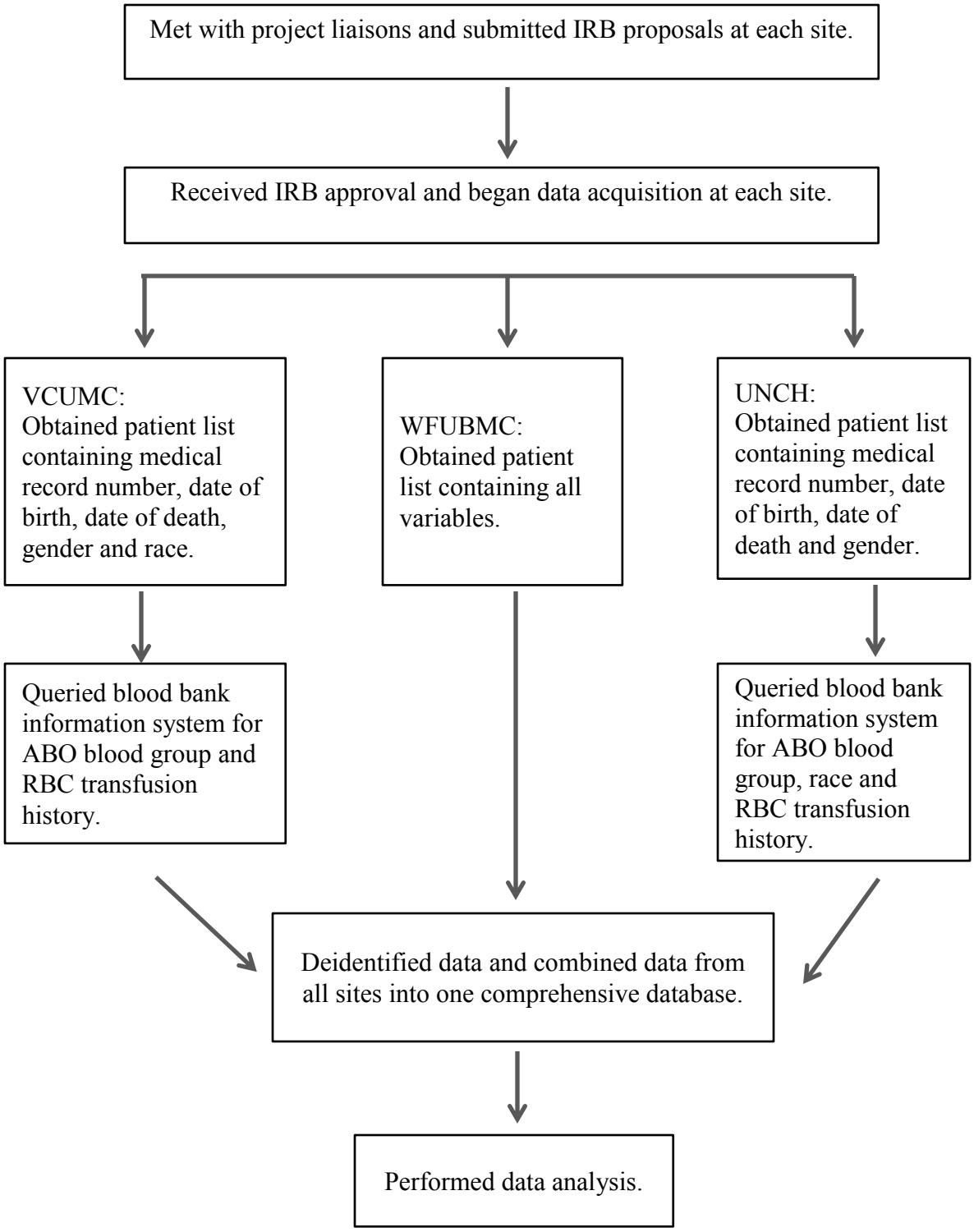

Figure 4. Data Collection Flowchart

Table 10.

Project Timeline

Project Activities	Months 1-4	5-8	9-12	13-16	17-20	21-24	25-28
Planning and Preparation							
Liaison meetings. Prepared management plans.	X						
Prepared IRB proposals for 3 sites.	X						
Submitted IRB proposals.	X						
Received IRB approval.	X (VCUMC)	X (WFUBMC, UNCH)					
Requested access to information systems.		X	X				
Data Collection and Analysis							
Prepared comprehensive database.		X					
Implemented project plans. Begin data collection.			X				
Ongoing data collection. Received electronic files.			X	X			
Ongoing data collection. Retrieved ABO blood groups.			X	X	X		
Ongoing entry of data into comprehensive database.			X	X	X		
Data analysis.					X	X	
Conclusions							
Prepared written results and discussion.							X

UNCH data required another request to access protected health information for research purposes from the medical records department. The types of information obtained from each facility on

the initial list of deceased patients differed among sites and is summarized in Table 11. WFUBMC supplied the comprehensive spreadsheet which included all the data needed for the study (date of birth, date of death, race, gender, ABO blood group and RBC transfusion history. Once the initial lists were received, the blood bank information systems at UNCH and VCUMC were used to query and record any other data. UNCH data was collected on site at UNCH and VCUMC data was queried remotely. Once the data collection was complete, the patient information was de-identified.

Table 11.

Initial Data Received from Each Site

	Variable					
Site	Date of Birth	Date of Death	Gender	Race	ABO Blood Group	Transfusion History
WFUBMC	✓	✓	✓	✓	✓	✓
VCUMC	✓	✓	✓	✓		
UNCH	✓	✓	✓			

The ABO blood groups, race and transfusion histories which were queried from VCUMC and UNCH were entered in a comprehensive database using Microsoft Excel 2010 alongside the corresponding data. The raw data and comprehensive database were stored on the principle investigator's laptop computer with security enabled. The data was backed up on a single secured flash drive. Both the computer and the flash drive were stored in a locked file cabinet in the principle investigator's office when not in use. Once all data was collected and entered into the single database, statistical analysis was performed using ANCOVA. Data analysis used the information directly from this database and SPSS 21.0 software.

Data analysis. Because ANCOVA is an extension of ANOVA, an initial description of ANOVA is provided. The primary purpose of ANOVA is to test for differences between means

of two groups for statistical significance (Tabachnick & Fidell, 2007). When comparing two means, ANOVA will give the same results as the t test. When there are three or more levels for a nominal variable (as in the case of ABO blood groups), a simple approach is to run a series of t tests between all pairs of levels. However, the number of t tests (pairwise comparisons) will increase geometrically as a function of the number of groups leading to an increased probability of committing a type I error. A more powerful approach is to analyze all the data at once using ANOVA. The model is the same, but the test statistic is the F ratio. ANOVA puts all the data into one number (F) and provides one p value for the null hypothesis.

In ANOVA, to test for statistical difference between means, the variances are compared. Variance is computed as the sum of squared deviations from the overall mean, divided by n-1 (sample size minus one) (Tabachnick & Fidell, 2007). Given a certain n, the variance is a function of the sums of squares (SS). Variances can be partitioned. For example, the total SS can be partitioned into the SS due to within-group variability and the variability due to differences between means of two groups. The within-group variability is referred to as error variance (SS Error). This term denotes the error that cannot be readily explained or accounted for it in the design of the study. However, the variance due to differences between means (SS Effect) of the groups can be explained by group membership.

Significance testing is based on a comparison of the variance due to the between-groups variability (called Mean Square Effect, or MS_{effect}) and with the within-group variability (called Mean Square Error, or MS_{error}) (Tabachnick & Fidell, 2007). Under the null hypothesis (that no mean differences exist between groups in the population), some minor random fluctuation in the means for the two groups are expected when taking small samples. Therefore, under the null hypothesis, the variance estimated based on within-group variability should be approximately the

same as the variance due to between-groups variability. The two estimates of variance are compared via the F test, which tests whether the ratio of the two variance estimates is significantly greater than 1. Therefore, in ANOVA, total variance is partitioned into the component that is due to true random error (in other words, within-group SS) and the components that are due to differences between means. These latter variance components are then tested for statistical significance. If significant, the null hypothesis that there are no differences between means is rejected and the alternative hypothesis that the means (in the population) are different from each other is accepted.

ANCOVA is similar to ANOVA, but allows for the addition of adjustment of the differences associated with one or more CVs. The main effects and interactions of IVs are assessed after DV scores are controlled for the effects of one or more of the CVs (Tabachnick & Fidell, 2007). As described by Tabachnick and Fidell (2007), ANCOVA could be used for two purposes in this project: (1) to increase sensitivity of the test of main effects and interactions by reducing the error term, and (2) to adjust the means on the DV themselves to what they would be if all subjects scored equally on the CVs. The second use of ANCOVA occurs commonly in nonexperimental situations, as in the present study, when subjects cannot be randomly assigned to treatments or groups. In this case, ANCOVA is used as a statistical matching procedure to adjust group means to what they would be if all subjects scored identically on the CV(s) (Tabachnick & Fidell, 2007). The differences between subjects on CVs are removed so that they only differences that remain are related to the effects of the IVs.

With ANCOVA, the focus becomes one of determining the effects of study factors on the DV adjusted for the presence of the CVs in the model. In ANCOVA, validity is achieved by adjusting for confounding variables, and therefore, obtaining an estimate of association that

would have been distorted if the CV(s) of interest had been ignored in the analysis. In the ANCOVA model, it is assumed that there is no interaction of CVs with study variables, although this assumption must be assessed in the analysis.

ANCOVA procedures are the same as ANOVA in that variance in scores is partitioned into variance due to differences among groups and variance due to differences within groups (Tabachnick & Fidell, 2007). Variance is partitioned by summing and squaring differences between scores and various means. The total sum of squared differences between scores on the DV and the grand (overall) mean is partitioned into two components: sum of squared differences between group means and the grand mean; and sum of squared differences between individual scores and their respective group means (error). In ANCOVA there are two additional partitions. First the differences between CV scores and their grand mean are partitioned into between- and within-groups sums of squares. Similarly, the covariance (the linear relationship between the DV and CV) is partitioned into sums of products associated with covariance between groups and sums of products associated with covariance within groups. This results in estimates of variance attributable to different sources: main effects of IVs, interactions among IVs, and error. Ratios of variances are used for tests of hypotheses about the effects of IVs on the DV. The difference between ANCOVA and ANOVA is that in ANCOVA, the regression of one or more CVs on the DV is estimated first (Tabachnick & Fidell, 2007). Subsequently, DV scores and means are adjusted to remove the linear effects of the CV(s) before analysis of variance is performed on these adjusted values. When a main effect is associated with a DV, then the next step is to quantitate the amount of variance in the adjusted DV that is attributed to the IV. This is referred to as effect size and is assessed by calculating the ratio of group sums of squares to group sums

of squares plus error sums of squares or, partial η^2 (partial eta squared) (Tabachnick & Fidell, 2007).

This project examined ABO group differences in length of life where age at the time of death was the measure of lifespan. The four ABO blood groups (A, B, O and AB) formed the IV. Three variables expected to vary with lifespan were gender, race and RBC transfusion history and were added to the analysis as CVs. A fourth CV that was considered was site since subjects will come from three different tertiary care centers. The statistical analysis tested the null hypothesis that lifespan does not differ with ABO blood group after adjusting for gender, race, transfusion history and site. Due to the non-experimental nature, this analysis did not allow for the implication that a greater lifespan is caused by one or more ABO blood groups. Instead, the results provide descriptive model building and enhanced prediction of the DV. Because similar data from 2004 reflected a bimodal distribution of age at the time of death (with one peak in the lowest age group and one peak around 51-60 years), it was predicted that examination of subgroups might be necessary. For example, one would expect that the disease processes or reasons for death in the lowest age group differ from those in the older age groups. Therefore, since the 2010 data from this study produced a bimodal curve, the data were also analyzed without the lowest age group included. Additionally, any differences between the 2004 and 2010 findings were further examined descriptively and by site.

In this project, ANCOVA was used to test for differences in the mean lifespans. The DV was age at the time of death, or lifespan. IVs were the four blood groups. In order to control for the effects of race, gender, site and transfusion history, these four variables were treated as CVs. ANCOVA is an extension of analysis of variance in which the main effects and interactions of IVs are assessed after DV scores are adjusted for differences associated with one or more CVs

(Tabachnick & Fidell, 2007). With ANCOVA, a more precise look at the IV – DV relationship after removal of the effect of CV(s) was obtained.

The optimal set of CVs was considered since several were available. If too many CVs are included in an ANCOVA and they are correlated with each other, a "point of diminishing returns in adjustment of the DV is reached" (Tabachnick & Fidell, 2007, p. 211-212). Power is decreased because numerous correlated CVs subtract degrees of freedom from the error term but do not remove corresponding sums of squares for error. Using the statistical analysis, a small set of CVs can be identified which are uncorrelated with each other but correlated with the DV. Theoretically, selection should be done by choosing CVs that adjust the DV for predictable but undesirable sources of variability. It may be possible to pick the CVs on theoretical grounds or on the basis of knowledge of the literature regarding important sources of variability that should be controlled. If theory is unavailable or the literature is insufficient to suggest important sources of variability in the DV, the statistical results can be used to select CVs. If the sample size is large and power adequate, it may still be worthwhile to find a small set of CVs for the sake of parsimony (in other words, the least complex explanation) (Tabachnick & Fidell, 2007).

When choosing CVs, all CVs should be correlated with the DV and none should be substantially correlated with each other. If they are, they do not add significantly to the reduction of error and can cause computational difficulties such as multicollinearity. The goal is to obtain the maximum adjustment of the DV with minimum loss of degrees of freedom of the error term since each CV costs 1 degree of freedom for error. When there is substantial correlation between the CV and the DV, the gain in terms of power often offsets the loss of power due to reduced degrees of freedom. The utility of each CV is established by using significance tests to assess their value in adjusting the DV. Multiple CVs enter the equation as a

set and within the set, each CV is evaluated as if it entered the equation last. Overlapping variability with other CVs is eliminated so that only the unique relationship between a particular CV and the DV is assessed (Tabachnick & Fidell, 2007). In addition to the significance levels for each CV, correlations between each CV and the DV and correlations among CVs are used to interpret the worth of CVs. As further runs are made, CVs are eliminated resulting in a small set of valuable CVs. The analysis with the smallest set of CVs will be reported, but mention will be made in the Results section of the discarded CV(s) and the fact that the pattern of results did not change when they were eliminated.

The statistical research question addressed was, are mean differences among ABO blood groups on the adjusted DV (lifespan) likely to have occurred by chance? Specifically, the analysis answered the question, is lifespan affected by ABO blood group, after holding constant race, gender, site and transfusion site? The statistical test did not ensure that changes in the DV were caused by the IV. The inference in causality is a logical rather than statistical problem that depends on the manner in which subjects are assigned to levels of the IV(s), manipulation of levels of the IV(s) by the researcher, and the controls used in the research. Because the IV and CVs used in the project could not be manipulated, inference depended on the controls used and the rigor of the research design.

ANCOVA has been used successfully in many nonexperimental studies. For example, Brambilla and colleagues (2003) examined differences in a nonexperimental study of amygdala volumes of 24 adults diagnosed with bipolar disorder and 36 adults without bipolar disorder. Amygdala volumes were measured blindly as the DV. Bipolar disorder status was the IV and the authors included age, gender and intracranial volume (ICV) as CVs. Bipolar patients had significantly larger left amygdala volumes compared with controls (mean volumes+/-SD =

2.57+/-0.69 vs. 2.17+/-0.58 mL, respectively; age, gender, ICV as CVs; $F = 4.42$, $df = 1/55$, $p = 0.04$). The volumes of the other temporal lobe structures did not differ significantly between the two groups (age, gender, and ICV as CVs, $p > 0.05$) (Brambilla et al., 2003). Additionally, serum cholesterol studies were examined in violent and non-violent female suicide attempters (Vevera, Zukov, Morcinek, & Papezova, 2003). ANCOVA with age as the CV was used to analyze differences in cholesterol levels in groups according to violence. The three groups were women with a history of violent suicide attempts, women with a history of non-violent suicide attempts and a non-suicidal comparison group. Violence was found to be a significant factor ($p = 0.016$). The Scheffé test was used as a post-hoc test to compare high violence suicide subjects, low violence suicide subjects and the control group. Scheffé tests revealed a significant difference ($p = 0.011$) between the group of violent and non-violent suicide attempters and between the violent suicide attempters and the control group. A statistically significant difference was not found between the non-violent and comparison group. The authors concluded that patients with a violent suicidal attempt have significantly lower cholesterol levels than patients with non-violent attempts and the control subjects.

Data screening procedures. After data collection and entry and prior to data analysis, the data was screened for accuracy with which the data were entered into the appropriate fields and any other factors that could produce distorted correlations. Univariate descriptive statistics were inspected for accuracy, plausible means and standard deviations, and univariate outliers. Stillborn infants and any patients with missing variables were eliminated from the study. Because the data set was large, this did not pose a problem (Tabachnick & Fidell, 2007). The fit between the data and the assumptions of the method was also assessed. Pairwise plots of the DV and CVs were checked for nonlinearity and heteroscedasticity.

Descriptive statistics were used to examine any missing data or extreme cases and their distribution was evaluated to ensure that they do not unduly influence or distort results. Nonnormal variables and univariate outliers were identified by checking for skewness and kurtosis. Because the assumption of normality applies to the sampling distribution of means and not raw scores, skewness by itself did not pose a problem. With the large sample size and the use of two-tailed tests, normality of sampling distributions of means was anticipated (Tabachnick & Fidell, 2007). Transformations of variables were considered to bring them into compliance with the requirements of the statistical procedure, but were not needed. Identification of multivariate outliers was conducted and variables were evaluated for multicollinearity and singularity. The four CVs (race, gender, transfusion history and site), were assumed to be reasonably consistent in reporting. Therefore, an adjustment in ANCOVA for unreliability of the CVs was not required.

Descriptive statistics were used to illustrate prevalence of the ABO blood groups in the study population as well as frequency of races, gender, transfusion history and the sample size at each of the three sites. It was expected that the populations at the collection site would be similar. Even if geographic locale had produced a difference in racial makeup, including race as a covariate would account for this dissimilarity. A discussion of the statistical analysis plan follows and is summarized in Figure 5.

Major analysis. The major analysis included main effects and effect sizes for all effects. Tests of interactions among IVs were not appropriate because the project contained only one IV, ABO blood group. For example, by evaluating main effects using the F statistic, one can test the null hypothesis that ABO blood group has no systematic effect on lifespan, thus determine if the association of the observed changes in lifespan and the IV is larger than expected through

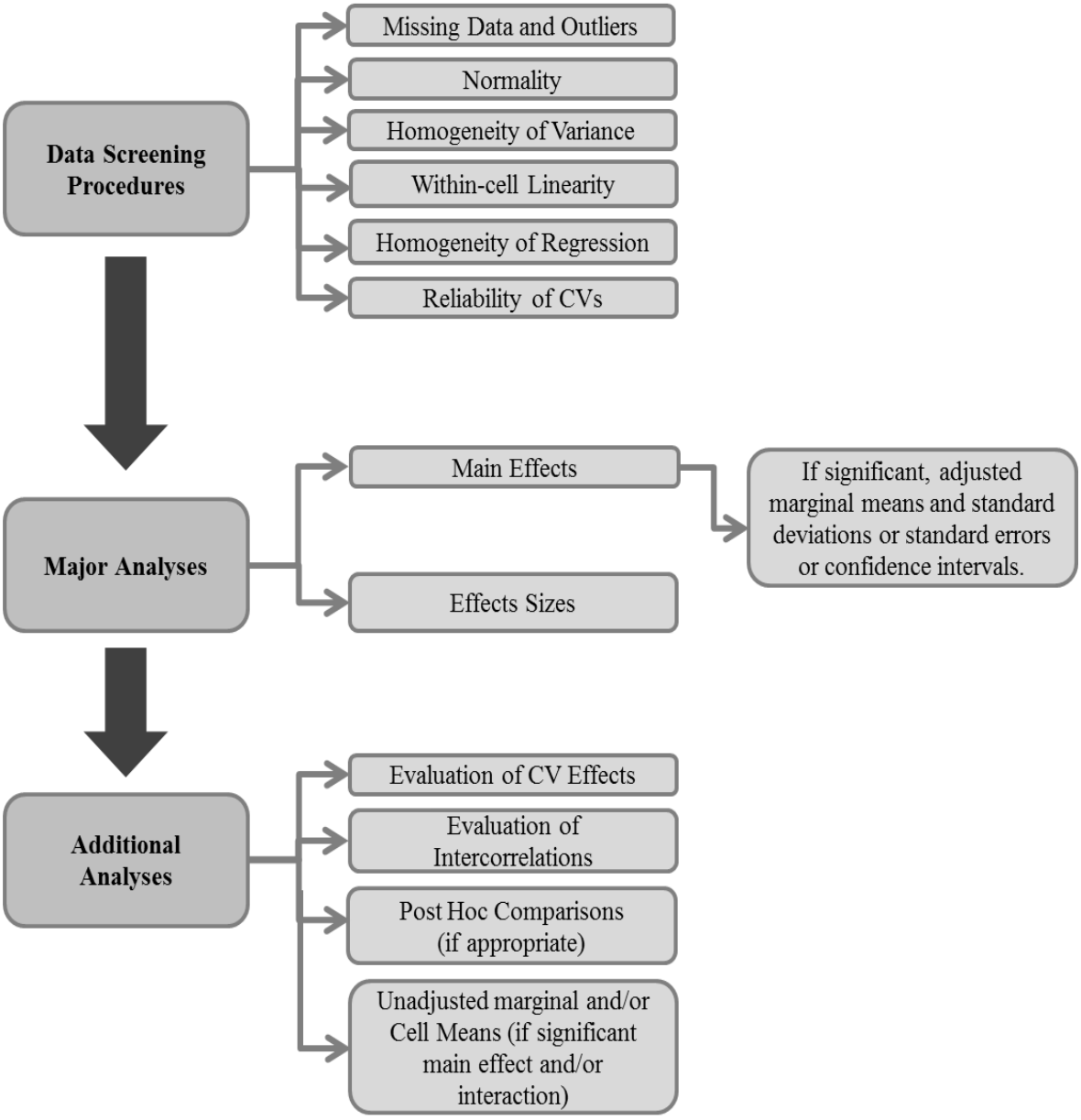

Figure 5. Summary of Plan for Statistical Analysis

random fluctuations occurring by chance. The procedures answered this question by testing the null hypothesis that ABO blood group has no systematic effect on lifespan. Since there are more than two categories of the IV, any finding of statistically significant effects would prompt an investigation of the nature of the differences. This is because the omnibus F test of a main effect gives no information as to which means are significantly different from which other means

(Tabachnick & Fidell, 2007). For example, it would be important to identify which ABO blood groups differ significantly from each other in post hoc analysis using additional statistical methods, such as the Scheffé test.

Additional analyses. The plan for additional analyses included the evaluation of CV effects, evaluation of intercorrelations, appropriate post hoc comparisons (such as Scheffé test for pairwise comparisons), and unadjusted marginal and/or cell means. ANCOVA is based on a linear regression between CVs and the DV; however, there is no guarantee that the regression is statistically significant (Tabachnick & Fidell, 2007). The regression should be evaluated statistically by testing the CVs as a source of variance in DV scores. For example, this analysis answered the question: to what extent is it possible to predict lifespan from ABO blood group, ignoring the effect of race? With multiple CVs, all CVs enter the multiple regression equation at once. Within the set of CVs, the significance of each CV is assessed as if it entered the equation last; only the unique relationship between the CV and the DV is tested for significance after overlapping variability with the other CVs, in their relationship with the DV, is removed. Thus, although a CV may be significantly correlated with the DV when considered individually, it may add no significant adjustment to the DV when considered last. When interpreting the utility of a CV, it is necessary to consider correlations among CVs, correlations between each CV and the DV and significance levels for each CV.

If the main effect of ABO blood group is reliably associated with changes in lifespan, the next step in the analysis would be to determine the amount of variance in the adjusted DV scores that is associated with the IV. Effect sizes (assessed using partial η^2) and their confidence intervals would be determined along with parameter estimates. Importance is usually assessed as the percentage of variance in the DV that is associated with the IV. If main effects were

statistically significant, estimated population parameters (adjusted mean and standard deviation or confidence interval) for each ABO blood group would be calculated. For example, if there is a main effect for ABO blood group, then what is the average adjusted lifespan in each of the ABO blood groups?

Because of the bimodal distribution in the 2004 pilot data, planned analyses also included conducting ANCOVA on the younger and older populations. Additionally, any discrepant results were further examined using descriptive statistics, *t* tests to compare means and Chi Square analysis to compare frequencies.

Validity and reliability of data. Overall, the variables were easily quantifiable and had face, content, criterion and construct validity, as well as reliability. Since existing databases were used (the hospital and blood bank information systems), we can anticipate that the same information would be extracted upon multiple attempts. However, because three different sites and information systems were used, it is possible that the policies for coding race at each site were different and could vary among hospital staff and the information available at the time of admission. Since the information was received or queried electronically, the potential for human error was minimized. It is possible that different researchers could extract data from different places in a database and have the potential to make errors as well. However, a single investigator was responsible for managing the data and developing the comprehensive database.

Summary

This non-experimental, retrospective study examined ABO blood group and lifespan in a Southeastern United States hospital population. Lifespan across ABO blood groups was determined by examining age at the time of death and ANCOVA was used to test for differences in the mean lifespans of patients based on ABO blood group. Gender, race, transfusion history

and site entered the analysis as CVs to control for any of their influences on lifespan. By using hospital data from three tertiary care centers and a population of deceased patients from a single year, this study provided an answer for the question "Is there a significant relationship between ABO blood group and lifespan in a hospitalized population?"

Chapter Four: Results

This chapter contains the results of the data analysis following the analytical plan described in Chapter 3. First, results of data exploration and cleaning are presented. Next, descriptive statistics are given followed by the results of the ANCOVA statistical analyses. All data was collected from a retrospective review of hospital and blood bank information systems and data analysis was conducted using SPSS 21.0 software.

Data Cleaning and Missing Data Analysis

Missing data. Data collection from the three sites (WFUBMC, VCUMC and UNCH) yielded a sample of 3316 patients who expired at the sites during the 2010 calendar year. Of the total sample, 890 (26.8%) patients did not have an ABO blood group on record or their race was unknown (Table 12). The race groups were classified as: White, Black, Hispanic, Asian, Native American and Other. All three sites used a group such as "Other" to indicate that the patient was not one of the five race groups provided. When the race field was left blank, it was classified as "unknown" during data collection; and the patient was ultimately removed from the data set. There was a higher percentage of missing patients than the 15% based on the 2004 UNCH data (N=871) collected for a similar project (Brecher & Hay, 2011). The percentage of missing data increased with the larger sample size and inclusion of more variables (such as race, gender and transfusion history) as expected. Removing the patients with missing data from this sample

Table 12.

Frequency of Missing Data by Site

Missing Variable(s)	WFUBMC *f*(%) N= 1494	VCUMC *f*(%) N=965	UNCH *f*(%) N=857	Total Sample *f*(%) N=3316
ABO Blood Group	466 (31.2)	212 (22.0)	36 (4.2)	714 (21.5)
Race	32 (2.1)	8 (0.8)	32 (3.7)	72 (2.2)
ABO Blood Group and Race	0 (0.0)	3 (0.3)	101 (11.8)	104 (3.1)
Total	498 (33.3)	223 (23.1)	169 (19.7)	890 (26.8)

Note: WFUBMC = Wake Forest University Baptist Medical Center, VCUMC = Virginia Commonwealth University Medical Center, UNCH = University of North Carolina Hospitals.

reduced the sample size to 2426. Still born infants were excluded from any analysis prior to missing data analysis.

Minimum and maximum values, mean, and standard deviation of age at the time of death were inspected for plausibility and determined to be appropriate. With regard to categorical variables (ABO blood group, race, gender, site, and transfusion history), outliers were identified by visually inspecting frequency distributions. Based on these analyses, no out-of-range values or univariate outliers were found.

Normality of Sampling Distributions

As in all ANOVA, it is assumed that the sampling distributions of means are normal within each group. Without knowledge of population values or production of actual sampling distributions of means, this assumption cannot be tested. In addition, the central limit theorem states that any statistic will have a normal or nearly normal distribution if the sample size is large enough (N>30) (Tabachnick & Fidell, 2007). Positive skewness was observed for the race variable in this study's population. However, skewness by itself poses no problem, since the

assumptions of normality apply to the sampling distribution of means, and not to the raw scores. In other words, the distribution of the sample means with large samples is normal regardless of the distribution of the original raw data, as long as the sample size is large. With the large sample size and use of two-tailed tests, normality of sampling distributions of means is anticipated (Tabachnick & Fidell, 2007).

Linearity. The ANCOVA model assumes a linear relationship between each CV and the DV as well as among pairs of CVs. Inspection of bivariate plots indicates no suggestion of nonlinearity between each of the CVs (race, gender, site, transfusion history) and the DV (age at the time of death). However, there is no need to check for linearity with gender or transfusion history because variables with two levels have only linear relationships with other variables (Tabachnick & Fidell, 2007).

Homogeneity of variance. It is assumed in ANCOVA that the variance of DV scores within each cell is a separate estimate of the same population variance. In ANCOVA the covariances are also evaluated for homogeneity of variance. The probability associated with Levene's test for equality of variances [$F(3, 2422) = 2.490$, $p=0.059$] is greater than alpha for diagnostic tests, 0.01 (Tabachnick & Fidell, 2007) . Therefore, the assumption of equal variances is satisfied.

Homogeneity of regression. Adjustment of scores in ANCOVA is made on the basis of an average within-cell regression coefficient. The slope of the regression between the DV and CV(s) within each cell is assumed to be an estimate of the same population regression coefficient, that is, that the slopes are equal for all cells. Therefore, the assumption of homogeneity of regression presumes that the CV-DV relationship is the same for all combinations of the IVs. Heterogeneity of regression implies that there is a different DV-CV(s)

slope in some cells of the design, or, that there is an interaction between IV(s) and CV(s). When the assumption of homogeneity of regression is not met, ANCOVA cannot proceed because the interpretation changes when the values of the CV differ (Tabachnick & Fidell, 2007). The assumption of homogeneity of regression is tested as an interaction that includes the CVs and the IV. If it is found to be statistically significant, the assumption is violated. The interactions of this model were not statistically significant at $\alpha = 0.05$ and the assumption of homogeneous regression slopes was satisfied (Table 13).

Table 13.

Evaluation of Homogeneity of Regression

Interaction	df	Error	F	p
ABO*Race	3	2418	1.190	0.312
ABO*Gender	3	2418	0.200	0.896
ABO*Transfusion History	3	2418	1.630	0.180
ABO*Site	3	2418	2.099	0.098

Reliability of covariates. It is assumed in ANCOVA that CVs are measured without error; the CVs are reliable (Tabachnick & Fidell, 2007). In this study, all data for all variables were extracted from the medical record rather than self-report. In the case of variables such as gender and race, the assumption of reliability can usually be justified. It is assumed that people are reasonably consistent in report of their own gender and race and that high reliability is likely (Tabachnick & Fidell, 2007). Additionally, as medical records are the official record for a health care event, the ABO blood group and transfusion history data can be used with sufficient confidence. Therefore, no adjustment in this ANCOVA was made for unreliability of CVs.

Results of evaluation of the assumptions of normality of sampling distributions, linearity, homogeneity of variance, homogeneity of regression and reliability of covariates were satisfactory. Additionally, no outliers were present.

Descriptive Statistics and Data Exploration

Characteristics of the ABO blood group distributions within the sample are provided in Tables 14-17 and Figure 6. They are tabulated by race, gender, site and transfusion history. The data presented have been adjusted for missing values as described previously. This resulted in a total sample size of 2426 from three sites: WFBMC (N=996), VCUMC (N=742), and UNCH (N=688). The sample was 56.3% male, 63.4% White, 31.0% Black and 2.1% Hispanic (Tables 14-15). Sixty-one percent of the sample had been transfused with red blood cells within one year of death (Table 16). WFUBMC patients made up the largest percentage of this group at 41.0%, followed by VCUMC (30.6%) and UNCH (28.4%) (Table 17). Lifespan, as measured by age at the time of death, ranged from 0 to 110 with a mean age of 58.7 years (Figure 7).

The racial distribution of the patients differed among the three sites with WFUBMC having the greatest number of White patients (77.2%) and VCUMC having the least number of White patients (47.8%) (Table 18). Conversely, WFUBMC had the least number of Black patients (20.0%) and VCUMC had the greatest number of Black patients (49.1%). UNCH had the greatest number of Hispanic (4.8%) and Asian (1.2%) patients as well as those classified as Other (5.4%).

In order to examine the length of life of those with differing ABO blood groups, it is necessary first to consider the distribution of the ABO blood groups within the study population. Cumulative frequencies of ABO blood group for the total sample across the three sites were compared to the known statistics for the general population (Table 3) (Garratty, Glynn, &

Table 14.

ABO Blood Group Distribution by Gender

	Gender	
ABO	Male N(%)	Female N(%)
O	614 (45.0)	517 (48.7)
A	518 (37.9)	375 (35.3)
B	181 (13.3)	131 (12.4)
AB	52 (3.8)	38 (3.6)
Total	1365 (56.3)	1061 (43.7)

Table 15.

ABO Blood Group Distribution by Race

ABO	White N(%)	Black N(%)	Hispanic N(%)	Asian N(%)	Native American N(%)	Other N(%)	Total N(%)
O	690 (44.9)	359 (47.7)	37 (71.2)	7 (53.8)	5 (50.0)	33 (54.1)	1131 (46.6)
A	667 (43.4)	192 (25.5)	10 (19.2)	3 (23.1)	4 (40.0)	17 (27.9)	893 (36.8)
B	131 (8.5)	167 (22.2)	3 (5.8)	3 (23.1)	1 (10.0)	7 (11.5)	312 (12.9)
AB	49 (3.2)	35 (4.6)	2 (3.8)	0 (0.0)	0 (0.0)	4 (6.5)	90 (3.7)
Total	1537 (63.4)	753 (31.0)	52 (2.1)	13 (0.5)	10 (0.4)	61 (2.5)	2426 (100.0)

Table 16.

ABO Blood Group Distribution by Transfusion History

ABO	Transfused N(%)	Not Transfused N(%)
O	689 (46.5)	442 (46.8)
A	548 (37.0)	345 (36.5)
B	192 (13.0)	120 (12.7)
AB	53 (3.6)	37 (3.9)
Total	1482 (61.1)	944 (38.9)

Table 17.

ABO Blood Group Distribution by Site

ABO	WFUBMC N(%)	VCUMC N(%)	UNCH N(%)	Total N(%)
O	460 (46.2)	354 (47.7)	317 (46.1)	1131 (46.6)
A	379 (38.1)	252 (34.0)	262 (38.1)	893 (36.8)
B	119 (11.9)	107 (14.4)	86 (12.5)	312 (12.9)
AB	38 (3.8)	29 (3.9)	23 (3.3)	90 (3.7)
Total	996 (41.0)	742 (30.6)	688 (28.4)	2426 (100.0)

Note: WFUBMC = Wake Forest University Baptist Medical Center, VCUMC = Virginia Commonwealth University Medical Center, UNCH = University of North Carolina Hospitals.

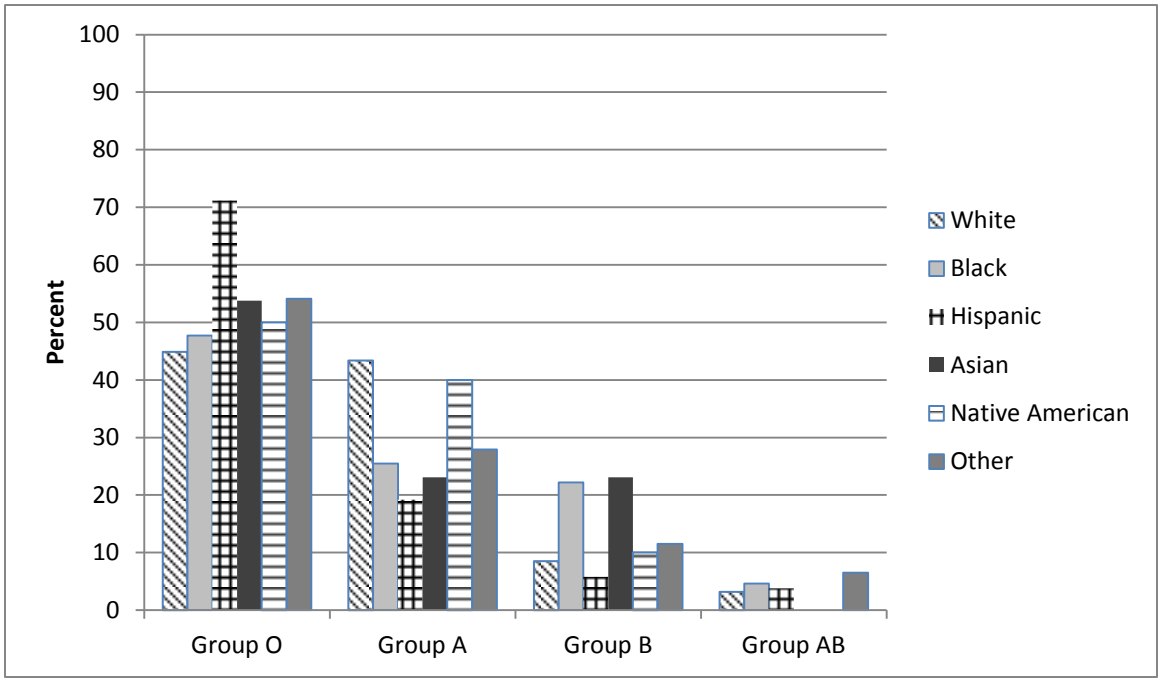

Figure 6. ABO Blood Group Distribution by Race

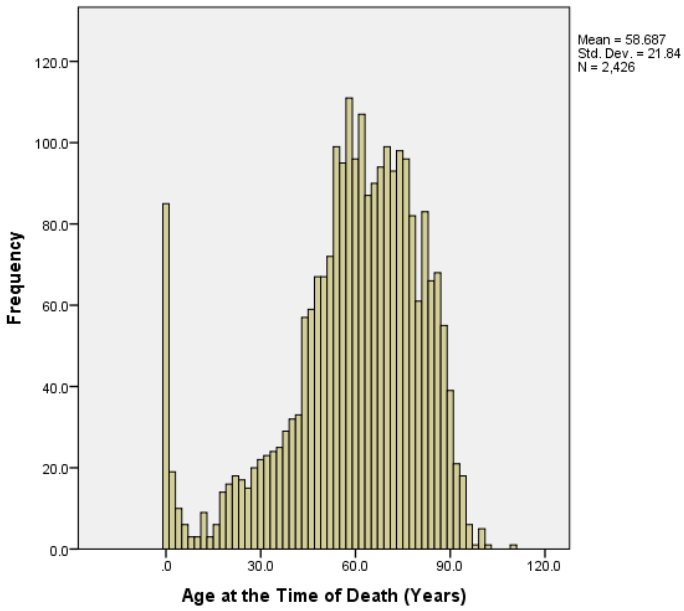

Figure 7. Age at the Time of Death

Table 18.

Racial Distribution by Site

Race	WFUBMC N(%)	VCUMC N(%)	UNCH N(%)	Total N(%)
White	769 (77.2)	355 (47.8)	413 (60.0)	1537 (63.4)
Black	199 (20.0)	364 (49.1)	190 (27.6)	753 (31.0)
Hispanic	19 (1.9)	0 (0.0)	33 (4.8)	52 (2.1)
Asian	4 (0.04)	1 (0.1)	8 (1.2)	13 (0.5)
Native American	2 (0.2)	1 (0.1)	7 (1.0)	10 (0.4)
Other	3 (0.3)	21 (2.8)	37 (5.4)	61 (2.5)
Total	996 (41.0)	742 (30.6)	688 (28.4)	2426 (100.0)

Note: WFUBMC = Wake Forest University Baptist Medical Center, VCUMC = Virginia Commonwealth University Medical Center, UNCH = University of North Carolina Hospitals.

McEntire, 2004). The 2426 hospitalized patients included in the study yielded a phenotypic distribution of ABO blood groups similar to the known frequencies for the United States population. Differences within the Hispanic and Asian races were noted. In both of these race groups, the study population had a higher prevalence of group O (14.7% and 14.0%, respectively) and a lower prevalence of group A (11.9% and 4.7%, respectively) compared to national data collected by Garratty, Glynn, and McEntire (2004).

The current study's population exhibited differences in the frequencies of ABO blood groups across the races (Table 15, Figure 6). However, regardless of ethnicity, group O made up the largest proportion of this sample (46.6%) followed by group A (36.8%), group B (12.9 %) and group AB (3.7%) (Table 15). The Hispanic population had the highest prevalence of group O (71.2%) compared to the other races and the total sample (Table 15, Figure 6). Additionally, a higher proportion of group B was found in the Black (22.2%) and Asian (23.1%) populations

when compared to the White population and the overall population values (Table 15, Figure 6). The AB blood group was not present in the current study's Asian and Native American populations.

Calculations of mean age at the time of death revealed differences among the groups of variables. Group A, White, female, non-transfused patients, and WFUBMC patients had the longest lifespans, while group AB, Hispanic, male, and transfused patients had shorter lifespans (Table 19).

Inspection of the distribution of age at the time of death (Figure 7) indicated a bimodal curve with the first peak in the very youngest patients and the second peak around 58 years of age. Because of the bimodal distribution and the likely differences in cause of death for these two populations, the data was divided into two subsamples: younger (patients who died at less than 10 years of age) and older (patients who died at age 10 or older). The younger subsample contained 123 patients from all sites, while the older subsample totaled 2303 patients. Characteristics of the ABO blood group distributions within each subsample are provided in Tables 20-28 and are tabulated by race, gender, site and transfusion history.

The younger subsample was 53.7% male, 42.3% White, 39.8% Black and 8.1% Hispanic (Table 20-21). Almost 80% of this group had been transfused with red blood cells within one year before death (Table 22). Eighty-five (69.1%) patients in this group died before 1 year of age (Figure 8). Group O made up the largest percentage of this population (52.0%) followed by group A (36.6%), group B (8.9 %) and group AB (2.4%) (Table 23). Like the larger total sample, group O made up a larger proportion of the Hispanic population's ABO distribution compared to that of the White population (Table 21). UNCH patients made up the largest

Table 19.

Mean Age at the Time of Death by ABO, Race, Gender, Site and Transfusion History

Variable		Mean Age at Death (Years)	N	Standard Deviation
ABO	O	57.8	1131	22.87
	A	59.8	893	21.35
	B	59.0	312	19.46
	AB	56.9	90	20.91
Race	White	62.1	1537	20.64
	Black	54.4	753	21.53
	Hispanic	36.0	52	23.30
	Asian	49.7	13	25.46
	Native American	53.0	10	16.11
	Other	49.1	61	29.11
Gender	Male	58.1	1365	21.22
	Female	59.4	1061	22.60
Site	WFUBMC	62.4	995	20.49
	VCUMC	56.1	742	21.02
	UNCH	56.1	688	23.77
Transfusion History	Transfused	56.2	1482	22.65
	Not Transfused	62.6	944	19.88

Note: WFUBMC = Wake Forest University Baptist Medical Center, VCUMC = Virginia Commonwealth University Medical Center, UNCH = University of North Carolina Hospitals.

Table 20.

ABO Blood Group Distribution of Younger Patients by Gender

	Gender	
ABO	Male N(%)	Female N(%)
O	33 (50.0)	31 (54.4)
A	29 (43.9)	16 (28.1)
B	3 (4.6)	8 (14.0)
AB	1 (1.5)	2 (3.5)
Total	66 (53.7)	57 (46.3)

Table 21.

ABO Blood Group Distribution of Younger Patients by Race

ABO	White N(%)	Black N(%)	Hispanic N(%)	Asian N(%)	Native American N(%)	Other N(%)
O	24 (46.2)	25 (51.0)	6 (60.0)	2 (100.0)	0 (0.0)	7 (70.0)
A	22 (42.3)	18 (36.7)	3 (30.0)	0 (0.0)	0 (0.0)	2 (20.0)
B	5 (9.6)	5 (10.2)	0 (0.0)	0 (0.0)	0 (0.0)	1 (10.0)
AB	1 (1.9)	1 (2.0)	1 (10.0)	0 (0.0)	0 (0.0)	0 (0.0)
Total	52 (42.3)	49 (39.8)	10 (8.1)	2 (1.6)	0 (0.0)	10 (8.1)

Table 22.

ABO Blood Group Distribution of Younger Patients by Transfusion History

ABO	Transfused N(%)	Not Transfused N(%)
O	51 (52.0)	13 (52.0)
A	37 (37.8)	8 (32.0)
B	8 (8.2)	3 (12.0)
AB	2 (2.0)	1 (4.0)
Total	98 (79.7)	25 (20.3)

Table 23.

ABO Blood Group Distribution of Younger Patients by Site

ABO	WFUBMC N(%)	VCUMC N(%)	UNCH N(%)	Total N(%)
O	20 (54.1)	18 (51.4)	26 (51.0)	64 (52.0)
A	15 (40.5)	13 (37.1)	17 (33.3)	45 (36.6)
B	2 (5.4)	4 (11.4)	5 (9.8)	11 (8.9)
AB	0 (0.0)	0 (0.0)	3 (5.9)	3 (2.4)
Total	37 (30.1)	35 (28.4)	51 (41.5)	123 (100.0)

Note: WFUBMC = Wake Forest University Baptist Medical Center, VCUMC = Virginia Commonwealth University Medical Center, UNCH = University of North Carolina Hospitals.

Table 24.

ABO Blood Group Distribution of Older Patients by Gender

	Gender	
ABO	Male N(%)	Female N(%)
O	581 (44.7)	486 (48.4)
A	489 (37.6)	359 (35.8)
B	178 (13.7)	123 (12.2)
AB	51 (3.9)	36 (3.6)
Total	1299 (56.4)	1004 (43.6)

Table 25.

ABO Blood Group Distribution of Older Patients by Race

ABO	White N(%)	Black N(%)	Hispanic N(%)	Asian N(%)	Native American N(%)	Other N(%)
O	666 (44.9)	334 (47.4)	31 (73.8)	5 (45.4)	5 (50.0)	26 (51.0)
A	645 (43.4)	174 (24.7)	7 (16.7)	3 (27.3)	4 (40.0)	15 (29.4)
B	126 (8.5)	162 (23.0)	3 (7.1)	3 (27.3)	1 (10.0)	6 (11.8)
AB	48 (3.2)	34 (4.8)	1 (2.4)	0 (0.0)	0 (0.0)	4 (7.8)
Total	1485 (64.5)	704 (30.6)	42 (1.8)	11 (0.5)	10 (0.4)	51 (2.2)

Table 26.

ABO Blood Group Distribution of Older Patients by Transfusion History

ABO	Transfused N(%)	Not Transfused N(%)
O	638 (46.1)	429 (46.7)
A	511 (36.9)	337 (36.7)
B	184 (13.3)	117 (12.7)
AB	51 (3.7)	36 (3.9)
Total	1384 (60.1)	919 (39.9)

Table 27.

ABO Blood Group Distribution of Older Patients by Site

ABO	WFUBMC N(%)	VCUMC N(%)	UNCH N(%)	Total N(%)
O	440 (45.9)	336 (47.5)	291 (45.7)	1067 (46.3)
A	364 (38.0)	239 (33.8)	245 (38.5)	848 (36.8)
B	117 (12.2)	103 (14.6)	81 (12.7)	301 (13.1)
AB	38 (3.9)	29 (4.1)	20 (3.1)	87 (3.8)
Total	959 (41.6)	707 (30.7)	637 (27.7)	2303 (100.0)

Table 28.

Mean Age at the Time of Death of Younger Patients by ABO, Race, Gender, Site and Transfusion History

Variable		Mean Age at Death (Years)	N	Standard Deviation
ABO	O	1.0	64	1.81
	A	0.7	45	1.76
	B	0.8	11	1.83
	AB	1.7	3	2.89
Race	White	0.7	52	1.46
	Black	0.7	49	1.90
	Hispanic	2.1	10	2.18
	Asian	1.5	2	2.12
	Native American	-	0	-
	Other	1.7	10	2.16
Gender	Male	0.9	66	1.80
	Female	0.9	57	1.82
Site	WFUBMC	0.8	37	1.67
	VCUMC	1.0	35	2.06
	UNCH	1.0	51	1.74
Transfusion History	Transfused	0.7	98	1.63
	Not Transfused	1.6	25	2.27

Note: WFUBMC = Wake Forest University Baptist Medical Center, VCUMC = Virginia Commonwealth University Medical Center, UNCH = University of North Carolina Hospitals.

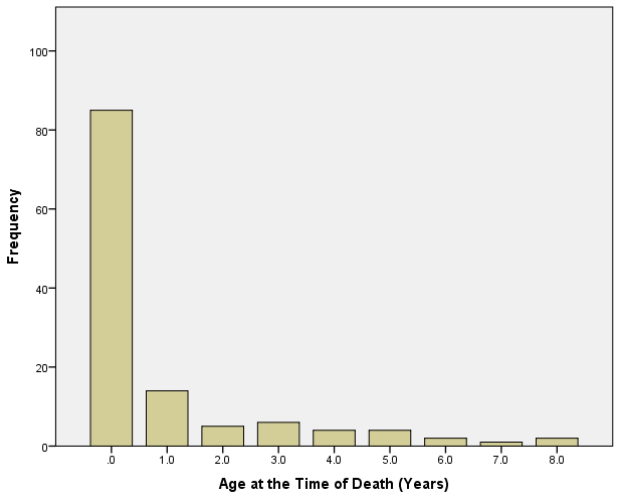

Figure 8. Age at the Time of Death of Younger Patients

percentage of this group at 41.5%, followed by WFUBMC (30.1%) and VCUMC (28.4%) (Table 23).

The older subsample was 56.4% male, 64.5% White, 30.6% Black and 1.8% Hispanic (Table 24-25). A smaller percentage (60.1%) of this group had been transfused compared to the the younger subsample (Table 22, 26). Group O made up the largest percentage of this sample at 46.3% followed by group A (36.8%), group B (13.1 %) and group AB (3.8%) (Table 27). As seen in the total sample and the younger subsample, group O made up a larger proportion of the Hispanic population compared to that of the White population (Table 25, Figure 9). Additionally, the Black and Asian populations had a higher percentage of Group B patients compared to the White population. WFUBMC patients made up the largest percentage of this group at 41.6%, followed by VCUMC (30.7%) and UNCH (27.7%) (Table 27). The greatest number of patients in this group died in their sixth decade of life, with a mean age at the time of death of 61.8 years (Figure 10).

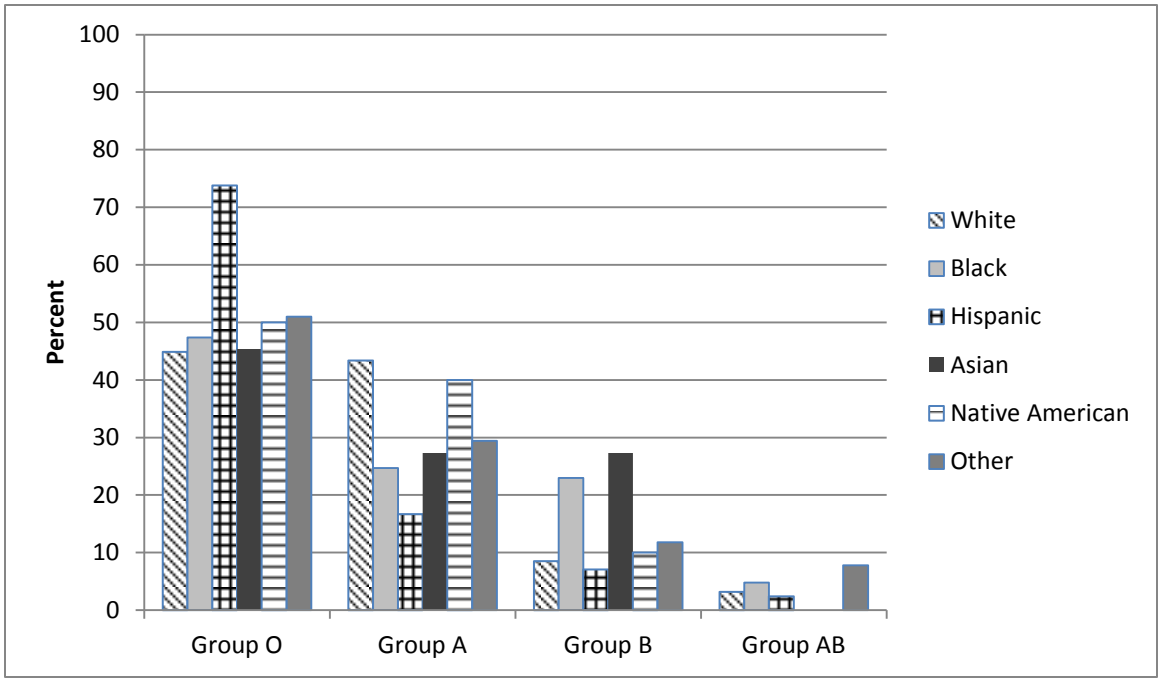

Figure 9. ABO Blood Group Distribution of Older Patients by Race

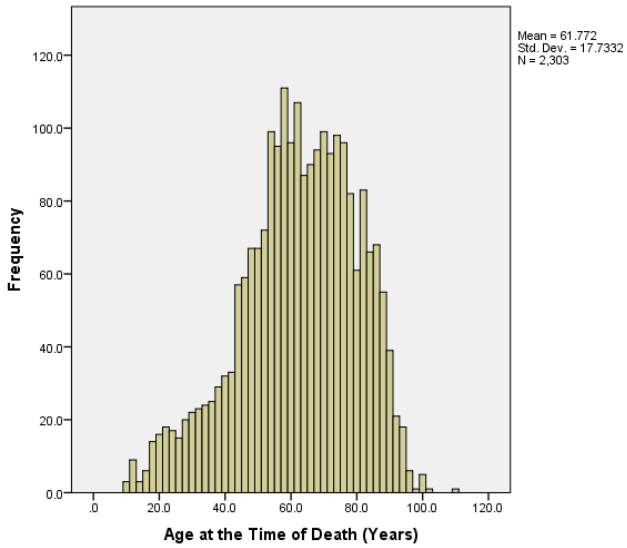

Figure 10. Age at the Time of Death of Older Patients

When comparing the demographics of the two subsamples, the gender distribution appears very similar. However, compared to the older subsample, the younger subsample contained fewer Whites, and more Blacks, Hispanics and Other. The younger subsample had been transfused more (79.7%) compared to the older subsample (60.1%). The older subsample consisted of a higher percentage of patients from WFUBMC (41.6%), whereas the younger subsample's highest frequency group was from UNCH (41.5%). When compared to the younger subsample, the older subsample had a smaller frequency of group O (46.3% vs. 52.0%) and a larger frequency of group B (13.1% vs. 8.9%).

The mean age at the time of death was calculated for both subsamples and by race, gender, site and transfusion history (Tables 28-29, Figures 11-20). In the younger subsample, lifespan was longest for group AB, Hispanic and those patients not transfused. The group AB patients represented only 2.4% of the younger subsample and all were hospitalized at UNCH. In the older subsample, lifespan was longest for group A, White, Female, not transfused and patients at WFUBMC. The pattern seen in the older subsample resembled that of the total sample (Table 19).

Multivariate Analysis

Initially, a one-way analysis of variance (ANOVA) was performed to determine if age at the time of death (DV) differed by ABO blood group (IV) without adjustment for CVs. The IV included the four ABO blood groups: O, A, B, and AB. The effect of ABO blood group on age at the time of death was not statistically significant, $F(3, 2422) = 1.625$, $p = 0.182$ (Table 30). The average amount of variation between the ABO groups ($MS_{effect} = 774.420$) was greater than that within the groups ($MS_{error} = 476.616$), which lead to an F-ratio greater than 1.

Table 29.

Mean Age at the Time of Death of Older Patients by ABO, Race, Gender, Site and Transfusion History

Variable		Mean Age at Death (Years)	N	Standard Deviation
ABO	O	61.3	1067	18.68
	A	63.0	848	16.86
	B	61.1	301	16.24
	AB	58.8	87	18.50
Race	White	64.2	1485	17.44
	Black	58.1	704	16.77
	Hispanic	44.0	42	18.10
	Asian	58.5	11	15.12
	Native American	53.0	10	16.11
	Other	58.4	51	21.86
Gender	Male	61.1	1299	17.27
	Female	62.7	1004	18.28
Site	WFUBMC	64.8	959	16.85
	VCUMC	58.8	707	17.47
	UNCH	60.5	637	18.63
Transfusion History	Transfused	60.1	1384	17.78
	Not Transfused	64.3	919	17.36

Note: WFUBMC = Wake Forest University Baptist Medical Center, VCUMC = Virginia Commonwealth University Medical Center, UNCH = University of North Carolina Hospitals.

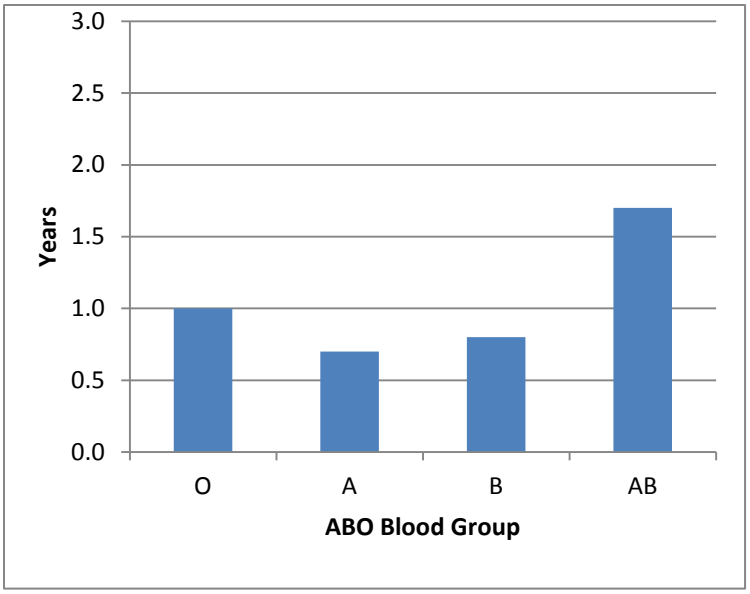

Figure 11. Mean Age at Death of Younger Patients by ABO Blood Group

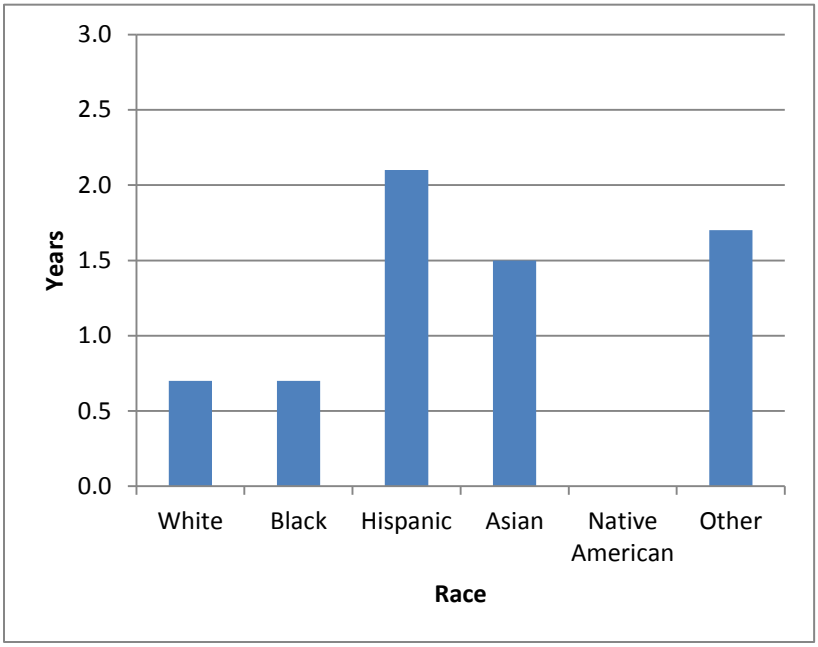

Figure 12. Mean Age at Death of Younger Patients by Race

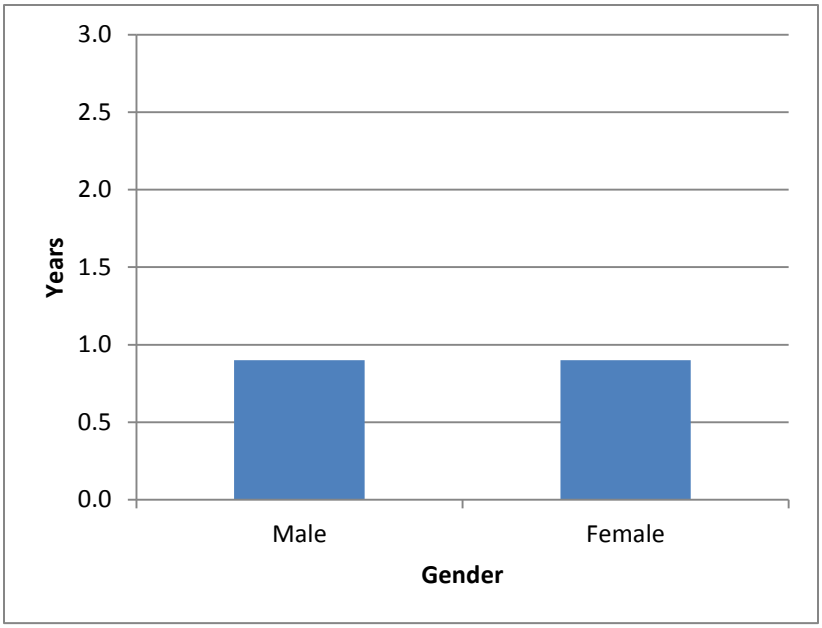

Figure 13. Mean Age at Death of Younger Patients by Gender

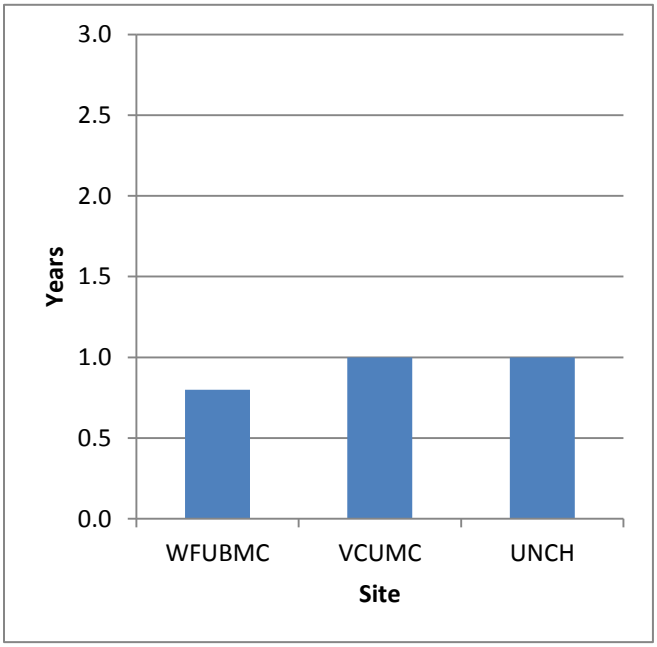

Figure 14. Mean Age at Death of Younger Patients by Site

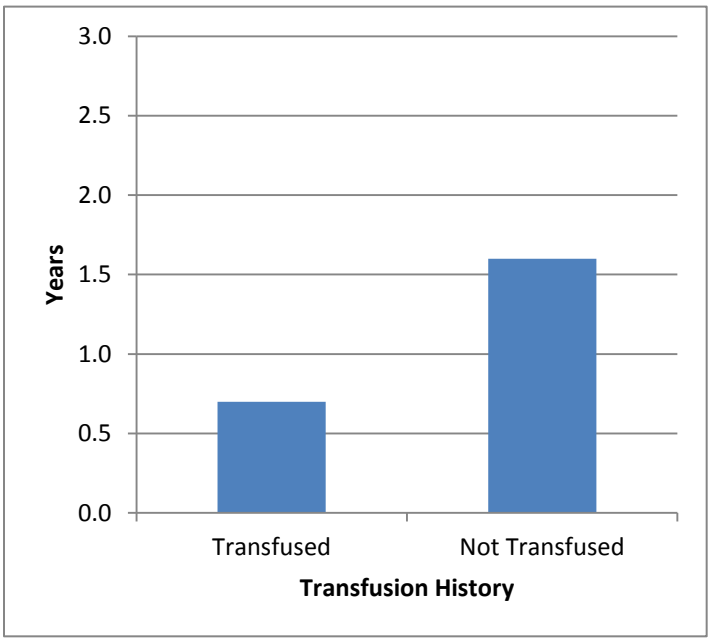

Figure 15. Mean Age at Death of Younger Patients by Transfusion History

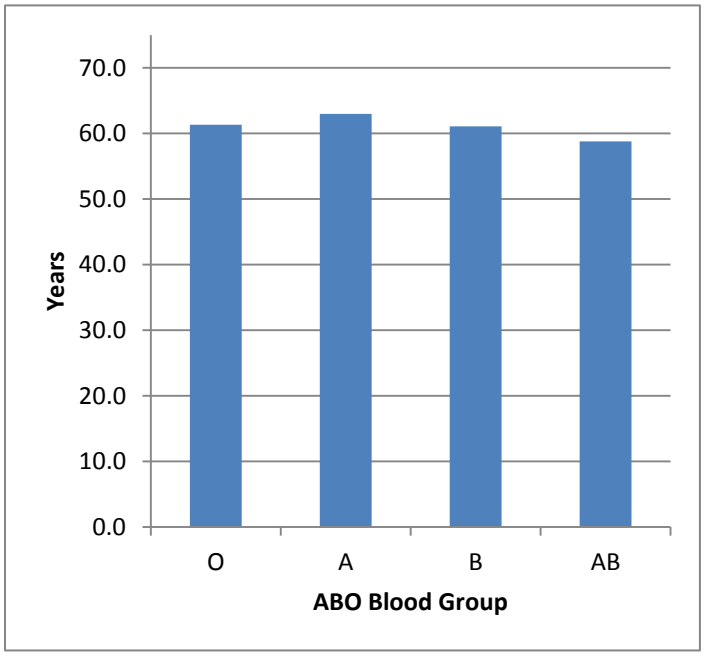

Figure 16. Mean Age at Death of Older Patients by ABO Blood Group

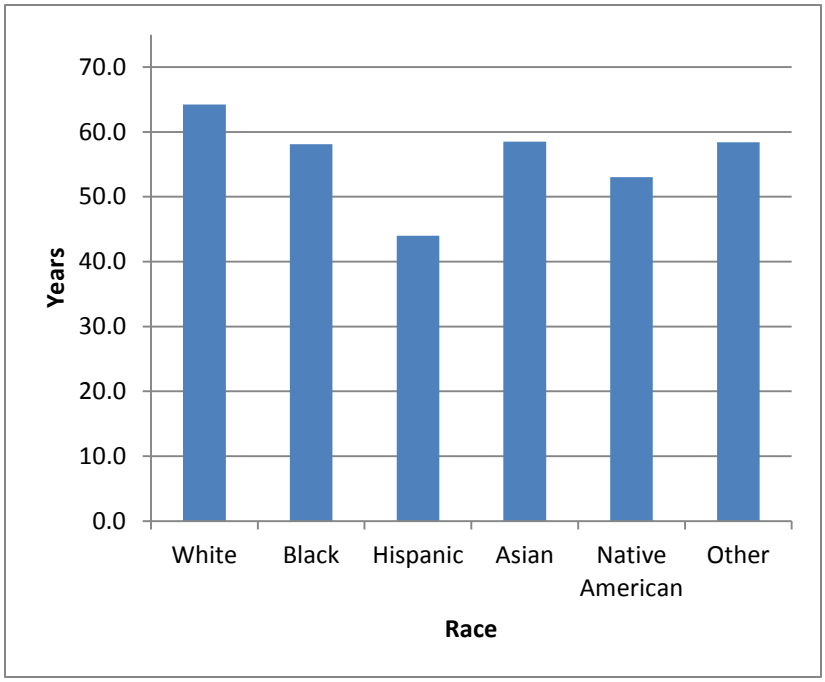

Figure 17. Mean Age at Death of Older Patients by Race

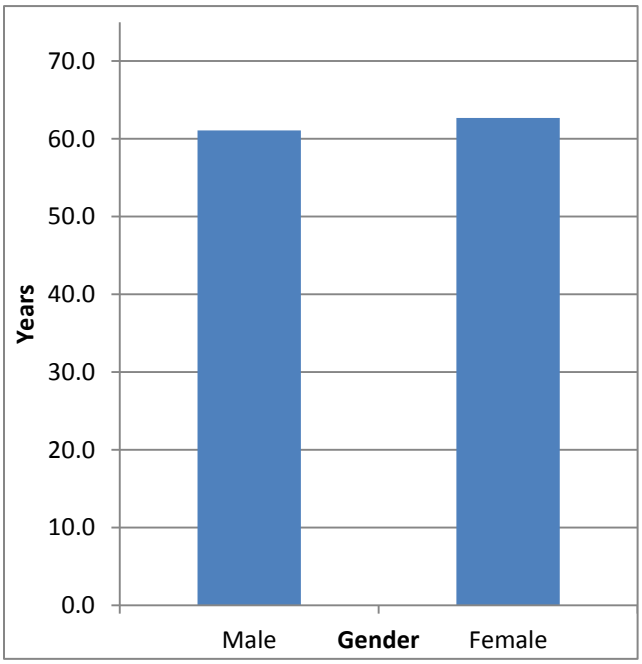

Figure 18. Mean Age at Death of Older Patients by Gender

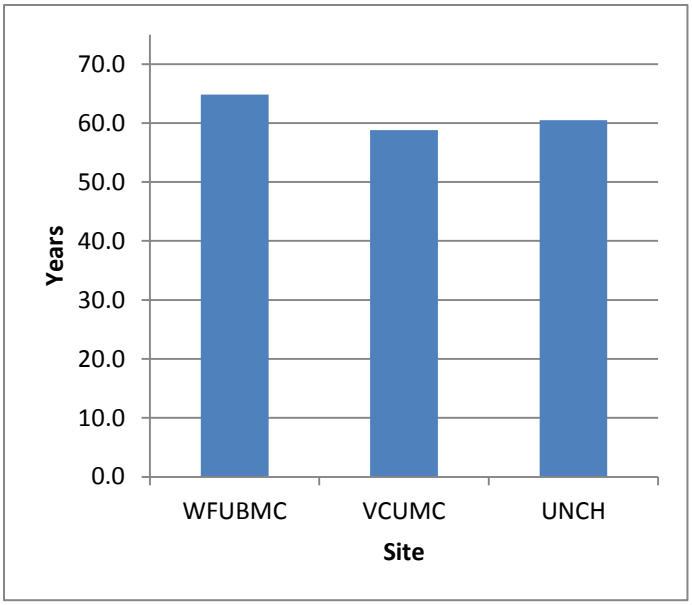

Figure 19. Mean Age at Death of Older Patients by Site

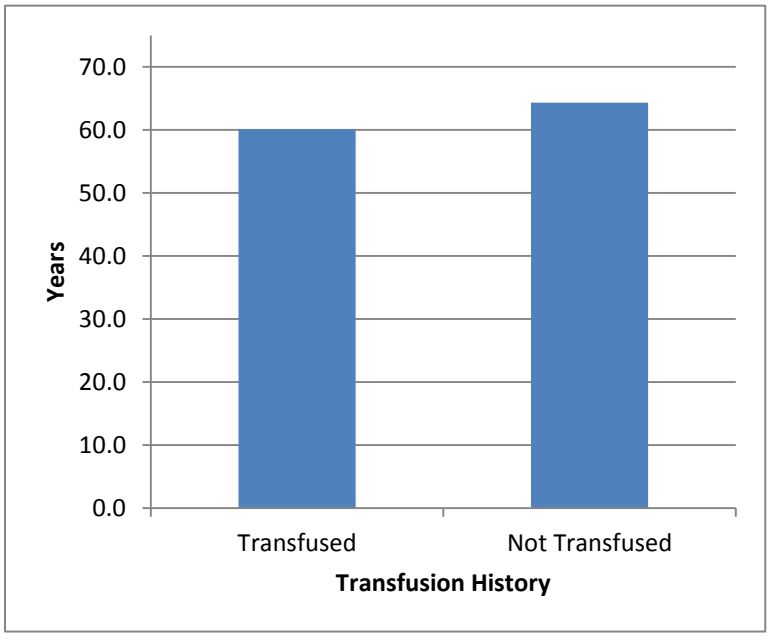

Figure 20. Mean Age at Death of Older Patients by Transfusion History

Table 30.

Analysis of Variance of ABO Blood in the Total Sample

Source	SS	df	MS	F	p
ABO	2323.260	3	774.420	1.625	0.182
Error	1154364.653	2422	476.616		
Total	9512152.000	2426			

Next, the CVs (race, gender, site and transfusion history) were entered into the model and ANCOVA was performed on age at the time of death for the total sample and separately for the two subsamples (younger and older). An alpha level of 0.05 was used for all statistical tests.

Analysis of the total sample. After adjustment by covariates, age at the time of death did not vary significantly with ABO blood group, F (3, 2418) = 0.954, p = 0.413 (Table 31). When covariates were added into the analysis, the average amount of variation between the ABO groups (MS_{effect} = 425.399) was less as was the variation within the groups (MS_{error} = 445.691). Covariates are included in the analysis with the expectation that they will reduce MS_{error} , increase the calculated F statistic and reduce the p value so that the null hypothesis can be rejected. However, the effect here was not enough to show statistical significance for the effect of ABO blood group on lifespan. Three CVs (race, transfusion history and site) significantly predicted age at the time of death with significance values less than 0.05. Race accounted for the most variation accounted for by the model (SS = 33993.468, partial η^2 = 0.031).

Analysis of younger subsample. Similar to the total sample, age at the time of death of the younger subsample did not vary significantly with ABO blood group after adjustment by covariates, F (3, 115) = 0.185, p = 0.907 (Table 32). In this analysis the relationship between transfusion and age at time of death approached statistical significance, but only one CV (race)

Table 31.

Analysis of Covariance of ABO Blood Group in the Total Sample

Source	SS	df	MS	F	p
Gender	1078.502	1	1078.502	2.420	0.120
Race	33993.468	1	33993.468	76.271	0.000
Transfusion History	22018.769	1	22018.769	49.404	0.000
Site	8586.484	1	8586.484	19.266	0.000
ABO	1276.197	3	425.399	0.954	0.413
Error	1077680.202	2418	445.691		
Total	9512152.000	2426			

Table 32.

Analysis of Covariance of ABO Blood Group in the Younger Subsample

Source	SS	df	MS	F	p
Gender	0.385	1	0.385	0.121	0.728
Race	13.757	1	13.757	4.341	0.039
Transfusion History	11.502	1	11.502	3.629	0.059
Site	0.268	1	0.268	0.084	0.772
ABO	1.756	3	0.585	0.185	0.907
Error	364.464	115	3.169		
Total	501.000	123			

significantly predicted age at the time of death with a significance value less than 0.05. Race accounted for the most variation accounted for by the model (SS = 13.757, partial $\eta^2 = 0.039$).

Analysis of older subsample. After adjustment by covariates, age at the time of death of the older subsample did not vary significantly with ABO blood group, F (3, 2295) = 1.590, p = 0.190 (Table 33). In this analysis all four CVs significantly predicted age at the time of death with a significance value less than 0.05. Similar to the younger subsample, race contributed the most variation accounted for by the model (SS = 12226.434, partial $\eta^2 = 0.017$).

Table 33.

Analysis of Covariance of ABO Blood Group in the Older Subsample

Source	SS	df	MS	F	p
Gender	1574.406	1	1574.406	5.242	0.022
Race	12226.434	1	12226.434	40.706	0.000
Transfusion History	9023.832	1	9023.832	30.043	0.000
Site	4603.919	1	4603.919	15.328	0.000
ABO	1432.684	3	477.561	1.590	0.190
Error	689324.195	2295	300.359		
Total	9511651.000	2303			

Comparison with UNCH 2004 pilot data. Similar data from UNCH was collected for a previous study (Brecher & Hay, 2011) and also used in a pilot analysis for this project. Like the 2010 sample, the 2004 UNCH sample (N=771) resulted in overall ABO frequencies similar to the general population (Table 7) and a bimodal distribution of age at the time of death (Figure 2). The 2004 sample consisted of 404 (52.4%) males and 367 (47.6%) females and the majority of the population had been transfused (76.1%). The racial composition was White (62.6%), Black (31.4%), Hispanic (3.0%), Asian (0.6%), Native American (0.8%) and unknown (1.6%). The estimated marginal mean age at the time of death was lowest for the group B patients at 48.0

years and highest for group AB at 62.5 years (Figure 3). Although this could be explained by a higher prevalence of group B Black persons, ANCOVA resulted in a statistically significant difference, $F(3, 763)$, $p=0.018$, in the age at the time of death among the ABO blood groups when controlling for race, gender and transfusion history.

Because the results of this 2010 sample disagreed with those of the 2004 pilot data collected at UNCH, the 2010 UNCH patients (N= 688) were extracted from the total 2010 sample and differences in variables were explored and analyzed using Chi Square analysis (Table 34). The frequencies of ABO blood groups were similar with the 2010 sample having a slightly higher proportion of group A patients and a slightly lower proportion of group O patients. The gender distributions were also very similar. Any differences in ABO blood group or gender frequencies were not statistically significant. However, the results indicated that the racial composition of the two samples was statistically different, $\chi^2(5, N=1459) = 22.39$, $p<0.001$. The 2004 UNCH sample contained more Black, and less Hispanic and Asian patients than the 2010 UNCH sample. Further, a higher proportion of patients in the 2004 sample was transfused (76.1% vs. 64.8%); this difference was also statistically significant, $\chi^2(1, N=1459) = 22.49$, $p<0.001$.

Given that the 2004 UNCH data provided evidence of a statistical difference in lifespan among the ABO blood groups, the frequency of variables among ABO blood groups were further compared (Table 35-37). The racial composition of the group O patients in the 2004 UNCH sample differed significantly from the of the group O patients of the 2010 sample [$\chi^2(5, N=706) = 26.93$, $p<0.001$], with a two-fold increase in the frequency of Hispanic group O patients and decreased frequency of White Group O patients. The 2004 UNCH pilot study suggested that group B was associated with a shorter lifespan compared to the other ABO blood groups. A

Table 34.

Comparison of 2004 and 2010 UNCH Distributions of ABO, Race, Gender and Transfusion History

Variable		2004 N(%)		2010 N(%)		χ^2	df	p
ABO	O	389	(50.5%)	317	(46.1%)	3.02	3	0.388
	A	264	(34.2%)	262	(38.1%)			
	B	92	(11.9%)	86	(12.5%)			
	AB	26	(3.4%)	23	(3.3%)			
Race	White	483	(62.6%)	413	(60.0%)	22.39	5	0.000
	Black	242	(31.4%)	190	(27.6%)			
	Hispanic	23	(3.0%)	33	(4.8%)			
	Asian	5	(0.6%)	8	(1.2%)			
	Native American	6	(0.8%)	7	(1.0%)			
	Other	12	(1.6%)	37	(5.4%)			
Gender	Male	404	(52.4%)	363	(52.8%)	0.02	1	0.890
	Female	367	(47.6%)	325	(47.2%)			
Transfusion History	Transfused	587	(76.1%)	446	(64.8%)	22.49	1	0.000
	Not Transfused	184	(23.9%)	242	(35.2%)			

Note: UNCH = University of North Carolina Hospitals.

Table 35.

Comparison of ABO Distribution among Racial Groups in 2004 and 2010 UNCH Data

	Race Frequency (%) 2004/2010								
ABO	White	Black	Native American	Hispanic	Asian	Other	χ^2	df	p
O	65.8/56.2	28.8/27.8	0.8/1.3	3.3/6.9	0.5/0.9	0.8/6.9	26.93	5	0.000
A	66.7/72.1	28.0/21.0	0.8/1.1	2.3/2.3	1.1/0.8	1.1/2.7	5.25	5	0.386
B	39.1/40.7	53.3/46.5	0.0/0.0	3.3/3.5	0.0/3.5	4.3/5.8	3.84	4	0.428
AB	57.7/47.8	26.9/30.4	3.8/0.0	3.8/8.7	0.0/0.0	7.7/13.0	1.97	4	0.741

Note: UNCH = University of North Carolina Hospitals.

Table 36.

Comparison of ABO Distribution by Gender in 2004 and 2010 UNCH Data

	Gender Frequency (%) 2004/2010				
ABO	Male	Female	χ^2	df	p
O	51.9/49.8	48.1/50.2	0.30	1	0.581
A	53.8/54.2	46.2/45.8	0.01	1	0.925
B	47.8/57.0	52.2/43.0	1.49	1	0.222
AB	61.5/60.9	38.5/39.1	0.00	1	0.962

Note: UNCH = University of North Carolina Hospitals.

Table 37.

Comparison of ABO Distribution by Transfusion History in 2004 and 2010 UNCH Data

	Transfusion History Frequency (%) 2004/2010				
ABO	Transfused	Not Transfused	χ^2	df	p
O	76.9/63.7	23.1/36.3	14.64	1	0.000
A	75.4/67.9	24.2/32.1	3.58	1	0.058
B	78.3/61.6	21.7/38.4	5.88	1	0.015
AB	65.4/56.5	34.6/43.5	0.40	1	0.525

Note: UNCH = University of North Carolina Hospitals.

closer look at group B reveals a higher frequency of Black group B patients in 2004 and a higher rate of transfusion in the group B patients in 2004 compared to the group B patients in 2010. However, only the difference in transfusion rate between the two samples was found to be statistically significant for group B patients, $\chi^2(1, N=178) = 5.88$, $p=0.015$. In blood group O patients, the transfusion rates were also statistically different and approached statistical significance for the group A patients. There were no statistical differences seen for gender distributions among the ABO blood groups. When mean age at death was compared among the ABO blood groups and between the 2004 and 2010 UNCH samples, a striking difference was seen in the lifespan of the group B patients (Table 38). The mean age at the time of death was 11.9 years longer in the 2010 group B patients, and this difference was statistically significant, $t(176) = -3.373$, $p = 0.001$. The differences in mean age at death between the 2004 and 2010 samples were not statistically significant for any other ABO blood group.

Table 38.

Comparison of 2004 and 2010 UNCH Mean Age at the Time of Death by ABO

	2004			2010					
ABO	Mean Age at Death (Years)	N	SD	Mean Age at Death (Years)	N	SD	t	df	p
O	52.9	389	25.76	55.0	317	24.83	-1.131	704	0.258
A	55.8	264	23.97	57.4	262	23.15	-0.786	524	0.432
B	46.4	92	26.07	58.3	86	20.46	-3.373	176	0.001
AB	60.3	26	24.58	47.2	23	26.02	1.821	47	0.075

Note: UNCH = University of North Carolina Hospitals, SD = Standard Deviation

Summary

This chapter summarized the results of the study, including data cleaning and missing data analysis, descriptive statistics, ANCOVA and comparisons of variable frequencies between populations. In terms of understanding whether ABO blood group is related to lifespan (age at the time of death), ANCOVA was conducted in an effort to compare age at the time of death while controlling for CVs (race, gender, transfusion history and site). Because the sample yielded a bimodal distribution of age at the time of death, the total sample population was divided into subsamples (death before 10 years of age, and death at 10 years old or later). The analyses were conducted on all three populations and each generated similar findings. For each population, the main effect of ABO blood group on lifespan did not reach statistical significance, while controlling for the CVs. Due to the lack of statistical significance in the main analyses, adjusted marginal means, standard errors, adjusted cell means, the evaluation of CV effects, and the evaluation of intercorrelations was not appropriate.

Because the current study's results disagreed with a pilot analysis of 2004 UNCH data in which group B was associated with a shorter lifespan, the two UNCH populations (2004 and 2010) were compared and analyzed. Interesting differences in racial composition and transfusion rates were noted. In particular, the mean age at the time of death for group B patients differed statistically between the two years of data collection. Interpretation of overall results and discordant findings will be reviewed in the next chapter.

Chapter Five: Discussion

The primary aim of this study was to determine if a relationship exists between ABO blood groups and lifespan in a hospitalized population in the Southeastern United States while controlling for race, gender, hospital site and history of RBC transfusion within a year of death. This chapter provides a review of the study's central findings, a discussion of the conclusions that can be drawn from the results and suggestions for the overall understanding of the relationship of ABO blood group to lifespan. It considers how these results compare to those of other studies and the pilot analysis. This chapter also indicates limitations of the study and possible areas for future investigation.

ABO Phenotypic Distributions

In order to examine the length of life of those with differing ABO blood groups, it is necessary first to consider the distribution and frequencies of the ABO blood groups. Worldwide and in the United States, group O is the most common, followed by group A, then group B with group AB as the least common (Cavalli-Sforza et al., 1994; Garratty, Glynn, & McEntire, 2004). However, the ABO frequencies within populations in the race group's country of origin where less racial admixtures occur may differ from these values. It should also be noted that the current study's data represent the racial categories designated by patients within the categories available at the three academic medical centers.

The current study confirmed the highest percentage of group O is found in the Hispanic population and that the Black and Asian groups have a higher percentage of group B compared to the other races. However, compared to the national findings of Garratty and colleagues (2004), higher frequencies of group O were observed among the Hispanic and Asian populations and 0% of the AB blood group was noted in the Asian and Native American samples. The ABO phenotype distributions between genders (Table 14), based on transfusion history (Table 16) and among the three sites (Table 17) demonstrated patterns similar to the overall population frequencies for the United States.

It is important to note that the present study provides frequencies of the ABO blood groups from two states in the southeastern United States (Virginia and North Carolina) while the United States data reported by Garratty and colleagues (2004) represents national frequencies using a much larger sample (N=3,086,215). Additionally, the sampling method and timeframe of data collection differed. While the present study represents one year (2010) of data collection, the United States data was collected over a 10-year period (1991-2000) (Garratty, Glynn, & McEntire, 2004). Timeframe could account for some variability; the difference in time between the two samples is at least 10 years. Additionally, the United States data represents healthy blood donors rather than the hospitalized patients used in the present study. Based on the multitude of reports that link ABO blood group to disease, it is plausible that the cause of death which placed the patients in the present study contributed to the different frequencies of ABO phenotypes in the study population. However, the results of this study provide no evidence of a relationship between ABO groups and lifespan.

Lifespan

Because the primary outcome of this research endeavor was as at the time of death or lifespan, it is important to consider the life expectancy of the pertinent population and how it may differ when other risk factors (such as gender or race) are included. The National Center for Health Statistics provides data on life expectancy in the United States by gender and for White and Black populations (Table 9). For example, life expectancy at birth has shown a disparity among race with 76 years for White males compared to 70 years for Black males and 81 years for White females compared to 76 years for Black females. (National Center on Health Statistics, 2007).

The age at the time of death for the study population ranged from 0 to 110 years with a mean age at death of 58.7 years (Figure 7). When the youngest subsample of patients was removed from the sample (less than 10 years old), the mean age at death increased to 61.8 years (Figure 10). This may more accurately represent the population relevant to the study's primary question as the youngest patients likely died from different diseases than those reported to have an association with ABO blood group. Since most of the diseases which have been associated with the ABO blood groups exert their morbidity and mortality relatively late in life, this seems reasonable. With life expectancy for the United States population reported to be 77.8 years (National Center on Health Statistics, 2007), it is clear that the mean age at death of the studied hospitalized population is much less. It is likely that this reflects poorer health of this population and their probable circumstance for being hospitalized at death. In a pattern similar to the reported national life expectancies, the study population also revealed shorter lifespans for Black and male individuals (Table 9, 19). WFUBMC had the highest mean age at death (62.4 years)

which may be related to a lower percentage of Black patients compared to the other sites (Table 18, 19).

Transfusion and lifespan. History of RBC transfusion within a year of death was included as a variable in the present study because the scientific literature provides robust evidence that transfusion presents risks beyond infectious disease and incompatibility and is associated with increased mortality (Kamper-Jorgensen et al., 2008; Kleinman, Marshall, AuBuchon, & Patton, 2004; Tynell, Norda, Shanwell, & Bjorkman, 2001; Tynall, Norda, Montgomery, & Bjorkman, 2005; Vamvakas & Taswell, 1994; Vamvakas & Moore, 1997; Wallis, Wells, & Chapman, 2004; Whyte, 1988). There is a substantial body of evidence demonstrating strong associations between transfusion and adverse outcomes in a range of clinical settings, including cardiac surgery and acute coronary syndrome, where transfusion is administered for reasons other than life threatening bleeding (Gerber, 2008; Marik & Corwin, 2008; Murphy et al., 2007; Reeves & Murphy, 2008; Taylor et al., 2006). Results are consistent in finding increased mortality among transfused patients (Vamvakas & Taswell, 1994, Vamvakas & Moore, 1997, Kleinman et al., 2004), and these findings make transfusion history an important factor to consider when examining lifespan or mortality in any group.

Both the transfused and not transfused patients demonstrated a mean age at death below the published life expectancies; however, a difference in the mean age at death was observed between those patients who were transfused and those who were not in this current study (Table 19). The mean lifespan of the transfused patients (56.2 years) was well below life expectancy (77.8 years). Some of this effect could be related to an increased need for transfusion among patients with more advanced disease, or patients who are at increased risk of dying from trauma or surgeries. Although the findings appear to show a shorter lifespan for transfused patients,

conclusions concerning a causal relationship between RBC transfusion and a shortened lifespan cannot be drawn from this study or the post-transfusion survival studies conducted to date as the research so far has been descriptive, not etiologic.

ABO blood group and lifespan. There have been many research endeavors investigating the associations of ABO blood groups with specific diseases, but fewer have examined an association of ABO blood groups with overall survival or lifespan. The limited research reports investigating ABO blood group and lifespan are disparate. While a Tokyo study of centenarians concluded that group B was associated with longevity (Shimizu et al., 2004), a United States study revealed conflicting results and suggested that group B was a marker for a shorter lifespan in their population of hospitalized patients (Brecher & Hay, 2011). The latter study (confined to a UNCH sample) did not include possible covariates, but the data was used in a pilot analysis for this project which was adjusted for race, gender, and transfusion history. This pilot analysis revealed similar findings suggesting a shorter lifespan for group B patients. Further contrasting findings were reported by Vasto and colleagues (2011) in their comparison of Sicilian centenarians and controls. The authors concluded that the frequencies of ABO blood groups in their two groups did not differ. To date, authors have not offered a biological explanation for their findings except to state that a particular ABO blood group may contribute to lifespan by some biochemical mechanism that allows that particular blood group to better survive disease.

This study was undertaken in an effort to examine the relationship of ABO and lifespan in a larger population while controlling for covariates. The data were analyzed as a whole and as subsamples of the younger (less than 10 years old at death) and older (greater than or equal to 10 years old at death) populations. In all analyses there was no statistical difference in lifespan

among the ABO blood groups even when controlling for race, gender, site and transfusion history. Because the results disagreed with the previous results found in the 2004 UNCH pilot data, the 2010 UNCH patients were extracted from the present sample and were analyzed separately. This resulted in the same findings, indicating that lifespan does not differ with ABO blood group.

Comparison of 2004 and 2010 populations. Although the statistical analysis controlled for covariates, it is important to note some key differences in the demographics of the 2004 and 2010 UNCH data. The 2004 UNCH sample had a slightly higher proportion of group A patients and a lower proportion of group O patients, but these differences were not statistically significant (Table 34). Overall, the 2004 UNCH sample had a higher number of Black patients, a lower number of Hispanic patients and these differences in the racial composition were statistically significant. Although the overall frequency of group B in both samples was similar, a higher percentage of the group B patients in the 2004 sample were Black (53.3% vs. 46.5%) (Table 35). This difference was not statistically significant. When the racial composition within each ABO group was compared between 2004 and 2010, statistically significant differences were evident only for group O which showed a higher frequency of White patients and a smaller frequency of Hispanic patients in the 2004 sample (Table 35). No differences in gender composition were significant.

Further examination revealed that the 2004 group O and B patients had a statistically significant higher rate of transfusion when compared to the 2010 sample (Table 37). The difference in transfusion rate for the group A patients also approached statistical significance. The 2004 sample had higher frequencies of patients with traits that are associated with shorter lifespans (such as race, and transfusion history). Inspection of the mean age at death by ABO

blood group exposed a dramatically lower lifespan for the group B patients of 2004 compared to the 2010 (46.4 years vs. 58.3 years) (Table 38). A difference in age at the time of death was statistically significant in the group B patients only.

While previous research reports (Shimizu et al., 2004; Hong et al., 2006; Brecher & Hay, 2011) and 2004 pilot data from UNCH suggested that ABO blood may be associated with lifespan, this larger and broader study of hospitalized patients in the Southeastern United States did not. When compared to the UNCH pilot data, the difference in timeframe (2004 vs. 2010) should be noted. It is possible that medical practice behaviors, prevalence and type of infectious diseases, or other effects of timeframe may have influenced the results. For example, transfusion practice is in transition; and there is robust evidence demonstrating strong associations between transfusion and adverse outcomes in many clinical settings where transfusion is administered for reasons other than life threatening bleeding (Gerber, 2008; Marik & Corwin, 2008; Murphy et al., 2007; Reeves & Murphy, 2008; Taylor et al., 2006). A meta-analysis of the effects of transfusion thresholds on the use of RBC transfusion and clinical outcomes indicated that restrictive transfusion strategies were associated with a statistically significant decrease in hospital mortality (Carson, Carless & Hebert, 2012). Due to these recent findings and because transfusion practice varies, the AABB (formerly, the American Association of Blood Banks) published a guideline to provide clinical recommendations about thresholds and other clinical variables that are appropriate triggers for red blood cell transfusions (Carson, et al., 2012). The AABB recommended adhering to a restrictive transfusion strategy in stable hospitalized patients and in patients with preexisting cardiovascular disease. The timing of these findings and consequential clinical recommendations provide one example of how transfusion practice may

have been evolving between 2004 and 2010. One can see evidence to support this by comparing the transfusion rates in the UNCH 2004 (76.1%) and 2010 (61.1%) samples.

It is also possible that the patterns and emergence of infectious diseases changed over the time period from 2004 until 2010. Cohen (2000) describes how changes in our society, technology and the microorganisms themselves influence the emergence of new diseases, the re-emergence of diseases once contained, and the development of antimicrobial resistance. It is not possible to point to one particular infectious disease that may have changed in prevalence during this time, but it is possible to highlight real examples of this phenomenon. For instance, during 2010, West Nile virus disease cases were reported from 40 states and the District of Columbia. The number of reported West Nile virus neuroinvasive disease cases increased 62% from the number reported in 2009 (Adams, et al., 2012).

Because the outcome variable is lifespan, an examination of the leading causes of death in 2004 and 2010 may provide insight on any changes in patterns or prevalence of diseases. The top four causes of death in all age groups in 2004 were: heart disease (27.2%), malignant neoplasms (23.1%), stroke (6.3%) and chronic lower respiratory disease (5.1%) (Heron, 2007). The frequencies were similar in 2010: heart disease (24.2%), malignant neoplasms (23.3%), chronic lower respiratory disease (5.6%), and stroke (5.2%) (Heron, 2013). Although still ranked at number one, the burden of heart disease has been decreasing since 1980 and similarly, deaths due to stroke have also seen a decline over time (Heron, 2013). Significant to the present study are the differences in cause of death that can be seen across age groups. The leading causes of infant death (less than 1 year old) in both 2004 and 2010 were congenital malformations (20.1% and 20.8%, respectively) and disorders related to short gestation and low birth weight (16.6% and 16.9%). In children aged one to four years old, death due to

unintentional injury becomes the number one cause, followed by congenital malformations. For populations 45 years old and over, chronic diseases accounted for more deaths than other causes. In both 2004 and 2010, cancer was first and heart disease was second for the 45 to 64 year age group; while for those 65 or older, heart disease was first and cancer was second. These data support the notion that the younger and older subpopulations of the present study died of different causes and therefore, should be analyzed separately.

Within this context, all race groups shared seven of the ten leading causes of death, but exhibited differences in relative disease burden (Heron, 2007; Heron, 2013). For example, heart disease and cancer were the first and second causes of death for White and Black populations in 2004 and 2010, but cancer was first and heart disease second for the Asian and Hispanic populations. While overall ABO frequencies were not significantly different between the 2004 and 2010 UNCH study populations, there were differences in the racial composition of the group O patients between samples. As previously noted, the group O patients of the 2010 sample had twice the frequency of Hispanic patients and a lesser prevalence of White patients. This may be relevant to the present study's findings since there have been many reports linking ABO blood group with heart disease (Medalie et al., 1971; Garrison et al., 1976; Rosenberg et al., 1983; Whincup, Cook, Phillips, & Shaper, 1990; Meade et al., 1994; Green, Jarrett, Ruth, Folsom, & Liu, 1995; Suadicani, Hein, & Gyntelberg, 2000; O'Donnell & Laffan, 2001; Ketch et al., 2008; Anvari et al., 2009). Although a consensus has not been reached, the most recent reports with larger sample sizes show an association with group O and heart disease (Suadicani, Hein, & Gyntelberg, 2000; O'Donnell & Laffan, 2001; Anvari et al., 2009). Variances in cause of death among age groups and race provide evidence that cause of death could be a confounding variable in this study and may have contributed to the difference in findings between the 2004 and 2010

populations. It is clear that there may have been changes in our environment (such as infectious disease patterns), changes in medical practice (such as transfusion thresholds), or variances in cause of death that affected outcomes in the 2004 and 2010 samples, but the design of this project did not allow for direct measurement of these effects.

An important variance to note regarding the 2004 and 2010 data is the method for extracting the transfusion history. "Transfused" 2004 patients included patients that had *any* record of RBC transfusion present in the UNCH blood bank information system at any time. In contrast, the look back for RBC transfusion for the 2010 patients only included one year prior to the date of the patient's death. Limiting the look back to one year was an effort to standardize the procedure among the three 2010 sites (due to any changes in information systems), but this difference may have affected the results of the study. It is possible that a further look back would have resulted in more patients being included in the "transfused" category for the 2010 sample.

There were inherent differences in the 2004 and 2010 UNCH samples that may have contributed to the discordant results. Dissimilarities in racial distribution and transfusion history were observed in the data. Although differences were noted for racial composition and transfusion history, a difference in the outcome variable (lifespan) was observed only for group B patients. It is possible that the group B patients of the 2004 UNCH sample had other unmeasured characteristics or more severe medical conditions (which required more transfusions) and put them at greater risk of death. For example, deep vein thrombosis and heart disease have been associated with group B (Jick, Dinan, Herman, & Rothman, 1978, Koster, Blann, Briet, Vandenbroucke, & Rosendaal, 1995, Robert et al., 2000, Green, Jarrett, Ruth, Folsom, & Liu, 1995, Medalie et al., 1971). Because both analyses controlled for race, gender,

and transfusion history, it appears that the difference in the two data sets is something that was not measured in this project.

It is also important to examine how the results of this project compare to previously published studies. The study participants in the research report of ABO blood group and lifespan (Shimizu et al., 2004; Hong et al., 2006; Vasto et al., 2011) differed from the present study's population with regard to geographic location, racial makeup and patient type. It should be noted that the geographic location and the low number of Asians in the present study (N=13), did not allow for a direct comparison of results with the investigation of Shimizu and colleagues (2004). Patients of previous studies were also limited to centenarians or liver transplant recipients rather than all patients who expired at an academic medical center (Shimizu et al., 2004; Hong et al., 2006; Vasto et al., 2011). Additionally, the sample from the present study did not result in a proportion of centenarians (N=3) that could be examined separately. Therefore, it is difficult to evaluate if these results are in direct contrast to other published findings.

The scientific literature provides a variety of data regarding the relationship of ABO phenotype with diseases and disorders, as well as overall length of life and survival. Reports of positive correlations with ABO blood group exist for: infectious disease, heart disease, pulmonary function, cancer, obesity, length of life, survival, and even behavioral traits (Table 6). It stands to reason that if a particular blood group provides a protective mechanism against serious illness, then that blood group should exhibit a longer lifespan. While the overwhelming majority of reports indicate an association with a particular ABO phenotype, it is important to note the potential for positive outcome bias. It is possible that studies indicating no association with ABO phenotype are less likely to be submitted for publication or to be accepted for publication. Additionally, the protective effect observed for ABO blood group does not

consistently point to one particular ABO group (Table 5, 6). Depending on the disease prevalence within a particular population or geographic location, one might predict that a particular ABO blood group would survive best based on previous reported associations. Because the current study population differed in geographic location and timeframe from previously reported studies, it is reasonable to suspect that changes or disparities in disease predominance in the populations may have contributed to divergent results. Additionally, by including three academic medical centers and thus, a wider geographic area, the effect of an association with a particular disease more prevalent in one location could have been diluted out by the larger sample size and geographic area.

Limitations

While this study is the largest to date to examine the relationship between ABO blood group and lifespan and provides valuable information, it has some limitations as well. The primary statistical test used in this analysis, ANCOVA, held theoretical limitations. If a relationship among ABO blood groups and lifespan had been elucidated, ANCOVA would not have assured that changes in lifespan were *caused by* ABO blood group. The inference of causality is a logical and scientific, rather than statistical, problem. Because this project was nonexperimental, causality is not justified.

There are inherent challenges and constraints when investigating any group of hospitalized patients and some of these existed with the other published studies in this area of interest. Specifically, there are limitations with the ability to generalize the results and it should be noted that the results can only be generalized to "hospitalized" patients. As "hospitalized" patients, the patients in this study were almost certainly different than the general population. Further, it is probable that these patients were in poorer health and had more complex medical

histories than patients who die in more rural hospital settings or assisted living facilities. It is possible that people who die in non-hospital settings are demographically different than the sample used for this project. Additionally, care must be taken when generalizing the results to populations other than those at academic medical centers which provide a large and disproportionate amount of indigent care and services for the most complicated cases that have been referred from other sites (Billi, Pai, Rothman, & Spahlinger, 2005; Moy, Valente, Levin, & Griner, 1996). It is possible that the patients in the sample for this project were older, had more complex disease processes or a different economic composition. Sampling from populations dying outside of a medical facility would present some challenges in data collection. It would likely be impossible to determine the ABO blood group, an accurate transfusion history and many other patient characteristics for the majority of people who die outside of a healthcare facility. For these reasons, using tertiary care centers provided the best opportunity to obtain a diverse sample population and access the data needed.

The mean age at death in the study population is earlier than the published life expectancy for the United States. This provides some evidence that this hospitalized population dying at academic medical centers is less healthy than those who die elsewhere. Therefore, it is possible that the relationship of poorer health and shorter lifespan to ABO blood group cannot be investigated without considering primary disease state, comorbidities or cause of death. These potential confounders were not included in the original design due to potential complexity, and ambiguity of the primary illness and/or cause of death in the medical record. Although it was not an objective of this study, without information regarding patient illness, it is not possible to tease out any associations between ABO blood group and disease.

It should also be noted that 27% of the original study population did not have ABO and/or race in their medical record and were excluded from the analysis. It is possible that these patients were different than the overall population. There may be some confounding factor that precluded obtaining an ABO blood group on these patients such as severity of illness, or religious affiliation.

While the study was intended to address the Southeastern United States, it represents only three academic medical centers and two states (Virginia and North Carolina) in the Southeast. In contrast to most other studies, this research was conducted with the explicit aim to discover relationships among variables for a specific group of people at one point in time. While generalizability to Virginia and North Carolina populations is inherent, the use of these results for other states or other countries is limited. Sampling more academic medical centers across a broader geographic region would have enhanced generalizability. Given that a previous study of contradictory results included an Asian population, one might question whether or not there was a sufficient Asian group within the study population. A very small proportion of the total study population was Asian (0.5%) and it is not appropriate to compare these findings directly with the findings of a population in another country. At the very least, differences in the health care system as well as differences in the presence or prevalence of certain disease patterns, make the results applicable to the populations of these two states only.

Due to the non-experimental nature of this study, concerns of internal validity must be explored. For example, the one-year timeframe of the study may have imposed a threat of history. Although unlikely, changes in the practice patterns of surgeons, new surgical procedures, changes in the delivery of health services or the availability of services over the year timeframe may have contaminated the results of the study. Differences in survival could also be

explained by the use of different transfusion triggers, population specific characteristics, variation in transfusion policy or differences in standards of medical care between countries, between hospitals, or over time. However, the threat of instrumentation was not a problem. The contents of the hospital or blood bank information systems, the format of their databases, and the usability of the databases were consistent among the three sites and over the study's timeframe.

Another confounding variable that was not measured was socioeconomic status as it is related to lifespan and virtually all health outcomes in most countries. Although Blacks, Native Americans, and Hispanics are disproportionately represented among the more socioeconomically disadvantaged groups in the United States, racial disparities do not simply reflect differences in income and the likelihood of poor or fair health are seen within each income group (Robert Wood Johnson Foundation, 2008). Therefore, it is possible that differences in this variable may have affected results.

Cognizance of the limitations is important as is realizing the limitations do not invalidate the utility of the findings. Several peer-reviewed articles have been published using smaller samples and less adjustment for covariates. This highlights the acceptance by the research community and the importance of the question. Communicating clearly what was found and not over generalizing the results is paramount. Even though this research will need to be replicated in other areas of the United States and other countries, the present project is the largest study to date in the United States and adds to the existing body of knowledge.

Future Research

Additional research is needed to advance this area of study. Future work should be done on expanded populations that cover a wider geographic area. Increasing the geographic area to include more areas of the United States would make the results more generalizable and may

increase the racial diversity of the sample. In addition, it may be necessary to specifically search for larger Asian populations or centenarians in order to more appropriately compare results with the findings of Shimizu and colleagues (2004) and Vasto and colleagues (2011). Data from previous studies may have been impacted by the use of individuals with advanced ages. Efforts to measure ABO blood group in persons of increased age could include the use of ambulatory geriatricians or other home health providers as sources for other aged populations. Because the older subpopulation in this study contained a wide range of ages at death (10 to 110 years old), further stratification of this group by decade may elucidate differences in the population in the oldest age groups. Furthermore, this study indicated that race accounted for much of the variability in outcome; however, it is possible that the differences due to racial disparities may decrease after age 65. United States data shows that differences in life expectancy for White and Black persons at 65 years of age are smaller than differences at birth (11.2 years versus 5.1 years) (U.S. Department of Health and Human Services, n.d.). Therefore, further examination of the oldest patients in this study (those greater than 65 years old at death), may provide results less affected by racial differences.

Additionally, future studies that include primary disease or comorbidities may provide more evidence for a functional role of ABO blood group. Even though the relationship of ABO blood group with disease or overall lifespan is not fully understood by the scientific community, the evidence suggesting associations between blood groups and particular diseases or disorders is expansive (Aird et al., 1953; Albert, 1996; Hein et al., 1998; O'Donnell & Laffan, 2001; Suadicani et al., 2007). Further, the collection of cause of death data may increase the ability to link ABO to disease and therefore, earlier death or prolonged survival. Advances in molecular genetics and cellular biochemistry may help to provide information regarding the structure of

blood group antigens which could be used in conjunction with epidemiological data to establish a link with ABO blood groups, disease and overall survival. Furthermore, the investigation of the biochemical mechanisms responsible for a longer lifespan in a particular blood group would allow for reflection and suggestions of how genetic inheritance and relative prevalence of ABO blood groups might be related to environmental factors.

Conclusion

Beginning with Landsteiner's discovery (1900), our understanding of the ABO blood group system has grown and scientists have been able to study one of the first human characteristics proven to be inherited. The ABO blood group antigens also appear to have been important throughout our evolution because the frequencies of different ABO blood types vary among different populations (Cavalli-Sforza, Menozzi, & Piazza, 1994), suggesting that a particular blood type conferred a selection advantage. Because the frequencies of ABO blood group phenotypes are not equal, it has been suggested that polymorphic genes provide intraspecies diversity which allows coping with diverse and rapidly evolving pathogens (Marionneau et al., 2001). The distribution of the four ABO blood groups (A, B, AB and O), varies in populations throughout the world (Cavalli-Sforza et al., 1994). Because environments are dynamic, it may be that a gene or trait that hindered survival and reproduction in the past, might aid in survival and reproduction today. In this way, natural selection and adaptation work to adjust the prevalence of traits so that they are suited for a particular environment and could influence frequencies and prevalence of ABO blood groups. Adding support to this theory, some research reports have shown that specific ABO blood types are associated with increased or decreased susceptibility to particular diseases (Albert, 1996; Cheng et al., 2005; Hein et al.,

1998; Kalayanarooj et al., 2007; Shimazu et al., 2000) and that ABO blood group antigens could have a physiological role previously unknown.

The primary purpose of this study was to examine the relationship between ABO blood group and lifespan in a hospitalized population in the Southeastern United States. In order to examine this relationship, the prevalence of ABO blood groups within the population and mean lifespan of each ABO blood group was determined. The analyses were adjusted for the effects of race, gender, transfusion history and site. Throughout this study, knowledge was gained about the distribution of ABO blood group in the hospitalized population and among race, the lifespan of those dying in the tertiary care centers, as well as the race, gender and transfusion history distributions among this population. The findings indicated that ABO blood group distribution differs among the race groups and lifespan differs most among the race groups. This study's sample size is the largest to date to examine this question and provided enough power to detect a difference in lifespan due to ABO blood group if a difference actually existed. However, the results showed that ABO blood group was not a predictor for lifespan in a hospitalized population in the Southeastern United States and that other variables have a stronger influence on lifespan.

There have been many research endeavors regarding the associations of ABO blood groups with specific diseases, but fewer have examined an association with ABO blood groups and overall survival or lifespan. Disparate conclusions concerning a relationship among ABO blood groups and lifespan supported the need for a larger, more comprehensive study to address this question. Given the plethora of existing research in the arena of ABO blood groups and disease correlation, the need for a large scale study to look at its overall influence on lifespan was important and relevant for healthcare. It is clear that consensus has not been reached at this

time, but the study of ABO blood groups in relation to disease and lifespan is one of evolutionary significance and may prove to reflect a functional role of the antigens on RBCs.

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Vita

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