Masters of Public Health (by Research)

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Evaluation of the morbidity and mortality associated with anti-nuclear antibodies: what is the predictive value of routinely-collected clinical diagnostic laboratory data?

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TABLE OF CONTENTS

INDEX OF TABLES

INDEX OF FIGURES

SUMMARY

Background: Antinuclear antibody (ANA) testing is used as a screening test for the detection of autoantibodies that bind to one or more constituents of the cell nucleus or cytoplasm (extractable antigens; ENA). These antibodies in turn are associated with particular diseases and may be useful to detect and to classify such diseases.

ANA detected by indirect immunofluorescence is a continuous variable detected at low level in some healthy individuals. At a higher level, ANA is more likely to be associated with certain autoimmune diseases. Nevertheless, ANA as a diagnostic test is reported as the highest dilution at which the antibodies can be detected or as a dichotomy – positive or negative. This study describes a clinical cohort tested for ANA and investigates the utility of quantitated ANA (measured in international units/ml (IU/ml)) as a screening test for related autoantibodies and diseases. This study also examines the relationship of ANA to mortality, cancer and inpatient morbidity.

Method: Data for incident ANA and for the individual autoantibodies assayed at PathWest Laboratory over the period 2000 – 2010 were linked to Western Australian, Department of Health, mortality cancer and inpatient morbidity data through the Western Australian Data Linkage System. The spectrum of clinical presentations and mortality associated with ANA level, pattern and follow on testing were investigated in a multivariate logistic regression or Cox regression analysis. The analysis was adjusted for one or more of the following variables: ANA outcome, age, gender, Indigenous status, country of birth, socio-economic status (Index of Relative Socio-Economic Disadvantage; IRDS) and residential remoteness (ARIA).

Results: ANA testing was requested more frequently for females compared to males and a higher proportion of females had an ANA above the reference value of <7IU/ml. Overall 17.5% of incident ANA results were greater than the reference value.

The most frequently reported ANA patterns were homogeneous (27.4%), speckled (21.4%), homogeneous plus speckled (21.4%) and nucleolar (19.5%). ANA >20IU/ml was associated with an increased risk of mortality. In addition, Cox regression analysis adjusted for age and gender showed an increased risk of mortality associated with the homogeneous, nucleolar, centromere and cytoplasmic patterns. The homogeneous plus

speckled pattern showed a reverse trend of decreased mortality with rising level of ANA: as ANA increased from 7-10IU/ml to >30IU/ml mortality fell from 12.7% down to 2.9%.

ANA level was a predictor of dsDNA antibody in the same serum. As ANA increased from <5 to >30IU/ml the OR for dsDNA antibody increased from 1.9 to 11.2.

ANA level was also a predictor of the detection of anti-RNP in the same serum. As ANA increased from <7 to >30IU/ml, the odds ratio (OR) increased from 1.0 to 163.5. For anti-SmD, the OR increased from 1.0 to 4.3. ANA was also predictive of SSA/Ro60, SSB/La, Scl70, Ro52 and histone antibodies.

In a multivariate logistic regression model adjusted for ANA level, age, gender, Indigenous Status, country of birth, ARIA, and IRDS, increasing ANA levels were significant predictors for Systemic Lupus Erythematosus, Sjogren's Syndrome, Systemic Sclerosis, mixed connective tissue disease and glomerular disorders (ORs ranged from 1.5 – 115.2). ANA level was associated with an increased risk of a number of gastrointestinal and myoneural disorders along with cancer of the oesophagus, non-Hodgkin's mature B cell lymphoma and diffuse large B cell lymphoma.

A comparison of ANA and dsDNA antibody associated with mortality data showed an increased frequency of dsDNA antibody positive cases in deaths associated with musculoskeletal and connective tissue and liver disease i.e. 22.2 and 20.3% respectively, compared to a frequency of 9.3% of the incident ANA and dsDNA antibody cohort for all cause death.

Conclusion: ANA were detected in a higher proportion of females compared to males. The majority of detected ANA were low level. In general an increasing level of ANA was associated with an increased likelihood of a dsDNA antibody and antibodies to RNP, SmD, SSB/la, Scl70, SSA/Ro60, Ro52 and CenpB. ANA had a higher predictive value for these autoantibodies in younger compared to older individuals.

This study has also demonstrated ANA pattern and quantitation to be significant predictors of morbidity and mortality particularly in musculoskeletal and connective tissue diseases.

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LIST OF ABBREVIATIONS

ABS	Australian Bureau of Statistics
ANA	Anti-Nuclear Antibody
APC	Antigen presenting cell
ARIA	Residential Remoteness
CenpB	Centromere protein B
CI	Confidence Interval
CIEP	Counter-immuno-electrophoresis
CMV	Cytomegalovirus
CTD	Connective Tissue Disease
df	Degrees of Freedom
DNA	Deoxyribonucleic acid
DoHWA	Department of Health, Western Australian
dsDNA	double stranded DNA
ELISA	Enzyme Linked Immunosorbent Assay
ENA	Extractable Nuclear Antigen
GPs	General practitioners
HEp	Human Epithelial cell line
HMDC	Hospital Morbidity Data Collection
HZV	Herpes Zoster Virus
ICD	International Classification of Disease and Health Related Problems codes
Ig	Immunoglobulin class
IIF	Indirect immunofluorescence assay
IRDS	Index of Relative Socio-Economic Disadvantage
IU/ml	International Units/ml
IL	Interleukin
LIS	Laboratory information system
LR	Logistic regression
Max	Maximum

MCTD	Mixed Connective Tissue Disease
Min	Minimum
N. Africa & M.E	North Africa and The Middle East
N.E. Asia	North-East Asia
N.W. Europe	North West Europe
OR	Odds Ratio
PAMPs	Pathogen associated molecular patterns
PRR	Pathogen Recognition receptor
RNP	Ribonuclear protein
S. & C. Asia	Southern and Central Asia
S.E. Asia	South-East Asia
S. & E. Europe	Southern and Eastern Europe
SEIFA	Socio-Economic Indexes for Areas
SLE	Systemic Lupus Erythematosus
SjS	Sjogren's Syndrome
SSc	Systemic Sclerosis
SD	Standard Deviation
T_H	T helper cells
USA	United States of America
WADLS	Western Australia Data Linkage System
WADoH	Western Australian Department of Health
WA	Western Australia

1. INTRODUCTION

1.1 BACKGROUND

In 1957 Holborow *et al* described antibodies in serum from Systemic Lupus Erythematosus (SLE) patients that bound constituents of cell nuclei (1). These antibodies were referred to as anti-nuclear antibodies (ANA) and they have since been associated with systemic rheumatic disease. The common screening test for ANA is an indirect immunofluorescence (IIF) assay which is reported as a pattern of staining and a level of fluorescence intensity (2). The highest dilution at which the antibodies can be detected in a patient with a disease such as SLE, myositis or rheumatoid arthritis varies between patients with fewer patients having high levels of the antibody (3). Subsequent studies have shown that antibodies that bind certain nuclear and cytoplasmic antigens are clinically important and disease specific, whereas others are not. (Reviewed in (4, 5)). ANA are also found in some individuals in the healthy population (5-7).

The IIF method for detection of ANA has been refined over time with testing methods shifting from a substrate of rat liver sections to clonal human epithelial type 2 cancer cells (HEp 2 or HEp 2000) (2, 5, 8, 9). A threshold level of ANA (generally set by the testing pathology laboratory) is ordinarily a prerequisite to further characterisation. Results are typically reported as detectable at a fixed dilution or the endpoint dilution of the patient serum i.e. the highest dilution at which clear fluorescence of the nucleus is visualised. A unique aspect of the study described in this thesis is that the results are reported in international units/ml (IU/ml) standardised against an international standard WHO66/233; an approach which is reported to reduce inter-laboratory and inter-assay variation (10, 11).

The research for this thesis has investigated the morbidity and mortality associated with a detectable ANA in samples from a clinical cohort collected over an 11 year period from routine testing at Clinical Immunology, PathWest Laboratory Medicine at the Queen Elizabeth II Medical Centre in Western Australia (WA). The incident ANA test result (i.e. first test result) for an individual has been linked through the Western Australian Data Linkage System (WADLS) to inpatient morbidity, mortality and socio-demographic data including age, sex, Indigenous Status, residential remoteness, social disadvantage and country of birth; extracted from the Department of Health Western Australia (DoHWA) administrative whole-of-population Hospital Morbidity Data Collection (HMDC) (12). Data collections were linked through the WADLS using best-practice probabilistic matching techniques reported elsewhere (12). The advantage of this approach is that it

reduces the selection bias associated with primary data collections methods such as self-reporting and allows longitudinal data collection and socio-demographic variables to be included in the analysis (13-16).

1.2 SIGNIFICANCE

The ANA test is a screening assay used to indicate the need for further investigation, to determine autoantibody specificity and thereby assist in the diagnosis of a possible systemic rheumatic disease. The benefit or otherwise of quantitation of the ANA has had mixed reports, Egner (8) concludes that "clinical importance cannot be extrapolated from ANA titre or pattern", Abeles *et al* (17) and Dinser *et al* (18) both comment on the low positive predictive value of an elevated ANA in the absence of clinical suspicion of autoimmune disease. Retrospective studies have also shown that ANA are detectable in patient serum three or more years before the diagnosis and two years before the onset of specific symptoms of autoimmune rheumatic disease (19, 20).

Retrospective epidemiological studies have investigated the autoantibodies, including ANA associated with connective tissue diseases (CTD) such as mixed CTD (MCTD) and SLE (21, 22). These studies have also considered the influence of sociodemographic factors such as gender, ethnicity and age on disease progression and severity (23, 24). However, autoantibodies including ANA are also detected in serum samples from the general population without clinical symptoms (5, 6, 18, 19, 21, 25).

ANA reporting is not standardised across testing laboratories (26, 27) and comparisons of ANA levels reported for a single sample exchange in the Royal College of Pathologists Australia Quality Assurance program shows considerable variation in outcomes (titres reported ranged from 1: 80 – 1:5120 for a single sample). The implication of this lack of standardisation and resulting breadth of possible results for a patient depending on testing sites brings into question the reliability of interpretation of an ANA result across testing laboratories.

The analysis of ANA and associated inpatient morbidity and mortality data collected for this study has demonstrated the utility of ANA testing with particular reference to its predictive value for screening and for clinical diagnosis of autoimmune disease and co-morbidities related to a detectable and quantified ANA.

Analysis of ANA data routinely collected over an 11 year period linked to whole of population WA health data through the WADLS (28, 29) has provided a unique opportunity to analyse the ANA data (including antibody level reported in IU/ml and pattern) in relation to sociodemographic variables and outcomes in terms of morbidity and mortality. In addition ANA outcomes have been reported quantitatively in IU/ml which provides a standardised approach to ANA reporting across the 11 years.

This research extends the knowledge of morbidity and mortality associated with ANA levels reported in IU/ml and ANA patterns by using whole-population information obtained from point-of-contact service linked to State death and cancer registries along with hospital morbidity.

1.3 AIMS AND OBJECTIVES OF THE STUDY

Analysis of ANA data from the PathWest data collections linked with WA inpatient morbidity, mortality and cancer registry data for the period 2000-2010 were used to investigate the following hypothesis:

That the IIF test for non-organ specific autoantibodies binding to intracellular components (ANA):

1. when precisely quantified in IU/ml, is an efficient screening test for diagnostically relevant autoantibodies and
2. ANA level, pattern and antigen specificity are all predictive of inpatient morbidity, cancer morbidity and mortality.

The aims of this study were to:

1. examine the relationship of ANA level and pattern to the occurrence of autoantibodies to particular nuclear or cytoplasmic components of the cell, for example U1 ribonucleoprotein (RNP), double stranded deoxyribonucleic acid (dsDNA) and centromere;
2. examine inpatient morbidity and mortality outcomes with respect to ANA level, pattern and antigen specificity: and

3. analyse the influence of age, gender, country of birth, Indigenous Status and socioeconomic status on associations between ANA level and inpatient morbidity and mortality.

1.4 STATEMENT OF CANDIDATE CONTRIBUTION

This thesis is my own account of my research and contains as its major content work that has not been submitted for a degree at any other university.

The ANA, dsDNA and extractable nuclear antigen (ENA) antibody data-sets used in this study were extracted from PathWest Laboratory Information System Database by Ms Charmaine Tonkin following Ethical Approval by the Human Ethics Research Committee for the DoHWA and consent from the data custodian of the PathWest Laboratory Medicine Information Database.

The whole of population inpatient morbidity, mortality and cancer data and data linkage were provided through the WADLS following ethical approval by the Human Ethics Research Committee (DoHWA).

Applications for Ethical Approval to conduct this research, access to the data through the WADLS and all the analysis undertaken for this study were conducted by the candidate. The manuscript has been prepared by the candidate in consultation with Prof David Preen and Clinical Associate Prof Peter Hollingsworth.

The candidate was responsible for conducting all analyses; writing up of results and, in conjunction with her supervisors, interpretation of the findings.

1.5 POSITION OF THE RESEARCHER

The researcher is a Clinical Scientist with 12 years of experience in the Autoimmunity and Immunopathology Diagnostic Laboratory where the ANA testing investigated in this study was performed. The researcher has been involved in the reporting and validation of results for ANA, dsDNA and ENA antibody characterisation assays that form the basis of the data investigated in this thesis.

1.6 STRUCTURE OF THE THESIS

This thesis is divided into nine chapters and includes five studies of ANA from a clinical cohort for which results were reported by a single Pathology service over the period 2000 to 2010.

Chapter 1 provides the background for the study; discusses the significance of the research and outlines the research hypothesis and aims of the study.

Chapter 2 reviews and critiques the literature related to autoantibodies, autoimmunity and ANA in the diagnosis of rheumatic disease.

Chapter 3 describes the study design, the study sample and the pathology and demographic variables used in the analysis. It also provides an overview of the data linkage process as well as ANA, dsDNA and ENA antibody testing protocols including changes in testing approaches and reporting that have occurred during the study period.

Chapters 4 to 8 comprise the five studies of the ANA variable; each chapter includes a brief introduction followed by details of the analysis relevant to the study. The chapters conclude with a discussion relating to the findings of the study. The specific studies undertaken in each of these chapters are as follows.

Chapter 4 looks at the descriptive statistics of age and gender for all ANA results in the clinical cohort reported in the study period. The number of tests/individuals, site of collection and difference in frequency of ANA outcome over the months and years of the study are also investigated.

Chapter 5 focuses on the incident ANA cohort (i.e. the first ANA test for an individual) linked to whole of population health data. It considers ANA level and pattern, age, gender, country of birth, Indigenous Status, socio-economic status and area remoteness as independent variables in a binomial regression analysis (pattern and level were not included in the regression analysis). This chapter also investigates the effect of ANA as a binary outcome or grouped into levels as predictor variables for mortality and cancer as outcomes in a binary regression analysis.

Chapters 6 and 7 describe the dsDNA and ENA antibody data associated with the incident ANA cohort along with a multivariate logistic regression (LR) analysis of mortality associated with the ANA outcome or level.

Chapter 8 analyses incident ANA linked to inpatient morbidity data in a multivariate LR analysis to determine the significance of ANA level and pattern on inpatient morbidity. This inpatient morbidity data includes the principal diagnosis and co-morbidities associated with hospital admission.

Chapter 9 summarises the findings of the thesis and discusses the significance of its findings in relation to current research and clinical practice. It also outlines recommendations for future research in relation to the use of ANA level and pattern as a screening test. The strengths and weaknesses of the study are also discussed in this chapter.

2 LITERATURE REVIEW

Autoimmunity is described as immune recognition and reaction against self-antigens (endogenous nuclear, cytoplasmic and surface membrane components of the cell) resulting from a breakdown in the highly regulated process of immune tolerance (30). Major factors thought to contribute to the initiation and progression of autoimmunity include inflammation, infection, the environment and programmed cell death (apoptosis) (31). Both humoral (B cell) and cellular (T and NK cells) immune responses are associated with autoimmune disease.

Autoantibodies can be associated with specific tissues e.g. pancreatic islet cells which are associated with Type 1 diabetes mellitus and thyroid peroxidase in the thyroid follicular cells which is associated with autoimmune thyroid disease. They can also be associated with a systemic autoimmune response in which a number of tissues are affected i.e. autoantibodies directed against endogenous intracellular nuclear and cytoplasmic components of the cell such as double stranded DNA, nucleoprotein particles and transfer RNA synthetases collectively referred to as ANA.

2.1.1 MECHANISMS OF AUTOIMMUNITY

An important function of the immune system is discrimination between self and non self in order to defend the body against and eliminate aberrant cells or pathogens that can cause inflammation, malignancy and infection (32). Non-reactivity to self is referred to as tolerance and is achieved under normal conditions by a number of tightly regulated processes of the innate and adaptive immune response involving numerous cells and molecules. Maturation and proliferation of B cells responsible for antibody production is regulated at several checkpoints during the process of maturation. Immature B cells with high affinity for self-antigen are eliminated in the early stages of development in the bone marrow. Mature B cells with self-reactive antigen receptors will undergo reconfiguration or elimination in secondary lymphoid follicles depending on their avidity and affinity for self-antigens and their response (or lack thereof) to co-stimulatory signals from T cells and cytokines (33).

The collective effect of the various control mechanisms results in a balance between a competent immune response to defend the host against aberrant cells or pathogens and autoimmunity.

The immune system comprises an innate and adaptive immune response (34-37). The initial immune response to a pathogen is initiated by the innate arm of the immune response which relies on recognition of specific components of the pathogen referred to as pathogen associated molecular patterns (PAMPs), molecules (proteins, carbohydrates and nuclear material) expressed by the microorganism but not by the host's own cells. The PAMPs are recognized by pattern recognition receptors (PRRs) on a number of cell types including; macrophages, dendritic cells and neutrophils. Ligation of PRR on neutrophils due to microorganisms or damaged tissue leads to the activation of the effector function of the neutrophils including the production of lytic enzymes and antimicrobial activity. They also interact with macrophages, dendritic cells, T, B and NK cells and modulate differentiation, proliferation and cytokine production (38). Macrophage and dendritic cell recognition of pathogens by PRRs results in engulfment and processing of the pathogen or microorganism leading to the generation of antigens which are then displayed on the surface of antigen processing and presenting cells (APC). APCs travel to the secondary lymphoid tissues where they present the antigen to T and B cells thereby initiating an adaptive immune response.

The adaptive immune response is highly specific, targeting distinct molecules (antigens) and has the ability to retain memory of antigen exposure allowing a rapid response to repeated exposure (34). In the adaptive immune response T and B cell recognition of the antigen presented by the APC leads to differentiation of the B and T cells into effector cells capable of eradicating the pathogen. T cells can be broadly divided into i. cytotoxic T cells, ii. helper cells (T_H)s which regulate the immune response or iii. memory cells which are able to react more rapidly and efficiently to a subsequent exposure to the antigen. The T_H cells have a role in regulating the immune response initially to promote proliferation and activation and subsequently to dampen the response when the antigen burden has been reduced.

In a number of autoimmune diseases there is reactivity between the self-antigens e.g. endogenous stress-induced self-molecules and the PRR, and in the presence of additional co-stimulatory signals an immune response to self-antigen is initiated (reviewed in (39)).

Failure to regulate or "turn off" the T or B cell response may lead to tissue destruction and exposure of self-antigens at elevated levels which may be sufficient to initiate an autoimmune response. Studies have investigated the role of deregulation of apoptosis in

the induction of autoimmune disease (40-43). Apoptotic cells were shown to be more antigenic and bound antibodies directed against a number of autoantigens including Jo-1, centromere protein B (CenpB) and ribonucleoprotein (RNP) more readily in cells under apoptotic conditions compared to living cells.

Both T and B cells are involved in autoimmune disease, however as this study is looking at a humoral autoimmune response further discussion will be limited to B cells and development of an autoantibody response.

2.1.2 AUTOANTIBODIES AND AUTOIMMUNE DISEASE

Autoantibodies have been placed into three broad categories:

i) those directly involved in the initiation and pathogenesis of the autoimmune disease (20),
ii) markers of disease but have no apparent role in pathogenesis,
iii) autoantibodies that react with self-molecules (predominantly Immunoglobulin class (Ig) M) which may prevent or dampen a further antibody mediated response to the respective antigen and are therefore not associated with disease (reviewed in (20, 44, 45)).

The frequency of autoantibodies in healthy individuals and disease cohorts has been investigated in a number of studies (21, 25, 46-49). These studies have demonstrated in the general population a background level of both ANA and numerous tissue autoantibodies (e.g. smooth muscle, parietal cell and thyroid peroxidase antibodies). The frequency was increased in older individuals and often higher in females in the absence of clinical symptoms (5, 46, 50, 51). Autoantibody frequency has been investigated in disease groups and healthy individuals across a range of age groups. Moulias *et al* (1984) reported an absence of ANA in healthy individuals between 20 and 50 years of age compared to a prevalence of 10% in individuals aged 70 and above (52). However a study of 4,754 individual in a health and nutrition survey in the United States of America (USA) showed a prevalence of ANA ranging from 11.2% to 19.2% in individuals aged 12-19 years of age up to 70+ years of age respectively (53).

Furthermore, studies have shown that antibodies can precede the onset of autoimmune disease. A retrospective analysis on a cohort of rheumatoid arthritis patients using stored

sera demonstrated that anti cyclic citrullinated antibodies or IgM antibodies against IgG (rheumatoid factor) were detectable in patient serum up to 13.8 years prior to the onset of disease (54). Similarly, in a 10 year follow-up study Arbuckle *et al* (19) demonstrated that autoantibodies preceded the onset of the autoimmune disease SLE and concluded that there was a predictable progression from normal immunity to benign then pathogenic autoimmunity followed by clinical illness (19, 54).

Recent studies have shown that immune function deteriorates with advancing age together with an increased susceptibility to infection, cancer and autoimmune disease, with a decline in T and B cell generation along with a change in the expression of co-stimulatory molecules and the anti-inflammatory cytokine interleukin (IL)10 production (55, 56).

Environmental and epigenetic factors can also contribute to an autoimmune response. Nutritional status and exposure to factors such as tobacco, drugs and ultraviolet rays have been implicated in the pathogenesis of autoimmune disease (57, 58). Molecular mimicry, tissue damage and antigen leakage arising from infection may contribute to the onset of an autoimmune response by the host (e.g. viral and bacterial infections such as Epstein Barr virus have been implicated in SLE, Hepatitis C Virus in autoimmune hepatitis and Helicobacter pylori in autoimmune gastritis(51)).

A number of factors including sex hormones and differences in the immune response in males and females have been proposed to explain the gender difference in the incidence of autoimmune disease, however the mechanisms for the gender differences remain inconclusive (51). Cooper *et al* report the prevalence of various autoimmune diseases to be between 0.4 (chronic active hepatitis) and 1,151.5 per 100,000 (Grave disease/hyperthyroidism) in the population with a female predominance between 32 and 95% (24). The most striking gender differences with an increased incidence in females are noted in Thyroiditis (95%) Addison's Disease (93%), Sjogren's Disease (94%), SLE and Primary Biliary Cirrhosis (89%)(24).

As mentioned previously, autoimmune diseases may be grouped as systemic or tissue specific disorders. Systemic autoimmune diseases are characterised by the presence of one or more ANAs. ANAs can be detected in the serum of patients with rheumatic disease (5, 59) and autoimmune diseases of the liver (60, 61), lung (62, 63) and thyroid (64) as well as other disorders including chronic infections (65-67) and inflammatory

myopathies (68). Accurate and early detection of an ANA is important given that the prognosis for autoimmune diseases is favourable when managed with immune modulating therapies (reviewed in (5, 69)).

Autoimmune disease can also affect pregnancy outcomes at all stages (57). Autoantibodies to Ro52 (an E3 ubiquitin ligase) found in patients with Sjogren's Syndrome (SjS), SLE and other rheumatic diseases transfer across the placenta and can lead to congenital heart block in the foetus (70, 71).

The presence of autoantibodies in the absence of clinical features of the disease does not infer the presence of autoimmune disease as observed with the incidence of autoantibodies in the general population. However it is important to consider the positive predictive value of an autoantibody in the presence of other clinical features.

2.2 ANTI-NUCLEAR ANTIBODIES

In a strict definition, ANAs are a heterogeneous group of antibodies directed against components (autoantigens) in the cell nucleus (5, 72, 73). Frequently however, the term also refers to autoantibodies binding to antigens in the cell cytoplasm (74). The antigens are common to all nucleated cells and have functions in transcription or translation, the cell cycle or as structural proteins (reviewed in (75)). A number of these molecules or complexes which include proteins and nucleic acids have been well characterised (76-80). Antibodies which act against these antigens, including SSA/La, SSB/Ro, Scl70, Sm, double stranded DNA (dsDNA) and the U1-RNP complex are considered specific markers of autoimmune diseases such as SLE, SjS, MCTD and Systemic Sclerosis (SSc) (81-83) and reviewed in (5, 72, 84)). ANAs directed against other nuclear antigens are less clearly associated with particular diseases (reviewed in (4, 5)).

The first step in the detection of ANA is a screening test by IIF which detects antibodies in patient sera that bind to components of the cell nucleus or cytoplasm visualised using a fluorescently labelled anti human detection antibody (85). ANA levels are quantified and reported either in international units or as the highest dilution at which they are detectable (titre) (84). The predictive value for various autoimmune disorders increases with an increase in the ANA level detected in the patient serum (84).

ANA are also classified according to the staining pattern observed by IIF on a cell substrate; examples are shown in Figure 2.1. The ANA pattern loosely predicts the target structure or complex bound by the ANA, and the antigenic target in turn predicts the disease. For example, a high level ANA with a speckled pattern is likely to be associated with antibodies to U1-RNP which is associated with Mixed Connective Tissue Disease (86), however the same pattern may also predict the presence of anti-SmD antibodies which are specific for SLE (84, 87). Both U1-RNP and Sm are components of ribonucleoproteins (82).

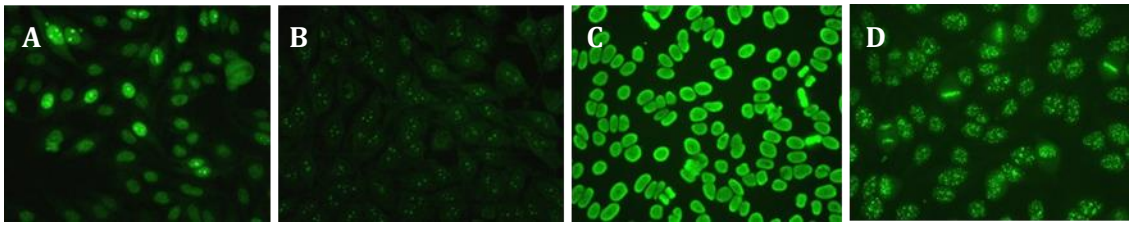


FIGURE 2.1 ANA CLASSIFIED ACCORDING TO IIF STAINING PATTERN ON HEP-2000 CELLS. **A.** RO60 ANTIBODY **B.** NUCLEOLAR PATTERN **C.** HOMOGENEOUS PATTERN **D.** CENTROMERE ANTIBODY PATTERN.

In diagnostic laboratory practice, when a sample is considered ANA positive, an effort is made to identify the antigen bound by the antibodies. Table 2-1 lists less common ANA antigenic targets. Various technologies have been used to identify the target antigens including immunodiffusion, counter-immuno-electrophoresis (CIEP), enzyme linked immunoassay (ELISA) or immunoblot (48, 88). Some ANA are putatively identified by the pattern alone e.g. nuclear dots and centriole. Furthermore, there are other ANA patterns which do not have a characterised target antigen or disease association (48). The predictive value of these unidentified autoantibodies is currently unclear, and future research is warranted.

As previously indicated the ANA test is found to be positive in approximately 3-8% of the apparently healthy population (46, 89, 90), and in some publications this level has been reported to be as high as 32% (7, 46). The 95th percentile of ANA by IIF in the general adult population is 7 U/ml (10) and Hollingsworth *et al* (73) have previously shown that where ANA is < 7IU/ml the target antigen will not be identified. In routine practice identification is not attempted on these samples.

TABLE 2-1 AUTOIMMUNE DISEASES ASSOCIATED WITH THE LESS COMMON ANA PATTERNS.

IIF Pattern	Clinical diagnosis
CenpB	Limited Scleroderma
	Liver disease
Lamin	Anti-phospholipid syndrome
Mitotic Spindle	Arthritis
Proliferating Cell Nuclear Antigen	SLE
Nuclear Dots	Liver disease
	SLE/SjS
	Rheumatoid Arthritis

Adapted from Roberts Thomson et al (48)

2.3 LINKED DATA

The WA Data Linkage System provides the opportunity to analyse data from a number of health record and administrative databases using computerised probabilistic matching (12, 91). This study has utilised routinely-collected ANA data linked with administrative data from WA statutory hospital, cancer and death registries to investigate disease associations with ANA antibody levels and ANA target antigens in a cohort of individuals who have had a serum sample submitted for ANA testing at PathWest Laboratory Medicine during the 11 years from 2000 to 2010. The use of linked data allows retrospective analysis of death, cancer and inpatient morbidity associated with an ANA result in a large WA cohort. Clark *et al* have evaluated the WA socio-demographic indicators relative to other Australian states and shown that WA was representative of all Australian jurisdictions (92). The Australian territories were shown to be the least representative of the sociodemographic and health economic indicators.

The approach this thesis has taken ensures that in a large WA clinical cohort, inpatient morbidity and mortality outcomes have been collected in a standardised manner and that the outcomes of the study are likely to be representative of other states of Australia.

2.4 PRIOR RESEARCH

Detection of an ANA in a patient's serum is important in the preliminary diagnosis of SLE. Epidemiological studies reviewed by Pons-Estel *et al* (14) and Reeves (93) have shown that gender, ethnicity and age are factors that affect the frequency and long-term outcomes for SLE. However, they do not discuss the level of ANA detected in patient serum. Studies described by Myckatyn and Wijeyesinghe (13, 94) investigate a group of 62 ANA positive individuals without clinical symptoms of CTD in the presence of a positive ANA result at the time of testing. Follow-up studies of the cohort using serological analysis and a telephone interview to establish a self-reported change in autoimmune status showed that a further 6% were diagnosed with CTD five years after the initial testing, and 7.4% 10 years after the initial testing. In addition, a study by Dinser *et al* (18) in which 76 patients were followed up over three years with a questionnaire, serological testing and a clinical evaluation showed that ANA had a low predictive value for progression to autoimmune disease. These studies have relied in part on self-reported assessment of participants' current status, however the validity and reliability of this approach has previously been questioned (15).

A number of studies have been reported which analyse large clinical cohorts of >10,000 individuals (48, 95-97), the frequency of a positive ANA in these studies ranged from 12.3– 28.3%. The objective of the studies varied and consequently gender, age distribution and frequency of ANA patterns and morbidity were not reported in all the studies.

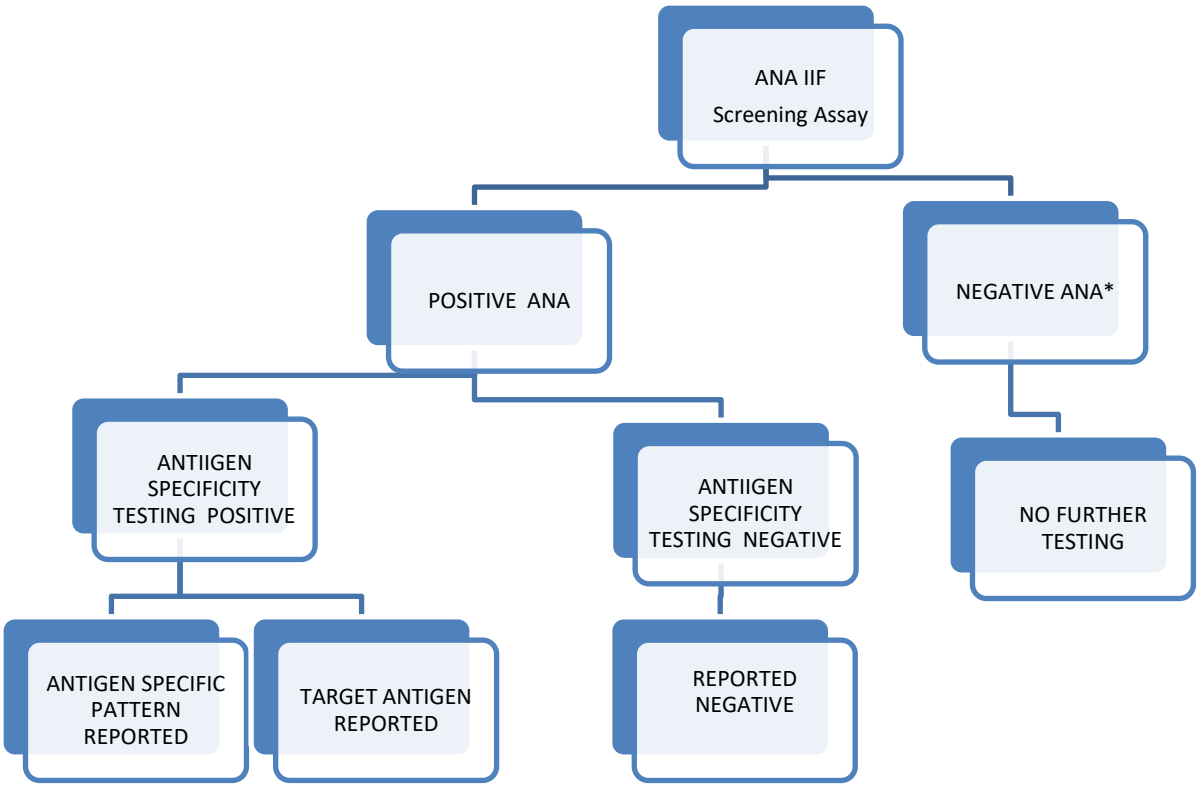
This thesis investigates a clinical cohort of >85,000 individuals with an ANA result (including level and pattern) and analyses the ANA outcome adjusted for age, gender and socio-demographic variables in a multivariate LR analysis. It also uses inpatient morbidity, mortality and cancer registry data to investigate clinical outcomes associated with an ANA.

3 METHODS

3.1 STUDY DESIGN

This investigation comprised a retrospective longitudinal cohort study using routinely-collected administrative data available through the WADLS linked to PathWest ANA test records for the period 2000-2010.

ANA have been assayed in PathWest Laboratory Medicine for the public sector for the last 30 years, an average of 11,000 results were reported annually over the study period (data shown in Chapter 4). Figure 3.1 is a diagrammatic representation of the testing protocol and result reporting for ANA testing at PathWest Laboratory Medicine.



*A clinical request for further testing on a negative sample will require it to be sent for characterisation

FIGURE 3.1 THE TESTING PROTOCOL FOR ANA REQUESTS RECEIVED IN THE DIAGNOSTIC LABORATORY.

3.1.1 STUDY SAMPLE

The study sample comprised a clinical cohort of all individuals residing in WA who had an ANA test through PathWest in the period 1st January 2000 to 31st December 2010. PathWest is responsible for ANA testing requested in public hospitals and metropolitan

and regional PathWest collection centres. The limitations placed on eligibility of patients tested for ANA for inclusion in the study were that:

- the request was not registered as a clinical trial sample,
- the patient's postcode at the time of ANA testing was in mainland WA,
- the date of birth was recorded (2 cases excluded).

The patient cohort used in the analyses for Chapters 5 – 8 was restricted to individuals who had not had an ANA or associated diagnostic test prior to 2000 (WA Incident ANA Clinical Cohort).

The 11-year observation period was chosen to maximise the opportunity of detecting associations between ANA and morbidity and mortality.

Data collected from June 1997 to the 31st December 1999 were also retrieved from the PathWest laboratory information system and used to identify patients whose first ANA test was reported prior to 1st January 2000 to allow exclusion from the study. Demographic data including age, sex, Indigenous Status, residential remoteness, social disadvantage and country of birth were extracted from the DoHWA administrative whole-of-population HMDC (12) and the PathWest Laboratory Medicine laboratory information system (LIS). Data collections were linked through the WADLS using best-practice probabilistic matching techniques reported elsewhere (16).

PathWest Laboratory Medicine is the only public pathology service provider in WA. It performs ANA testing for all regional public hospitals, metropolitan and rural PathWest collection centres, the two largest of the State's three teaching hospitals (Sir Charles Gairdner Hospital and Royal Perth Hospital) and other community pathology service providers in WA. Data available from the Australian Government Department of Human Services, Medicare Benefits Schedule showed that in the period from 2000-2010 352,225 claims were processed for an ANA test (Medicare item 71097) in WA (98). This is likely to be an underestimate of the total number of ANA tests requested due to Medicare coning rules which limits the number of tests that can be billed on a single pathology request (99). The information provided above suggests that this study of 120,997 requests represents approximately one third of the ANA tests carried out on the WA population over the 11 year period (98).

3.1.2 ANA TESTING

ANA testing was performed on a serum sample and the results reported as a level in IU/ml ranging from <2 to >30IU/ml (100). ANA data were also categorised into 5 groups according to ANA level:

- <7IU/ml (negative test result),
- 7 - <10IU/ml,
- 10 - <20IU/ml,
- 20 – 30IU/ml,
- >30IU/ml.

The IIF pattern of staining was routinely recorded if the ANA was ≥ 7IU/ml and if the pattern identified was for antibodies against cellular elements such as the Golgi apparatus and centriole (101, 102) which were reported independent of the intensity of the staining.

3.2 WA DATA LINKAGE SYSTEM DATA

The DoHWA WADLS systematically links WA population health data from a number of sources including administrative and clinical data to be integrated. The data collections linked by the WADLS relevant to this study were: cancer registrations, hospital admissions and death records. Records from these data collections were extracted for patients identified from the ANA testing cohort as outlined above.

Variables collected through the WADLS (WA mortality, inpatient morbidity and cancer registry) are outlined in Table 3-1.

TABLE 3-1 SPECIFIC VARIABLES TO BE EXTRACTED AND LINKED BY THE WADLS FOR THE PATHWEST STUDY SAMPLES FROM 1997-2010.

Variable	Item
Cancer Registry	Date of registration
	Cause of death ICD-10-AM codes
	Cancer type Standard tumour information including: morphology, site and grade
Morbidity	Date of birth (mm/yyyy)
	Gender Indigenous Status Country of birth
	Principal diagnosis (ICD-10-AM Coding specified)
	Co-diagnoses (up to 20 available) Additional diagnoses (ICD-10-AM Coding specified)
	Principal diagnosis 5 years prior to first recorded ANA test result
	Additional diagnoses 5 years prior to first recorded ANA test result
Mortality	Date of Death
	Cause of Death
	Age at time of Death
	Multiple causes of death ICD codes
	Indigenous Status
	Country of Birth

3.2.1 LOSS TO FOLLOW UP

The WADLS data are restricted to the WA population, the potential loss to follow-up due to population migration out of WA will vary over the time frame of this study, however as an indication of the level of change that may occur; in 2008-2009 approximately 59,500 people (2.65%) migrated interstate or overseas from WA (92, 103). The age and gender distribution of interstate and overseas migrants is available through the Australian Bureau of Statistics (ABS) and can be used to assess whether there is an age and gender bias due to systematic differences in those lost to follow up.

3.2.2 MORBIDITY AND MORTALITY DATA

Inpatient data were extracted from the hospital morbidity data collection containing information concerning all inpatient discharge summaries from all public and private (licensed by WA Health) acute and psychiatric hospitals and private day surgeries in WA (104) for an episode of care. Data were available for 127,677 individuals in the study sample.

Mortality data available for linkage were limited to deaths that occur in WA and for the period of this study the data were collected from the Registrar General and the Australian Bureau of Statistics (ABS)(105). Accordingly mortality analysis was limited to individuals who were resident in WA at the time of incident ANA request and at the time of death.

Survival times were calculated from the time of collection of the incident ANA to the time of death or the censor date of 20th February 2012.

Morbidity and mortality data collected through the DoHWA WADLS were reported as World Health Organisation International Classification of Disease and Health Related Problems Codes (Version 10 with Australian Modifications: ICD-10-AM) (106). The ICD is a standardised classification system applied to the written clinical notes of hospital admission patients and coding for cause of death.

All-cause mortality and cause of death at the first and second tiers of the ICD codes were analysed.

Morbidity data were reported on an episode basis. The diagnostic classifications for each episode included a principal diagnosis and up to 20 additional co-diagnosis of episode care. In the data analysis, morbidity data were expressed as the incidence of a broad disease category for both the principal diagnosis and co-diagnosis of an episode of care.

Deaths were reported for 9,002 individuals with incident ANA data; however ICD-10 coded deaths descriptions were not available for 1,165 individuals (12.9%).

3.2.3 CANCER DIAGNOSIS

Cancer data supplied through the WADLS were recorded on an individual (case) basis listing multiple events where they occurred.

Cancer is defined in the Health (Notifications of Cancer) Regulations 1981(107, 108) as "any malignant growth of human tissue which if unchecked is likely to spread to adjacent tissue and beyond its site of origin and includes —

(a) all *in situ* neoplasms;

(b) all malignant neoplasms of the skin other than primary basal cell carcinoma and primary squamous cell carcinoma; and

(c) all neoplasms of the brain, spinal cord and cranial nerves, and any other intracranial neoplasms, whether benign or malignant."

Doctors and pathologists are legally required to report all cancers diagnosed in WA (109). Information is also received about Western Australians who have been diagnosed elsewhere. These data are used to compile the Cancer Registry (104). Prior to 1996, only invasive tumours were subject to compulsory notification. However, since February 1996 Health Regulations (109) have required notification of:

- all tumours
- all non-melanoma skin cancers other than basal cell and squamous cell carcinomas
- all intracranial and central nervous system tumours whether benign or malignant

Variables collected through the WADLS (WA mortality, inpatient morbidity and cancer registry) are outlined in Table 3-1 above.

3.3 PATHWEST DATA EXTRACTION

Data extracted from the PathWest Laboratory Medicine Information System included all results for ANA level and pattern as well as demographic details as described in Table 3-2. An ANA result recorded in the PathWest LIS ULTRA comprised 3 variables: i) the quantitation of the antibody (IU/ml), ii) the presence or absence of antibodies to the SSA/Ro antigen which is a specific pattern observed on the Hep2000 ANA test substrate and, iii) a code to identify other patterns observed on the substrate (e.g. nucleolar, centromere etc.). ANA patterns reported include: homogeneous, speckled, homogeneous plus speckled, centromere, multiple nuclear dots and nucleolar. Patterns seen with a frequency of less than 1% were grouped together as 'other' in this study.

TABLE 3-2 SPECIFIC VARIABLES EXTRACTED FROM THE PATHWEST DATABASE FOR THE STUDY SAMPLE FROM 1997-2010.

PATHWEST DATA		
Variable	Item	Output
	De-identified Sample ID	Numerical
	Gender	M/F
	Postcode	
	Date of Birth	DD/MMM/YYYY
	Collection Centre	Coded Text
ANA IIF	IIF Pattern	Description (e.g. nucleolar, speckled)
	Quantitation (Level)	IU/ml
	Ro60 specific ANA pattern	Detected/Not detected
	Comment Fields	Text
ENA Screen	Immunodiffusion	Detected/Not Detected
	CIEP	Detected/Not Detected
	ELISA Screen Result	Positive/Negative
ENA Characterisation	SSA Ro (52 and 60)* SSB/La Sm (D and B)* RNP (A,C and 70)* Jo-1 Topoisomerase (Scl70) Histones § Centromere B (CenpB) § Ribosomal P§	Detected/Not Detected Negative/Positive/Equivocal§
	Anti dsDNA antibodies	IU/ml

*additional data collected using the immunoblot: RNP separated into RNP A,C and 70, SSA Ro52 and Ro60 reported separately and Sm reported as SmD and SmB. §Data collected from immunoblot only

The ANA assay is a screening test used to flag samples which are likely to have one or more clinically relevant autoantibodies present and require further testing. Clinicians are therefore advised that ANA samples with a result ≥7IU/ml or with a specific ANA pattern (centromere and SSA/Ro60) has been reported and the sample should be sent for follow-

up testing to identify the antigen bound by the antibody (as indicated in Figure 3.1). A panel of up to 13 target antigens were included in the characterisation by immunoblot. These results were classified as i) detected, ii) equivocal and iii) negative. An equivocal result was assessed as 66 – 100% of the level of the cut-off control used in the assay and was considered negative in the analysis.

Antibodies against dsDNA are frequently associated with a homogeneous ANA pattern and are reported separately to other ENA and cytoplasmic antigen antibody responses. The presence of a dsDNA antibody is not excluded in samples with an ANA ≥ 5 IU/ml and further testing is recommended for these samples.

3.4 DSDNA ANTIBODY TESTING

DSDNA antibody levels were measured by two variations of the Farr radioimmunoassay (RIA; reported in IU/ml) (110). Reference ranges varied both between and within methods with three changes made over the 11 year period. The appropriate ranges (shown below) have been applied to the relevant time periods in the data and results categorised as positive or negative accordingly:

- Method 1 1.01.2000 2000 – 11.02.2000- dsDNA antibody Reference value< 5IU/ml
- Method 2 11.02.2000 - 2000 - 06.12.2000- dsDNA antibody Reference value < 3IU/ml
- Method 3 6.12.2000 – 28.05.2002-dsDNA antibody Reference value < 5IU/ml
- Method 4 28.05.2002 onwards -dsDNA antibody Reference value < 7IU/ml.

For analysis, dsDNA antibody results were grouped as:

- Negative i.e. less than the reference value (92.8% of individuals),
- Ref <10IU/ml (4.5% of individuals)
- 10 – 30IU/ml (1.6% of individuals)
- >30 – 70IU/ml (0.6% of individuals)
- >70IU/ml (upper reporting limit of assay without dilution of patient sample; 0.5% of individuals).

3.5 ENA ANTIBODY TESTING

ANA characterisation by a secondary test (excluding dsDNA antibody) results were merged with the ANA data. The total number of cases in the merged dataset was 42,761 which comprised 34,921 individuals of which 4,975 had repeat testing. The maximum number of repeat tests for characterisation was 23. Results for a screening test for the first request were not available for 108 cases.

Comparisons of ENA and cytoplasmic antibody outcomes and ANA were based on the first ANA test for each individual followed by:

a. an ENA/cytoplasmic antibody screening test,
b. an ENA/cytoplasmic antibody characterisation result.

3.5.1 ENA ANTIBODY SCREENING ASSAY

Over the 11 year period covered in this study three screening methods were implemented:

- Immunodiffusion (prior to June 2000) using the Ouchterlony diffusion method in which a positive result is reported when a line of precipitation is visible between the patient serum and soluble antigen in an agarose gel.
- CIEP (May 2000 to August 2006, after which dual testing was carried out for ENA/cytoplasmic antibody positive results up to September 2010). Briefly, this method involves an initial immunodiffusion step using a crude soluble antigen extract and patient serum followed by an electrophoresis step. The crude antigen sources were rabbit thymus and K562 cell line. A positive result was reported when a line of precipitation between patient serum and antigen is detected.
- Enzyme Linked Immunosorbant Assay (ELISA) - a screening assay (a solid phase assay that includes recombinant or highly purified antigens; RNP, Sm, SSA (Ro52 and Ro60), SSB, Scl70 and Jo1. A positive result was determined using a manufacturer assigned cut off value of 20 Units (results were reported using this method from August 2006 onwards).

Screening results were reported as positive or negative. A positive screen result was followed up with testing against a panel of target antigens included in the screening assay and with known clinical relevance to identify ENA antibodies present in the serum. If specifically requested by the clinician a sample with a negative screening result would also progress to characterisation.

3.5.2 ENA ANTIBODY DETECTION ASSAY

Three characterisation methods have also been adopted over the 11 year study period:

- Immunodiffusion (January – October 2000) in which characterised ENA antibody sera are tested in parallel with patient serum using a crude soluble antigen extract to identify a line of identity between the two samples.
- CIEP (October 2000 – 2 February 2003) occasional samples were tested by CIEP post Oct 2003. This method is similar to the immunodiffusion method however it includes an additional electrophoresis step.
- Immunoblot (INNO-LIA ANA Update, Fujirebio Europe NV, Belgium; Feb 2003 onwards).

In addition, the antigens available for autoantibody detection by each of the characterisation/detection methods have not been consistent over the testing period; detection of autoantibodies targeting Ro52, CenpB, histones and Jo-1 were added with the introduction of the immunoblot method.

3.6 OTHER MODIFYING VARIABLES INCLUDED IN THE ANALYSIS

3.6.1 SOCIO-ECONOMIC DISADVANTAGE

The Index of Relative Socio-Economic Disadvantage (IRDS) was used to divide the study population into quartiles based on the Socio-Economic Indexes for Areas (SEIFA) assigned to areas of residence at the postcode level (111). The variables used to determine the IRDS were low income, low educational attainment and high unemployment. SEIFA is based on questions asked in the Australian Census that indicate low income, low educational attainment, high unemployment, jobs in relatively unskilled occupations (e.g., labourer, production, clerical, sales or service workers), and other indicators of disadvantage such as public rental housing, and separated/divorced or single parent households (112).

The SEIFA scores were assigned according to the year that the ANA test sample was collected. For samples collected before 2004, the 2001 Census SEIFA weightings were

assigned. For samples collected from 2004 onwards, the 2006 Census SEIFA weightings were assigned (112, 113). SEIFA quartile ranges were determined and the IRDS variable expressed as the quartile within which the SEIFA value fell. The first quartile represented the greatest degree of disadvantage.

Data for which a SEIFA value was not available were assigned a dummy value and not included in the ranking of the SEIFA codes to determine the limits for the IRDS quartiles.

3.6.2 RESIDENTIAL REMOTENESS

To investigate the effect of geographical disadvantage, the Accessibility/Remoteness Index of Australia (ARIA) was used. This index measures access in terms of physical distance from services and is grouped into five categories: very accessible, accessible, moderately accessible, remote, and very remote (114). The ARIA scores were assigned at the postcode level according to the scores available through the Australian Medicare website (115).

3.6.3 COUNTRY OF BIRTH

The country of birth variable was limited to DoHWA linked data with 21,228 (24.5%) of individuals having an undefined country of birth classification. Country of birth was categorized into the major groups of the Standard Australian Classification of Countries (2011, Version 2.2 (116)). This broadly categorised patients country of birth into i) Australia, ii) Oceania and Antarctica (excluding Australia), iii) North-West Europe (N.W. Europe) iv) Southern and Eastern Europe (S.& E. Europe), v) North Africa and The Middle East (N. Africa & M.E.), vi) South-East Asia (S.E. Asia), vii) North-East Asia (N.E. Asia), viii) Southern and Central Asia (S. & C. Asia), ix) Americas and x.) Africa (excluding North Africa). This variable was also evaluated in a subset analysis of patients with known country of birth.

3.6.4 INDIGENOUS STATUS

Individuals included in the study were grouped according to their Indigenous classification reported as a variable from the DoHWA data. Aboriginal not Torres Strait Islander, Aboriginal and Torres Strait Islander and Torres Strait Islander have been grouped

together for the purposes of this study. Additional categories were Non Indigenous (Not Aboriginal or Torres Strait Islander) and Ungrouped (all individuals for which data were not available).

3.7 STATISTICAL ANALYSIS

Descriptive statistics were initially calculated for age, gender, Indigenous Status and country of birth for the Incident ANA Clinical Cohort. ANA, dsDNA antibody, ENA antibody, ANA pattern and cytoplasmic antibody were dichotomised into positive and negative groups. ANA and dsDNA antibody variables were also grouped by level of antibody detected.

Sociodemographic factors were compared as grouped variables. .Crude comparisons were performed using a Chi-square (χ^2) test for categorical data and independent-samples t-tests for continuous variables. Where multiple groups were compared an analysis of variance (ANOVA) was performed for continuous variables.

In addition multivariate LR was used to estimate the odds ratio (OR) and 95% confidence interval (95%CI) for each pathology test outcome with adjustment for age, gender, Indigenous Status, country of birth, ARIA and IRDS. Degrees of freedom (df) in the χ^2 and multivariate LR analysis are reported in each table of results

An additional category for each covariate with missing data (incomplete cases) was created and then included in the analysis for regression modelling (n = 21,089, 24.6% of the incident ANA clinical cohort). Individuals without DoHWA linked health dated accounted for 14,962 (70.9%) of incomplete cases, country of birth and Indigenous Status data were missing on an additional 5,967 (28.3%) and 4,226 (20.0%) cases respectively. Sensitivity analyses were performed between the population with complete data on all variables and the data with the inclusion of the imputed categorical variable. Effect estimates did not change for ANA outcome data; as such the complete data along with imputed values were included in the analysis.

ANA data were grouped according to antibody level and subdivided into observed IIF patterns with:

i. identified antigenic targets
ii. no identified antigenic target

Statistical analysis included a comparison of the proportion of high and low antibody levels detected for the various IIF patterns.

Multivariate LR analysis adjusted for ANA, age, gender, country of birth, ARIA, IRDS an Indigenous Status was performed for:

i. dsDNA antibody outcome
ii. ENA and cytoplasmic antigenic target outcomes.
iii. hospital admission ICD-10 codes
iv. diagnosis of specified cancer
v. all cause and specified cause of death

Significant differences in survival rates for ANA, dsDNA, ENA and cytoplasmic antibody positive and negative outcomes were determined by the log rank test and/or Cox regression analysis. Survival times were calculated from the time of collection of the incident ANA to the date of death or the censor date of 20th February 2012.

The specific analysis performed for each of the five separate studies comprising this thesis is further described in Chapters 5.8.

SPSS v21 and v22 (117, 118) were used for the statistical analysis with statistical significance set at $p<0.05$.

3.8 DATA CLEANING

PathWest ANA data collected from June 1997 to 31 Dec 2010 were reviewed and entries were excluded from the analysis if:

- date of birth was incomplete (n = 191/154,708; 0.12%),
- the postcode recorded was not on the mainland or within WA. Christmas Island and the Cocos Islands were excluded from the analysis (n = 1,603/154,708; 1.03%).
- the ANA record was incomplete (n = 10,550; 6.8%)

- data were identified as a research trial request for ANA testing (n = 441/154,708; 0.28%).

Data up to the 31st Dec 1999 were only used to identify individuals with a previous ANA result (n = 21,312; 13.8%).

To prepare the Incident ANA Clinical Cohort, ANA, ENA and dsDNA antibody datasets were ordered by individual and sequenced by date of sample collection. Individuals with one of these tests reported prior to 1 Jan 2000 were excluded from the incident ANA cohort.

All results for the ANA testing cohort were ranked by date of test and only the 1st test result was included in the analysis. The Incident ANA Clinical Cohort had 17 entries for which gender was not specified, following the preliminary analysis of gender and ANA and dsDNA antibody results these entries were excluded from subsequent analysis.

3.9 ETHICAL APPROVALS

Following approval from the DoHWA Human Research Ethics Committee, a data extraction was requested from the PathWest Data Custodian for all ANA, dsDNA and ENA antibody records collected for the period 1997 to 2010 in the PathWest ULTRA databases relating to ANA testing and the associated variables outlined in Table 3-1. Ethics approval was also gained from The University of Western Australia Human Ethics Committee (WA Health Department HREC Approval 2010/50 and UWA Ethics Approval number RA/4/1/4818).

4 DEMOGRAPHICS OF ANA TESTING AT PATHWEST QEII MEDICAL CENTRE, WESTERN AUSTRALIA

4.1 INTRODUCTION

Detection of ANA as a first line screening test for systemic autoimmune disease is reported to have a high sensitivity. It is used firstly to determine if additional testing is required (level of ANA detected) and secondly to direct more specific follow up testing to characterise the target antigen (dsDNA, cytoplasmic or nuclear antigens). The results of ANA and follow up detection method in conjunction with clinical findings are used in the diagnosis rheumatic disease (95).

However, ANA is reported to have a poor positive predictive value for rheumatic diseases and there is a high background rate of ANA positivity in the general population (17). ANA are associated with active or latent connective tissue disease and have been shown to be present 3 or more years before diagnosis and a mean of 2.2 years before the onset of clinical symptoms (19).

In this chapter the descriptive statistics of age, gender and source of the requests are summarised for the PathWest ANA data collected over 11 years. The data includes multiple tests for individuals.

4.2 METHODS

Study Population: The cohort investigated in this chapter includes all individuals with an ANA reported during the period 1 January 2000 to 31 December 2010 as described in Section 3.1.1. Individuals have been grouped according to their ANA result i. <7IU/ml and ii. ≥7IU/ml (Section 3.1.2).

Analysis: Descriptive statistics have been calculated for age and gender in:

i. the whole cohort
ii. ANA grouped as <7 and ≥7IU/ml.

The frequency of ANA<7IU/ml and ≥7IU/ml results were compared across the years and months of collection and differences compared by χ^2analysis.

The sample collection centre details were extracted with the ANA result. Collection centres were grouped as:

i. non-hospital collection,

ii. hospital collection (location specified for tertiary hospitals and larger collection sites) and

iii. other (locations with small collection numbers grouped together (4.4% of all samples collected).

4.3 RESULTS

4.3.1 GENDER AND AGE

The source data restricted to a Western Australian postcode used for analysis in this study included 120,997 test results. Females were tested more frequently than males: 62.4% v 37.6%, and if tested were more likely to have an ANA ≥ 7IU/ml; 27.4% v 14.7% (Table 4-1).

The mean age of females with an ANA ≥ 7IU/ml was 48.8 (SD=17.5) years compared to 52.5 (SD= 17.5) years for males as shown in Table 4-2.

TABLE 4-1 AGE AND GENDER IN THE STUDY COHORT.

Gender	Frequency (N)	%	Mean Age (years)	Median Age (years)	Age Range (years)	% ANA ≥7IU/ml
Female	75,487	62.4	48.0 (17.6)	48.0	0.0 - 101	27.4
Male	45,493	37.6	49.7 (17.9)	50.0	0.0 - 100	14.7
Undefined	17	<0.1	33.1 (12.0)	34.0	7.0 - 52	11.8
Total	120,997	100.0	48.7 (17.7)	48.0	0.0 - 101	22.6

TABLE 4-2 AGE AND GENDER OF SUBJECTS WITH ANA <7IU/ML AND ≥7IU/ML OVER THE 11 YEARS ASCERTAINED BETWEEN 2000 AND 2010.

	ANA < 7					ANA ≥ 7					Total				
	AGE (years)					AGE (years)					AGE (years)				
	Count	Mean	SD	Min	Max	Count	Mean	SD	Min	Max	Count	Mean	SD	Min	Max
Male	38,810	49.3	17.9	0.0	100.0	6,683	52.5	17.5	0.0	96.0	45,493	49.7	17.9	0.0	100.0
Female	54,841	47.7	17.6	0.0	101.0	20,646	48.8	17.5	0.0	101.0	75,487	48.0	17.6	0.0	101. 0
Undefined	15	31.4	11.5	7.0	50.0	2	45.5	9.2	39.0	52.0	17	33.1	12.0	7.0	52.0
Total[§]	93,666	48.4	17.7	0.0	101.0	27,331	49.7	17.6	0.0	101.0	120,997	48.6	17.7	0.0	101.0

Abbreviations: Minimum (Min), Maximum (Max),

4.3.2 SOURCE OF SAMPLES

The collection centre at which the sample was collected was recorded in the LIS at the time of collection. Samples from tertiary teaching hospitals in the metropolitan area represent 39.9% of the samples in the study. Non-hospital patients accounted for 49.6% of cases and are likely to be requests from both General Practitioners (GPs) and private specialists. The remaining 10.5% of samples were from regional and non-tertiary hospitals. Collection centre information is shown in Table 4-3.

TABLE 4-3 FREQUENCY OF ANA TEST REQUESTS SENT FROM TERTIARY HOSPITALS, METROPOLITAN AND REGIONAL HOSPITALS AND COLLECTION CENTRES.

Collection Centre	Count	% of samples
Non Hospital Patients	60,032	49.6%
Royal Perth Hospital (RPH)	22,514	18.6%
SCGH	21,083	17.4%
SCGH Clinics	3,899	3.2%
Fremantle Hospital	485	0.4%
PMH	182	0.2%
KEMH	96	0.1%
Albany	653	0.5%
Armadale	1,163	1.0%
Bunbury	1,093	0.9%
Geraldton	638	0.5%
Kalgoorlie	1,266	1.0%
Port Hedland	736	0.6%
Rockingham	585	0.5%
Swan Districts	1,242	1.0%
Other	5,330	4.4%

4.3.3 VARIATION IN ANA TESTING BY YEAR AND MONTH

NUMBER OF ANA TESTS

The total number of ANA requests each year is displayed in Figure 4.1. These results show an increase in the number of ANA tests performed annually over the 2000 – 2007 period. The decline in 2008 is associated with a redistribution of samples to a second testing site (requests from the tertiary hospital RPH were redirected to the Fremantle Hospital testing laboratory). The increased number of tests requested during the 2008 to 2010 period reflects an overall increase in workload in the QEII PathWest laboratory.

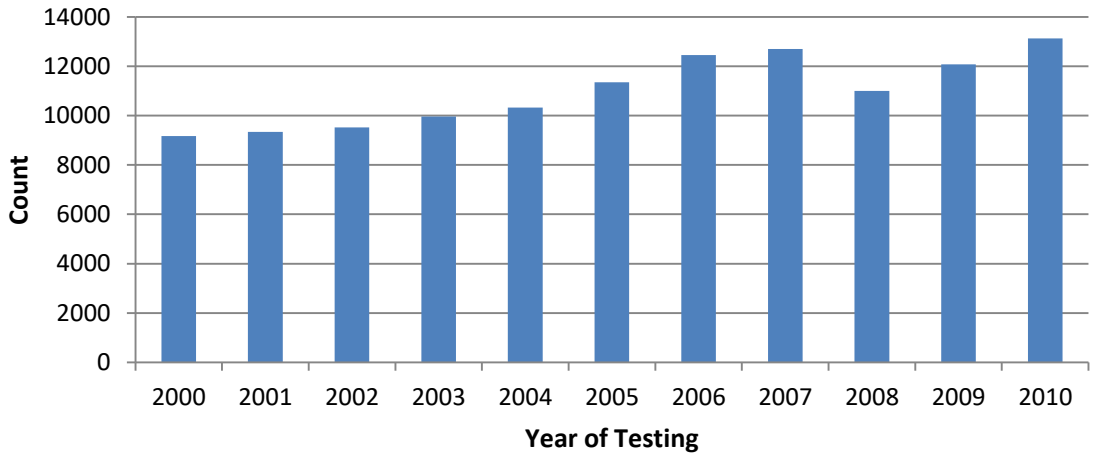


FIGURE 4.1 TOTAL NUMBER OF ANA REQUESTS PER ANNUM IN THE PERIOD 2000 TO 2010.

The average number of ANA tests reported in each month across the 11 year period is shown in Figure 4.2 below.

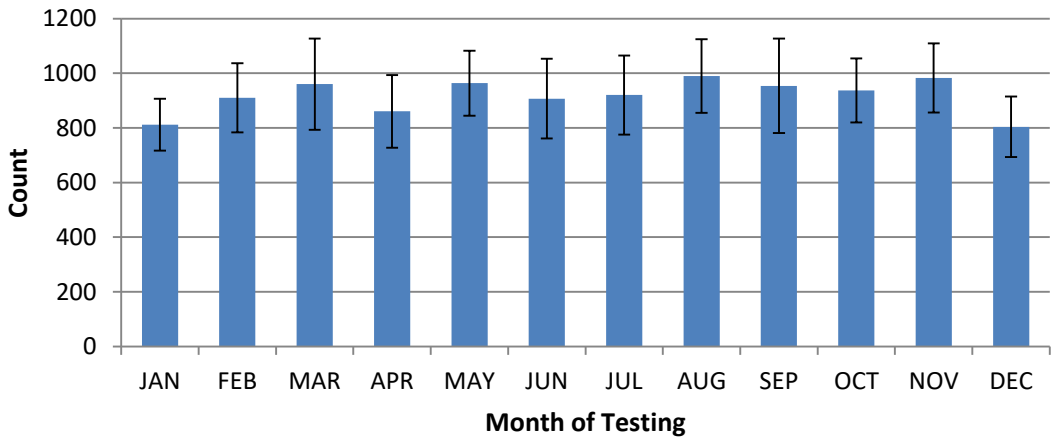


FIGURE 4.2 MEAN NUMBER (AND SD) OF ANA REQUESTS FOR EACH MONTH OVER THE 11 YEARS 2000 TO 2010.

A χ^2 analysis of the distribution of tests across the 12 months indicates that the distribution of tests is not equal ($p<0.001$). The months in which the frequency is at its lowest are December and January. These months are typically associated with a lower level of activity and clinic closure periods over the Christmas and New Year holiday period.

PROPORTION OF POSITIVE TESTS BY YEAR AND BY MONTH

ANA was been measured by IIF and reported in IU/ml as described on Sec 3.1.1. An ANA result of ≥7IU/ml is considered positive and requesting clinicians are advised that follow up ENA or dsDNA antibody testing should be considered. The proportion (%) of tests that produced a result of ≥7IU/ml in the entire cohort for each year is shown in Figure 4.3 and for each month in Figure 4.4.

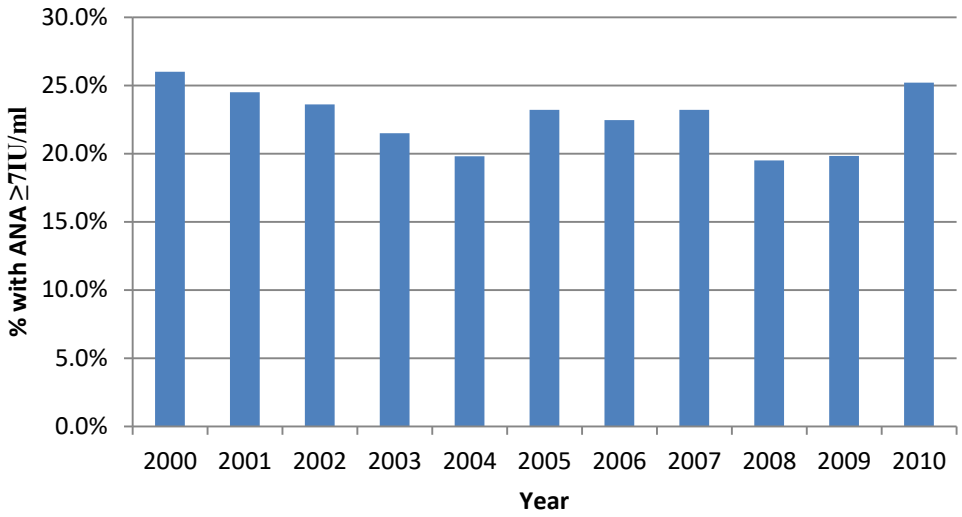


FIGURE 4.3 PERCENT OF REQUESTS WITH AN ANA >7IU/ML OVER THE PERIOD 2000 TO 2010.

A χ^2 analysis of year of collection and ANA outcome showed a significant difference between year of collection and ANA outcome; <7IU/ml and ≥7IU/ml (χ^2 = 306.2, n = 120,997, $p<0.05$). Over the 11 year period of data the proportion of ANA ≥7IU/ml reported annual ranged from 19.5 – 26.0%. Data are shown in Figure 4.3.

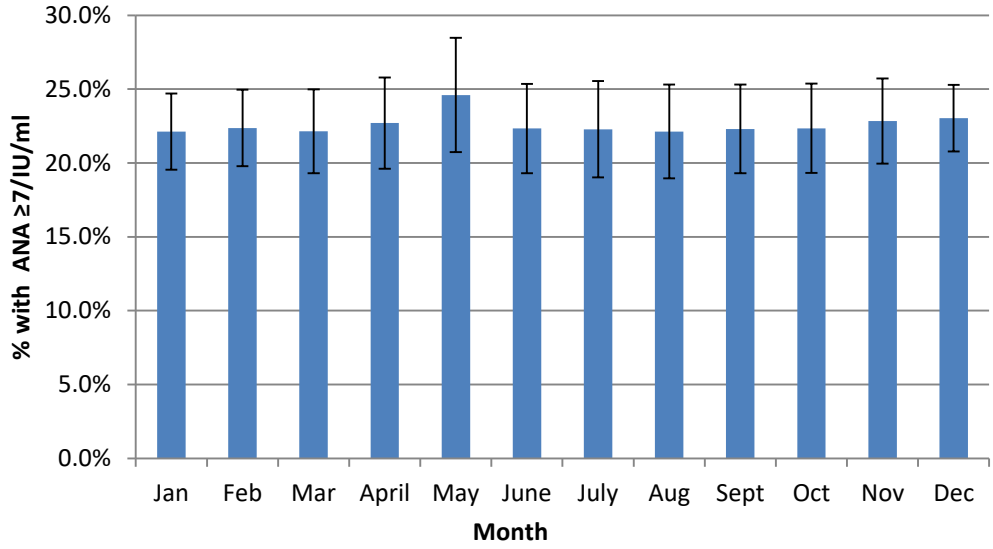


FIGURE 4.4 AVERAGE PERCENT OF ANA TESTS ≥7IU/ML IN EACH OF THE MONTHS OVER THE 11 YEARS FROM 2000 TO 2010.

χ^2 analysis of ANA status (<7IU/ml v ≥7IU/ml) and month of testing showed an increased proportion of ANA ≥7IU/ml results reported in the month of May compared to other months, χ^2 = 35.0, n=120,997, p<0.001, and an OR = 1.15 (95%CI 1.072 -1.226) for an ANA result of ≥7IU/ml in the month of May.

4.3.4 MULTIPLE TESTS ON INDIVIDUALS

Patients with a diagnosis of an ANA associated autoimmune disease will often have repeat ANA and/or other serology testing requested by the Clinician for various reasons (i.e. monitoring disease, change in clinical phenotype and in some cases lack of information about previous results). The study dataset represents 89,537 individuals, and 31,460 repeat tests on individuals. The maximum number of tests on a single individual over the 11 year period was 76 (median = 1 test/individual, mean = 1.4 tests/individual).

4.4 DISCUSSION

The mean age of all individuals of 48.6 years (age range = 0 – 101) and higher proportion of females (62.4%) compared to males (37.6%) tested for ANA is consistent with the expectation that there is a higher likelihood of ANA related autoimmune diseases in older individuals and in females (119, 120).

The data showed that of all samples from individuals on the mainland of WA 22.5% had an ANA with an intensity of ≥7IU/ml of which 75.5% were female (data shown in Table 4-1). Importantly, this data-set includes multiple tests for individuals (18.7% of individuals had repeat testing) and monitoring of patients with known rheumatic disease.

In the period 2000 to 2010 ANA testing increased by 43% while the Western Australian population increased by 22% (Source: Figures produced by the Epidemiology Branch, System Policy and Planning Division, DoHWA, data downloaded from the Epidemiology Branch web-site on 30 August 2013) indicating that ANA test requests were more likely to be a consequence of increased clinical investigation rather than population growth. The median and mean age of individuals tested for ANA was similar i.e. 48.0 and 48.7 years respectively. The percentage increase in the WA population of individuals in 40 – 70 years age bracket was 31% over the study period. This increase, and more specifically the proportion of the population cohort more likely to present to the GP with symptoms which could include rheumatic and other autoimmune conditions, would support the increase in the number of tests requested over the 11 year period.

The large number of requests (49.6%) for non-hospital patients is in line with the fact that ANA is a screening test for rheumatic diseases and it is likely that it will be requested by GPs as well as specialists in the preliminary investigations for rheumatic disease.

The information available for this study does not allow discrimination between biological factors and factors affecting clinical decisions to request the ANA test. In the 11 year period reviewed in this study the frequency of an ANA ≥7IU/ml was significantly higher in the month of May relative to other months. Vitamin D has been shown to have an important effect on a range of immune processes including an inhibitory effect on T and B cell proliferation and antibody production (121).

The body's ability to synthesize vitamin D is linked to sun exposure and summary data for mean monthly solar exposure at centres in the north (Broome), south (Albany) and east (Kalgoorlie) of WA and the metropolitan area (Perth) show declining levels of solar exposure in May which reaches a minimum in June (http://www.bom.gov.au/climate/data/index.shtml). Data are shown in Figure 4.5. Vitamin D insufficiency has been linked to a wide range of health issues including autoimmunity and seasonality of infections (122) and is seen as a global problem (123).

Perhaps it is not unexpected that an association can be seen between low sun exposure and an increase in the frequency of ANA ≥7IU/ml. Importantly this is a single variable that has been considered to account for the variation in ANA; other factors such as seasonal infections may also contribute to the variation independent of the vitamin D levels and cannot be ruled out as an explanation.

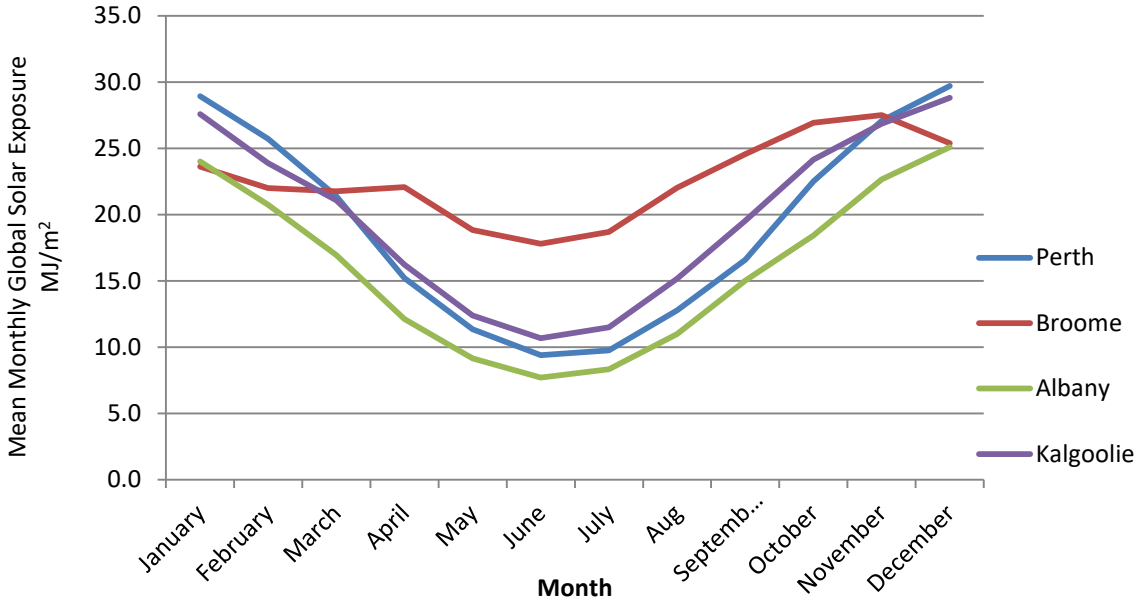


FIGURE 4.5 MEAN MONTHLY SOLAR EXPOSURE AT PERTH, BROOME, ALBANY AND KALGOOLIE CALCULATED OVER THE STUDY PERIOD 2000 - 2011.

Source: Data collected by the Commonwealth of Australia Bureau of Meteorology (124).

In summary the data shown in this chapter provides an overview of the demographics and trends in ANA data collected over an 11 year period for individuals living on the mainland of WA. However as the data-set contains repeat testing data for approximately 30% of individuals, subsequent chapters will consider data restricted to a single test for each individual.

5 INCIDENT ANA

5.1 INTRODUCTION

ANA is usually requested by clinicians as an initial screening assay for suspected systemic autoimmune disease (125). However, ANA has also been reported in 5 – 39% of the healthy population (7, 46, 126) with variations possibly due to the demographics of the population sampled (7). In addition, the wide variation in ANA-positive prevalence estimates in the non-diseased population can largely be explained by variations in the method of testing and reporting of results across different jurisdictions (7, 59, 125).

The IIF ANA screening method adopted in many diagnostic laboratories uses a commercially prepared immortalised human epithelial cell line (HEp 2 or HEp2000) as the substrate (101). It is used to determine both antibody level and pattern and thereby identifies samples that should undergo further testing to determine the target antigen of the autoantibody (53, 101).

The prevalence of ANA has been investigated in the general population and selected disease groups in a number of studies (7, 25, 46, 48, 95, 97, 127). In these studies, sample size varied from 120 to 10,550 participants. Five studies have looked at ANA levels in a large population (India (95), USA (127), Japan (46), Belgium (97) and South Australia(48)). However, previous studies have not quantified the level of ANA reported, or indicated whether the ANA result reported is the first or a subsequent ANA test result for a patient. This is an important consideration as treatment may affect ANA level.

This chapter describes the ANA level, pattern and demographics for incident ANA results over an 11-year period in a Western Australian cohort. ANA outcome is also related to linked mortality data.

5.2 METHODS

5.2.1 INCIDENT ANA COHORT

The approach to generating the incident ANA Cohort was described in Section 3.9. Briefly, ANA, ENA and dsDNA antibody datasets were linked by a unique identifying number. Entries for each individual were sequenced by date of collection; any individuals with one of these tests reported prior to 1 January 2000 were excluded from the incident ANA cohort to remove prevalent cases. In total 85,542 individual patients met the criteria

for inclusion in the incident ANA cohort. The incident ANA clinical cohort had 17 entries for which gender was not specified. Following the preliminary analysis of gender and ANA and dsDNA antibody results these entries were excluded from subsequent analysis.

ANA categorised as a binary outcome was analysed in a multivariate LR analysis adjusted for, age, gender, country of birth, ARIA, IRDS an Indigenous Status as described in Section 3.8.

5.2.2 ANA AND MORTALITY

ANA and Death registry data were linked as described in Section 3.3.2. Individuals without DoHWA Linkage data (n = 14,962, 17.5%) were excluded from the analysis of mortality as the outcome variable data were not available. An additional four cases were removed from the analysis as the date of death preceded the date of sample collection; it is likely that these samples had come from mortuary requests.

ANA was linked to a death record for 9,002 individuals. Cause of death was recorded as a text description without an ICD code in 1,165 individuals. To ensure consistent reporting these cases were excluded from specific cause of death analysis.

5.2.3 STATISTICAL ANALYSIS

Descriptive statistics were initially calculated for age, gender, Indigenous Status, country of birth, ARIA and IRDS for the Incident ANA Cohort. ANA dichotomised into <7IU/ml and ≥ 7IU/ml and ANA grouped by level as described in Section 3.3.2 were then analysed by these sociodemographic factors. Continuous variables were compared by independent-samples t-tests and crude comparisons were performed using a Chi-square test.

Multivariate LR analysis was performed to determine the effect of age, gender, country of birth, Indigenous Status, ARIA and IRDS on the likelihood of an individual developing an ANA ≥7IU/ml.

Frequency data were generated to investigate the association between ANA level and pattern and cause of death. These differences were compared using a Chi-square analysis of proportions.

The association between ANA and mortality (cause described by ICD code) was investigated by multivariate LR analysis using "Cause of Death" as a binomial outcome of censored versus deceased.

Cox proportional hazard regression modelling was used to investigate the effect of factors including age, gender, antibody level and time to death for the ANA patterns reported with the highest frequency.

5.3 RESULTS

There were a total of 85,542 individuals who had a first-time ANA test reported in the period January 2000 to December 2010. Of these, 59.6% (n = 50,954) were female. Gender was not available for 17 individuals. Mean (±SD) age for males and females at the time of the first ANA test differed significantly being 48.6 (±18.2) years and 47.1 (±18.2) years respectively ($p < 0.001$). Descriptive statistics of the incident ANA cohort are shown in Table 5-1.

Repeat ANA testing had been performed on 17.5% (n=14,968) of the sample population with the maximum number of repeat tests being 66 (median = 1).

The majority of individuals were non-Indigenous Australians (73.5%), 4.1% of the study group were Indigenous and 22.4% had unspecified Indigenous Status.

5.3.1 AGE AND GENDER

An ANA result of ≥7IU/ml was detected in 17.5% of individuals (n= 14,967) at the time of their first ANA.

The ratio of males:females for a positive ANA test (i.e. ANA ≥ 7IU/ml) was 1:2.5 compared to the ratio of 1:1.5 for individuals tested. The difference in the proportion of males and females with an ANA≥7IU/ml at the time of their first ANA test was statistically significant (χ^2=1043.6 $p<0.001$), with a higher than expected number of females and fewer than expected males with an ANA ≥7IU/ml (Table 5-1). When ANA≥7IU/ml individuals were grouped into 10-year age intervals there was a significantly higher proportion of women with a positive result compared to males in each age group (χ^2=141.068, $p < 0.001$; Figure 5.1).

In a multivariate LR analysis of ANA result (<7 or ≥7IU/ml) adjusted for age and gender, an ANA ≥7IU/ml was significantly associated with increasing age as a continuous variable, OR = 1.008 (95%CI 1.007-1.009; p<0.001), similarly there was an increased likelihood of an ANA≥7IU/ml in females compared to males; OR =1.893 (95%CI 1.822–1.968; p<0.001).

TABLE 5-1 GENDER AND AGE OF THE CLINICAL COHORT ACCORDING TO INCIDENT ANA STATUS.

	Total	ANA <7IU/ml	ANA ≥7IU/ml
Male (%)	34,571 (40.4)	30,283 (87.6)[a]	4,288 (12.4)[a]
Female (%)	50,954 (59.6)	40,276 (79.0)[a]	10,678 (21.0)[a]
Unspecified (%)	17 (0.02)	16 (94.1)	1(5.9)
Total	85,542 (100)	70,575 (82.5)	14,967 (17.5)
Mean Age years (± SD)			
Men	48.6 (18.2)[a]	48.1 (18.2)[c]	52.0 (18.3)[c,d]
Women	47.1 (18.2)[a]	46.7 (18.1)[b]	48.8 (18.6)[b,d]
Unspecified	32.3 (11.1)	31.9 (11.3)	39.0
All individuals	47.7 (18.2)	47.3 (18.1)	49.7 (18.6)

[a, b, c, d] Denotes comparison group with statistically significant difference p< 0.001. [b, c, d] Two way analysis of variance comparison of mean age in males and females with an ANA <7 or ANA ≥7IU/ml.

The greatest difference between the proportion of males and females with an ANA ≥7IU/ml was seen in the 20-29 year age group in which 19.9% and 9.3% of females and males respectively had an ANA ≥7IU/ml. There was a peak in the proportion of individuals with an ANA ≥7IU/ml in both males and females in the 10-19 year age group M = 10.9%, F = 20.0% (Figure 5.1). This early peak in the female group extended to the 20-29 year age group and then showed a continual increase from 40 years of age onwards. A similar trend was observed in the males from 50 years of age onwards, with a temporary dip after 80 years of age as shown in Figure 5.1.

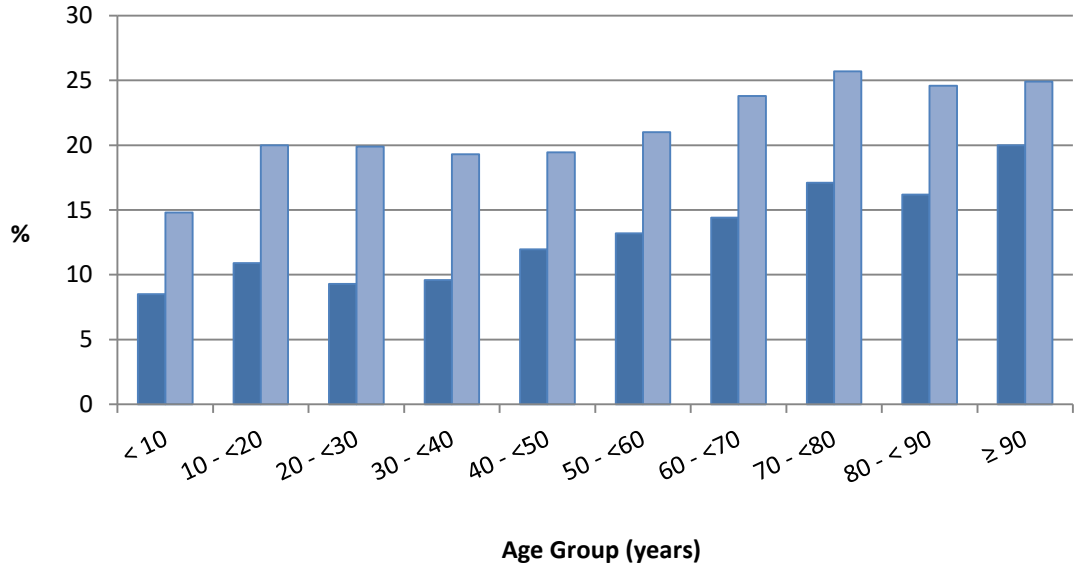


FIGURE 5.1 PERCENT OF INDIVIDUALS WITH AN ANA ≥7IU/ML BY DECADE OF AGE FOR MALES (DARK BARS) AND FEMALES (LIGHT BARS).

5.3.2 ANA LEVEL

ANA is reported from <2 to >30IU/ml. The distribution of ANA results is asymmetric and skewed in both males and females. The median and 95th percentiles are 2 and 15IU/ml respectively, for males and 2 and 25 IU/ml respectively for females, refer to Table 5-2.

For further analysis ANA results were subdivided into 5 groups <7, 7–10, >10-20, >20–30 and >30IU/ml (see Table 5.2 and Figure 5.2). At each level from 7-10 IU/ml and above the number and proportion of females was higher than males.

TABLE 5-2 FREQUENCY DISTIBUTION (%) AND THE 50TH, 95THAND 99TH PERCENTILES OF ANA REPORTED IN IU/ML IN MALES AND FEMALES.

	Frequency (%)					**Percentile**		
	< 7	**7 - 10**	**>10 - 20**	**>20 -30**	**>30**	50th	95th	99th
Males	87.60%	7.20%	3.00%	1.60%	0.50%	2	15	30
Females	79.00%	10.60%	5.20%	3.90%	1.30%	2	25	>30
All	82.50%	9.20%	4.30%	3.00%	1.00%	2	20	30

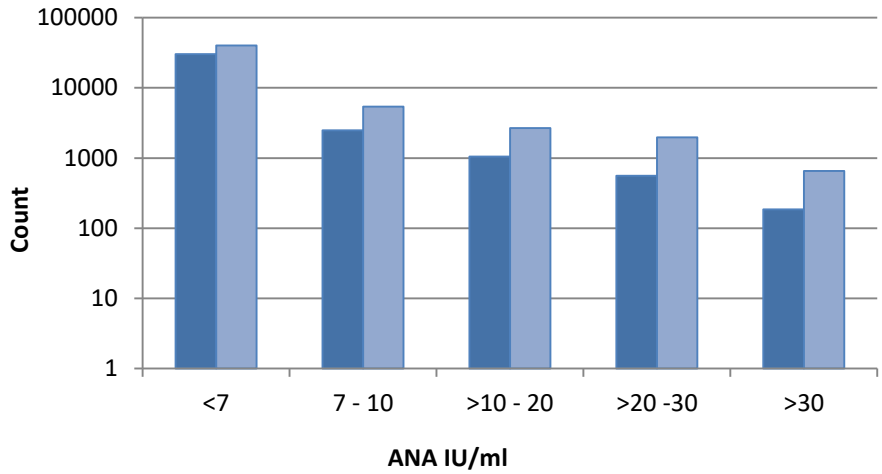


FIGURE 5.2 FREQUENCY DISTRIBUTION OF ANA IN IU/ML IN MALES (DARK) AND FEMALES (LIGHT).

5.3.3 IMMUNOFLUORESCENCE PATTERNS

The most prevalent ANA patterns reported in patients with an ANA ≥7IU/ml (n = 14,967) were: homogeneous (27.4%), speckled (21.4%), homogeneous plus speckled (21.4%), nucleolar (19.5%) and centromere (3.8%). A mixed pattern which included nucleolar with either an additional homogeneous or speckled nuclear pattern was reported in 3.8% of ANA ≥7IU/ml samples.

Approximately 77% of samples with an ANA ≥7IU/ml were reported as ≤20IU/ml (Figure 5.2), The median level for an ANA speckled and homogeneous plus speckled pattern (expressed as a group level) was 7–10IU/ml, the 95th percentile for these patterns was >30 and >20-30IU/ml respectively. This compared with a median level of >10–20IU/ml for samples with a homogeneous pattern (95th percentile; >30IU/ml). Data are shown in Table 5-4 and Figure 5.3.

TABLE 5-3 PERCENTILES OF ANA (IU/ML) FOR EACH OF THE PATTERNS; HOMOGENEOUS. SPECKLED AND HOMOGENEOUS PLUS SPECKLED PATTERNS.

Pattern	N	Median	95 th	99th
Homogeneous	4,104	>10–20IU/ml	>30IU/ml	>30IU/ml
Speckled	3,203	7-10IU/ml	>30IU/ml	>30IU/ml
Homogeneous plus Speckled	3,205	7-10IU/ml	>20-30IU/ml	>30IU/ml

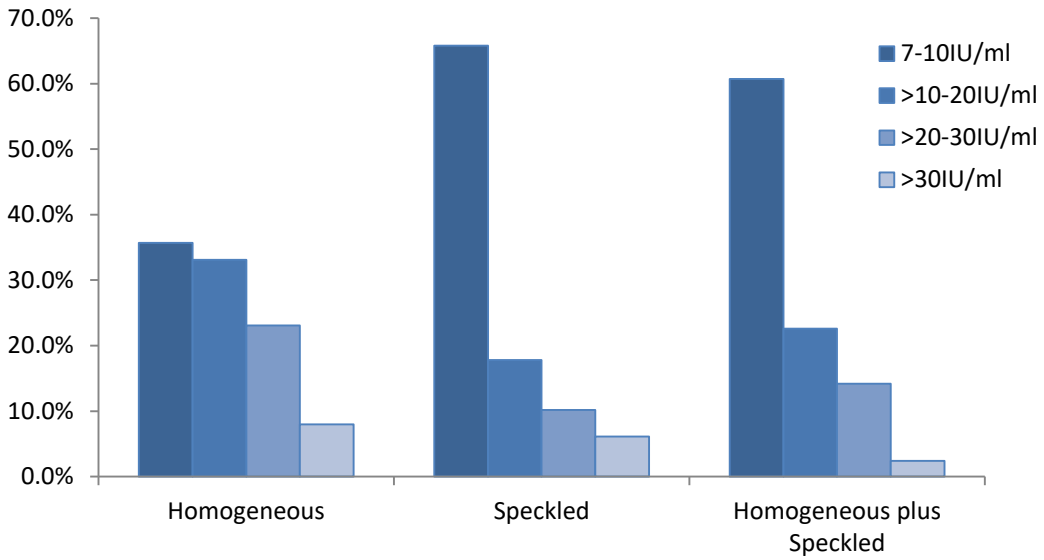


FIGURE 5.3 PERCENT FREQUENCY DISTRIBUTION OF ANA LEVELS (IU/ML GROUPED) ACCORDING TO ANA PATTERN: HOMOGENEOUS, SPECKLED AND HOMOGENEOUS PLUS SPECKLED.

Various patterns are routinely reported without a measure of intensity; these patterns are expressed as a percent of all tested individuals (n = 85,542) and include the nuclear matrix pattern (1.1%), 1 to 4 nuclear dots (0.7%), mitotic spindle apparatus (0.8%), and centriole (0.9%). Multiple nuclear dots (5–10) were reported in <0.1% of individuals. Other patterns (nuclear envelope, spindle apparatus, Golgi apparatus and nuclear pore) were collectively reported in 0.38% of all individuals. Cytoplasmic staining was reported in 6.4% of all samples tested (n = 5,446).

5.3.4 SSA DETECTION

Antibodies targeting SSA/Ro60 antigen were identified on the HEp2000 substrate as a specific pattern (nucleolar and speckled nuclear staining in 10 – 20% of the interphase nuclei) (9). In the presence of strong patterns including homogeneous and nucleolar, SSA/Ro60 antibody is reported as not excluded since it cannot be distinguished from the other dominant patterns.

In this study a pattern consistent with SSA/Ro60 antibody was detected in 826 individuals of which 65% (n=537) were reported in individuals with an ANA <7IU/ml, 19.9% (n = 164) were reported in the presence of an additional pattern reported as a low level positive (7–10 IU/ml). The remaining 15.1% (n=125) were reported in individuals with an ANA

>10IU/ml. Antibodies to SSA/Ro60 could not be excluded in 10,546 individuals of whom 28.7% had an ANA result <7IU/ml. The detection of anti-SSA/Ro60 will be further investigated in Chapter 6.

5.3.5 COMPARISON OF INDIGENOUS AND NON-INDIGENOUS GROUPS

The incident clinical ANA cohort categories for imputed data (complete) was analysed for gender, age and ANA levels. However the Indigenous Status variable was restricted to the DoHWA linked data and available for 66,354 individuals (74.9% of study sample). This variable was evaluated in a subset analysis of patients with known Indigenous Status (Tables 5.4 & 5.6). The frequency of ANA ≥7IU/ml in the non-Indigenous cohort was higher (18.1%) than that seen in the Indigenous cohort (13.5%; $\chi = 48.301$, $p<0.001$).

TABLE 5-4 PERCENT FREQUENCY OF ANA RELATED TO INDIGENOUS STATUS.

Status	Total	ANA <7IU/ml N (%)	ANA ≥7IU/ml N (%)
Indigenous	3,514 (4.1)	3,040 (86.5)[a]	474 (13.5)[a]
Non Indigenous	62,840 (73.5)	51,464 (81.9)[a]	11,376 (18.1)[a]
Ungrouped	19,188 (22.4)	16,071 (83.8)	3,117 (16.2)

[a] denotes a statistically significant difference between groups p< 0.001

Females represented 60.9 and 64.5% of the non-Indigenous and Indigenous individuals respectively in the clinical cohort; this difference was statistically significant ($p<0.001$). However, the proportion of both males and females with an ANA ≥7IU/ml was higher in the non-Indigenous group (13.1% and 21.3%) compared to the Indigenous group (10.6% and 15.1%), as shown in Table 5-5 (males $p=0.01$ and females $p>0.001$).

In a comparison between Indigenous and non-Indigenous groups, the frequency of males in each ANA level approached but did reach statistical significance ($\chi^2 = 8.38$, $p=0.078$). In contrast, a statistically significant difference was noted in the female groups ($\chi 2 = 57.4$ $p < 0.001$):

i. a higher proportion of non-Indigenous females had a ANA ≥ 7IU/ml (Non Indigenous = 18.1% v Indigenous = 13.5% v),
ii. a higher proportion of Indigenous females had an ANA >30IU/ml (Indigenous = 1.5% v Non Indigenous = 1.2%) as shown in Table 5-6.

TABLE 5-5 AGE (YEARS), INDIGENOUS STATUS AND GENDER RELATED TO ANA.

Status		ANA <7IU/ml					ANA ≥7IU/ml					All				
		N	Age				N	Age				N	Age			
			Mean	SD	Range	Median		Mean	SD	Range	Median		Mean	SD	Range	Median
Indigenous	Male	1,115	39.1	16.2	1-83	40.0	132	41.7	16.6	7-79	41.0	1,247	39.4	16.2	1-83	40.0
	Female	1,925	38.2	15.1	1-84	38.0	342	40.0	16.0	2-83	39.0	2,267	38.5	15.2	1-84	38.0
	Total	3,040	38.5	15.5	1-84	39.0	474	40.5	16.2	2-83	39.5	3,514	38.8	15.6	1-84	39.0
Non Indigenous	Male	21,357	51.4	18.0	0-101	52.0	3,222	54.9	17.9	1-96	56.0	24,579	51.9	18.0	0-100	52.0
	Female	30,107	48.9	18.0	0-101	48.0	8,154	50.7	18.6	0-101	51.0	38,261	49.3	18.1	0-101	49.0
	Total	51,464	49.9	18.0	0-101	50.0	11,376	51.9	18.5	0-101	52.0	62,840	50.3	18.1	0-101	50.0
Total	Male	22,472	50.8	18.1	0-100	51.0	3,354	54.4	18.0	1-96	55.0	25,826	51.3	18.1	0-100	52.0
	Female	32,032	48.2	18.0	0-101	47.5	8,496	50.3	18.6	0-101	50.0	40,528	48.6	18.1	0-101	48.0
	Total	54,504	49.3	18.1	0-101	49.0	11,850	51.4	18.5	0-101	52.0	66,354	49.7	18.2	0-101	49.0

TABLE 5-6 FREQUENCY DISTRIBUTION (%) OF ANA IN IU/ML RELATED TO INDIGENOUS STATUS AND GENDER.

Gender	ANA Level	Indigenous N (%)	Non Indigenous N (%)	Total N (%)
Males	<7IU/ml	1,115 (89.4%)	21,357 (86.9%)	22,472 (87.0%)
	7-10IU/ml	84 (6.7%)	1,850 (7.5%)	1,934 (7.5%)
	>10 - 20IU/ml	29 (2.3%)	803 (3.3%)	832 (3.2%)
	>20 -30IU/ml	14 (1.1%)	418 (1.7%)	432(1.7%)
	>30IU/ml	5 (0.4%)	151 (0.6%)	156 (0.6%)
	Total	1,247	24,579	25,826
Females	<7IU/ml	1,925 (84.9%)	30,107 (78.7%)	32,032 (79.0%)
	7-10IU/ml	177 (7.8%)	4,111 (10.7%)	4,288 (10.6%)
	>10 - 20IU/ml	77 (3.4%)	2,030 (5.3%)	2,107 (5.2%)
	>20 -30IU/ml	55 (2.4%)	1,536 (4.0%)	1,591 (3.9%)
	>30IU/ml	33 (1.5%)	477 (1.2%)	510 (1.3%)
	Total	2,267	38,261	40,528
Total	<7IU/ml	3,040 (86.5%)	51,464 (81.9%)	54,504 (82.1%)
	7-10IU/ml	261 (7.4%)	5,961 (9.5%)	6,222 (9.4%)
	>10 - 20IU/ml	106 (3.0%)	2,833 (4.5%)	2,939 (4.4%)
	>20 -30IU/ml	69 (2.0%)	1,954 (3.1%)	2,023 (3.0%)
	>30IU/ml	38 (1.1%)	628 (1.0%)	666 (1.0%)
	Total	3,514	62,840	66,354

5.3.6 ARIA AND ANA STATUS

The geographical remoteness variable was generated using postcode data collected at the time of the ANA specimen collection. Sixty one percent of individuals were resident in areas considered highly accessible compared to 14.9% living in remote or very remote areas (Table 5-8) at the time of their first ANA test. Chi-square analysis of ARIA and ANA results showed a statistically significant association between ARIA and ANA result (χ^2=86.62, p<0.001). There was a significantly higher than expected number of individuals with an ANA ≥7IU/ml in the highly accessible areas and significantly fewer than expected with an ANA ≥7IU/ml in very remote areas (Table 5-7). The ratio of males to females ranged from 1:2.0 to 1:2.9 for individuals with an ANA ≥7IU/ml.

TABLE 5-7 FREQUENCY OF ANA ≥7IU/ML RELATED TO GENDER AND ARIA SCORE (PERCENTAGES SHOWN IN BRACKETS).

	Total	ANA ≥7IU/ml N (%)		
ARIA		**Males and Females**	**Males**	**Females**
Highly Accessible	52,158	9,552 (18.3)[a]	2,789 (29.2)	6,762 (70.8.)
Accessible	10,843	1,842 (17.0)	471 (25.5)	1,371 (74.4)
Moderately Accessible	9,719	1,641 (16.9)	457 (27.8)	1,184 (72.2)
Remote	2,705	449 (16.6)	126 (28.1)	323 (71.9)
Very Remote	10,042	1,471 (14.7)[a]	441 (30.0)	1,030 (70.0)
Undefined	75	12 (16.0)	4 (33.3)	8 (66.7)
Total	85,542	14,967	4,288	10,678

[a] Statistically significant difference ($p < 0.05$)

5.3.7 COUNTRY OF BIRTH

Country of birth data were available for 75.5% of the clinical cohort (n = 64,613) of which Australia and N.W. Europe were the most frequently reported, 66.3% and 20.2% respectively of all individuals in the clinical cohort with data available.

ANA differed significantly by country of birth ($\chi 2 = 29.24$, $p = 0.001$ excluding cases were details were unavailable; Table 5-8). The country of birth associated with the highest frequency for a positive ANA was the Americas (24.2%).

TABLE 5-8 FREQUENCY OF ANA RELATED TO COUNTRY OF BIRTH.

Country of Birth	Total	ANA <7IU/ml N (%)	ANA ≥7IU/ml N (%)
Australia	42,819	35,309 (82.5)	7,510 (17.5)
Oceania and Antarctica (excluding Australia)	1,862	1,512 (81.2)	350 (18.8)
N.W. Europe	13,044	10,650 (81.6)	2,394 (18.4)
S. & E Europe	1,073	867 (80.8)	206 (19.2)
N. Africa & M.E	1,741	1,429 (82.1)	312 (17.9)
S.E. Asia	1,155	926 (80.2)	229 (19.8)
N.E. Asia	788	633 (80.3)	155 (19.7)
S. & E. Asia	566	460 (81.3)	106 (18.7)
Americas	368	279 (75.8)	89 (24.2)
Africa (Excluding N. Africa)	1,197	1,017 (85.0)	180 (15.0)
Not Available	20,930	17,494 (83.6)	3,436 (16.4)
Total	85,542	70,575 (82.5%)	14,967 (17.5%)

5.3.8 SOCIAL DISADVANTAGE

The frequency of a positive ANA result in each IRDS quartile is shown in Table 5-9. The Chi-square analysis of IRDS quartile and ANA was statistically significant (χ^2 = 36.46, p<0.001), with fewer than expected ANA results ≥7IU/ml in the 1st IRDS quartile (represents the highest level of disadvantage), in contrast to a higher than expected number of ANA positive individuals in the 4th quartile.

A comparison of IRDS and Indigenous Status showed an over-representation of Indigenous individuals (62.9%) in the lowest quartile of IRDS, and an under-representation in the highest quartile (least disadvantaged; 4.8%). In comparison the distribution of Non Indigenous individuals ranged from 21.7–27.6% across the four levels of IRDS.

The frequency of individuals with an ANA≥7IU/ml was lower across the IRDS quartiles in the Indigenous Group (10.8-14.3%) compared to the Non Indigenous (17.2-18.7%) and Ungrouped individuals (15.5–18.4%). The highest frequency of ANA≥7IU/ml in the Non Indigenous individuals was observed in the 4th quartile and the lowest frequency in the 1st quartile (Figure 5.4). The difference in frequency of an ANA ≥7IU/ml across the quartiles for the Indigenous group was not statistically significant ($\chi^2 = 1.464$, p = 0.691) in contrast to the Non Indigenous and Ungrouped categories ($\chi^2 = 16.484$, p = 0.006 and $\chi^2 = 12.296$, p= 0.001 respectively). In both cases there was a lower than expected frequency of ANA ≥7IU/ml in the 1st quartile and higher than expected frequency in the 4th quartile. ANA ≥7IU/ml was also reported more frequently in the 2nd quartile for the Non-Indigenous group, data shown in Figure 5.4.

TABLE 5-9 FREQUENCY OF ANA ≥7IU/ML RELATED TO INDEX OF RELATIVE SOCIO-ECONOMIC DISADVANTAGE (IRDS) QUARTILE.

IRDS Quartile	**Total**	**ANA ≥ 7IU/ml n (%)**
1st Quartile[§]	21,436	3,507 (16.4)[a]
2nd Quartile	21,473	3,800 (17.7)
3rd Quartile	21,225	3,694 (17.4)
4th Quartile (High)	21,165	3,928 (18.6)[a]
Undefined	243	38 (15.6)
Total	85,542	14,967

[§]Highest level of disadvantage

[a] a statistically significant difference between ANA outcome and IRDS quartile p<0.05.

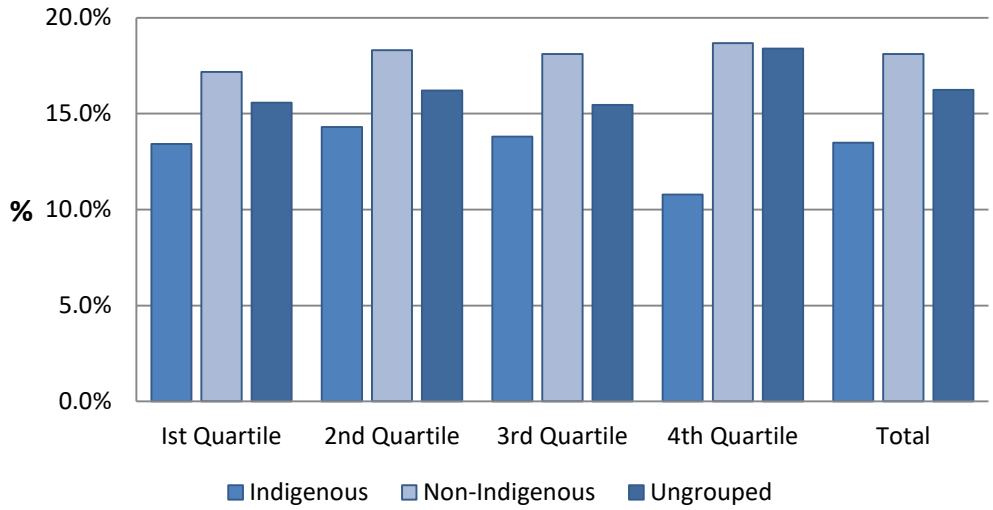


FIGURE 5.4 FREQUENCY OF ANA ≥7IU/ML RELATED TO IRDS QUARTILE AND INDIGENOUS STATUS.

5.3.9 PREDICTORS OF ANA ≥7IU/ML: A MULTIVARIATE LOGISITC REGRESSION ANALYSIS

The ORs and p values for all variables included in a multivariate LR analysis to determine predictors of an ANA≥7IU/ml are shown in Table 5-10. The variables significantly association with increased odds of an ANA ≥7IU/ml were: age (OR = 1.007, 95%CI 1.006–1.009), female gender (OR = 1.905, 95%CI 1.833–1.980) and the country of birth group Americas (OR=1.440, 95%CI 1.131–1.835).

A second multivariate LR model on the subset of individuals for which all variables were complete (n=64,453) showed no change in the significance of any of the factors included in the regression analysis.

The ORs indicated a reduced likelihood of ANA ≥7IU/ml in individuals with an Indigenous background, an African country of birth (excluding N Africa) and living in areas classified as accessible but not highly or moderately accessible. Data are shown in Tables 5-7 – 5-10.

When stratified by gender a LR analysis of ANA outcome including age, country of birth, IRDS and ARIA showed that age had a similar effect on ANA outcome in both males and females (OR=1.011 and 1.006 respectively). The country of birth variable was not significant in the regression analysis of males, however country of birth groups; Oceania and Antarctica (excluding Australia), South East Asia and the Americas were all significant and had OR's >1 in the analysis of females alone (Table 5-11).

TABLE 5-10 ODDS RATIOS FOR ANA ≥7IU/ML IN A MULTIVARIATE LR ANALYSIS ADJUSTED FOR GENDER, INDIGENOUS STATUS, COUNTRY OF BIRTH, AGE, IRDS AND ARIA.

VARIABLE	All Entries					All Variables Complete				
	N	OR	OR 95% C.I. Min	OR 95% C.I. Max	p value	N	OR	OR 95% C.I. Min	OR 95% C.I. Max	p value
GENDER										
Males (Reference)	50,954	1.000				25,026	1.000			
Females	34,571	1.905	1.833	1.980	<0.001	39,427	1.824	1.745	1.907	<0.001
Unspecified	17	0.478	0.063	3.610	0.474					
INDIGENOUS GROUPS										
Non Indigenous Reference)	62,840	1.000				60,966	1.000			
Indigenous	3,514	0.814	0.733	0.904	<0.001	3,487	0.823	0.739	0.916	<0.001
Ungrouped	19,188	0.987	0.867	1.123	0.841					
COUNTRY OF BIRTH										
Australia (Reference)	42,819	1.000				42,700	1.000			
Oceania & Antarctica (excl. Australia)	1,862	1. 100	0.975	1.240	0.121	1,860	1.100	0.975	1.240	0.121
N.W. Europe	13,044	0.956	0.906	1.008	0.095	13,010	0.961	0.910	1.014	0.142
S.E. Europe	1,073	1.066	0.912	1.246	0.422	1,072	1.067	0.912	1.247	0.419
N. Africa and the M.E.	1,741	0.933	0.822	1.058	0.280	1,740	0.932	0.821	1.059	0.280
S.E. Asia	1,155	1.111	0.958	1.289	0.165	1,153	1.113	0.959	1.291	0.159
N.E. Asia	788	1.031	0.861	1.233	0.741	788	1.035	0.865	1.238	0.710
S. & C. Asia	566	1.035	0.835	1.283	0.754	566	1.037	0.836	1.284	0.743
Americas	368	1.441	1.131	1.836	0.003	368	1.445	1.134	1.841	0.003
Africa (Excluding N. Africa)	1,197	0.797	0.678	0.937	0.006	1,196	0.799	0.680	0.939	0.007
Not Available	20,929	1.021	0.901	1.157	0.746					

TABLE 5-10 CONTINUED: ODDS RATIOS FOR ANA ≥7IU/ML IN A MULTIVARIATE LR ANALYSIS ADJUSTED FOR GENDER, INDIGENOUS STATUS, COUNTRY OF BIRTH, AGE, IRDS AND ARIA.

	All Entries					All Variables Complete				
VARIABLE	N	OR	OR 95% C.I.)		p value	N	OR	OR 95% C.I.		p value
			Min	Max				Min	Max	
Age		1.007	1.006	1.009	<0.001		1.007	1.006	1.008	<0.001
IRDS										
4th Quartile (Reference)	21,165	1.000				17,027	1.000			
3rd Quartile	21,225	0.980	0.931	1.032	0.446	15,983	1.008	0.951	1.068	0.796
2nd Quartile	21,473	0.976	0.925	1.030	0.380	15,997	1.014	0.955	1.077	0.655
1st Quartile	21,436	0.945	0.895	0.997	0.039	15,446	0.961	0.904	1.022	0.208
Undefined	243	0.849	0.561	1.285	0.439					
ARIA										
Highly Accessible (Reference)	52,158	1				42,870	1.000			
Accessible	10,843	0.903	0.852	0.958	0.001	7,347	0.880	0.821	0.943	<0.001
Moderately Accessible	9,719	0.914	0.859	0.971	0.004	6,141	0.935	0.868	1.006	0.071
Remote	2,705	0.934	0.839	1.041	0.218	1,854	0.931	0.819	1.059	0.277
Very Remote	10,042	0.853	0.798	0.911	<0.001	6,241	0.835	0.769	0.907	<0.001
Undefined	75	1.159	0.555	2.424	0.694					

TABLE 5-11 SIGNIFICANT ODDS RATIOS FOR ANA ≥7IU/ML IN MALES AND FEMALES IN A MULTIVARIATE LR ANALYSIS ADJUSTED FOR INDIGENOUS STATUS, COUNTRY OF BIRTH, AGE, IRDS AND ARIA.

	Males					Females				
	df	p value.	OR	95% C.I for OR		df	p value	OR	95% C.I for OR	
				Lower	Upper				Lower	Upper
Age	1	<0.001	1.011	1.008	1.013	1	<0.001	1.006	1.004	1.007
ARIA (Reference: Highly Accessible)	4	0.087				4	0.001			
Accessible	1	0.087	0.891	0.780	1.017	1	0.001	0.873	0.805	0.947
Very Remote	1	0.014	0.824	0.706	0.962	1	<0.001	0.835	0.757	0.921
IRDS (Reference: 4th Quartile)	3	0.702				3	0.154			
1st Quartile	1	0.447	1.044	0.934	1.168	1	0.045	0.927	0.861	0.998
Indigenous Grouping	1	0.706	0.962	0.788	1.175	1	<0.001	0.778	0.684	0.883
Country of Birth (Reference; Australia)	9	0.906				9	<0.001			
Oceania and Antarctica (excluding Australia)	1	0.842	0.978	0.786	1.217	1	0.043	1.161	1.005	1.341
South-East Asia	1	0.619	0.929	0.695	1.242	1	0.047	1.194	1.002	1.421
Americas	1	0.292	1.280	0.809	2.026	1	0.004	1.520	1.140	2.025
Africa (Excluding N. Africa)	1	0.352	0.868	0.643	1.170	1	0.009	0.773	0.638	0.937

5.3.10 CHANGE IN ANA RESULT

The ANA data has been analysed based on the first ANA result for each patient, however as indicated in Section 5.3 repeat ANA testing had been performed on 17.5% (n=14,968) of the individuals in the study.

A comparison of incident and maximum ANA level showed an increase in level for 1,832 individuals, In 41% of the cases (n=746) there was a change from <7IU/ml to a low level positive group (7–10IU/ml) as shown in Table 5-12A.

In addition the change in ANA level between the first two tests was investigated; median time between tests was 278 days (range 0–3,980 days). In 10% of cases (n=1,568) there was a decrease in ANA level; an increase was reported in 9% of individuals (n=1,326). The decrease between the 1st and 2nd test was limited to one ANA interval in 84% of individuals. The change from >30IU/ml to <7IU/ml was reported in 2 individuals (Table 5-12B).

TABLE 5-12 A. HIGHEST ANA RELATED TO INCIDENT ANA IN INDIVIDUALS WHO HAD AT LEAST ONE REPEAT TEST. B. COMPARISON OF ANA RESULT BETWEEN FIRST TWO TESTS FOR AN INDIVIDUAL.

A.		**ANA IU/ml At Incident Test**					
		<7	7 - 10	>10 – 20	>20 -30	>30	Total
Maximum ANA Result IU/ml	<7	10,076	0	0	0	0	10,076
	7 - 10	746	1295	0	0	0	2,043
	>10 - 20	184	249	74	0	0	1,174
	>20 -30	76	86	2,239	647	0	1,048
	>30	31	15	41	164	376	627
	Total	11,115	1,645	1,021	811	376	14,968

B.		**ANA IU/ml At Incident Test**					
		<7	7 - 10	>10 – 20	>20 -30	>30	Total
2nd ANA Result IU/ml	<7	10,362	687	116	28	2	11,195
	7 - 10	584	688	276	66	3	1,617
	>10 - 20	109	213	424	195	39	980
	>20 -30	46	52	189	424	156	867
	>30	14	5	16	98	176	309
	Total	11,115	1,645	1,021	811	376	14,968

An increase in ANA of 1 level between the first two tests was reported in 82% of individuals (n=1,084). The greatest increase (ANA<7IU/ml to >30IU/ml) was reported in 14 individuals (Table 5-12B).

Multivariate LR of maximum ANA adjusted for gender, Indigenous Status and country of birth showed each variable to be a significant predictor ($p < 0.05$). Being female and born in N.W. or S. & E. Europe or the Americas was associated with increased odds of an ANA ≥7IU/ml. Data are shown in the Appendix Table 10.1.

5.3.11 ANA AND MORTALITY

Mortality data collected through the data linkage unit was merged with the incident ANA data to investigate death rates with reference to i. ANA as a dichotomous variable and ii. ANA grouped into levels as previously described.

Deaths were reported in 12.4 % of incident ANA <7IU/ml individuals compared to 14.3% of those with an incident ANA ≥7IU/ml (Table 5-13). This difference was significant in a Chi-square analysis of ANA and an outcome of death v censored ($\chi^2 = 30.822$, p value < 0.001).

TABLE 5-13 FREQUENCY OF DEATH RELATED TO ANA.

ANA IU/ml	Deceased N	Total N	Deceased %
<7	7,208	57,986	12.4%
≥ 7	1,794	12,588	14.3%
Overall	9,002	70,574	12.8%

A summary of ANA, gender, age at time of incident ANA and age at time of death is shown in Table 5-14. The mean and median age at death in ANA ≥7IU/ml individuals was approximately 2 years greater that in ANA<7IU/ml individuals. At the time of the incident ANA test, individuals with an ANA ≥7IU/ml were also older by approximately 2 years (Table 5-14). This indicates that individuals with an ANA <7IU/ml and ANA ≥7IU/ml had similar survival following the incident ANA test.

Interestingly, the mean and median age of the censored incident ANA cohort at the time of testing was approximately 20 years less that the age at incident ANA of the deceased cohort. This difference was noted for both ANA <7IU/ml and ANA≥7IU/ml (Table 5-14).

TABLE 5-14 GENDER AND AGE AT TIME OF INCIDENT ANA AND DEATH RELATED TO ANA.

		LIVING (Censored) COHORT				**DECEASED COHORT**			
		ANA Negative		ANA Positive		ANA Negative		ANA Positive	
		Males	Female	Males	Female	Males	Female	Males	Female
	n	20,137	30,641	2,807	7,987	3,952	3,256	764	1,030
Age at Time of Incident ANA (years)	Mean	47	46	50	47	66	69	68	70
	Median	47	46	51	47	69	73	71	74
	SD	17	17	17	17	15	16	14	15
	Minimum	0	0	0	0	0	14	17	7
	Maximum	98	96	94	96	100	101	96	101
Age at Time of Death (years)	Mean					69	72	71	74
	Median					72	76	74	77
	SD					15	16	14	15
	Minimum					1	15	18	10
	Maximum					101	105	97	103

5.3.12 MULTIVARIATE LOGISITC REGRESSION ANALYSIS OF ANA AND MORTALITY DATA

The predictor variables ANA, age at time of incident ANA and gender were significantly associated with death (Table 5-15) however only ANA ≥7IU/ml, an Indigenous background, age and a country of birth of N.W. Europe were associated with increased odds of mortality, female gender was associated with a decreased likelihood of mortality relative to males.

TABLE 5-15 MULTIVARIATE LR ANALYISIS OF MORTALITY ADJUSTED FOR ANA OUTCOME, GENDER, AGE AT TIME OF ANA TESTING, COUNTRY OF BIRTH.

Variables in the Equation	N	df	OR	95% C.I.		P Value
				Lower	**Upper**	
ANA						
<7IU/ml (Reference)	57,986					
≥7IU/ml	12,588	1	1.100	1.032	1.173	0.003
INDIGENOUS STATUS						
Non Aboriginal or TSI (Reference)	62,835	2				<0.001
Not Grouped	4,225	1	0.101	0.069	0.148	<0.001
Aboriginal or TSI	3,514	1	4.145	3.71	4.632	<0.001
AGE		1	1.087	1.085	1.089	<0.001
COUNTRY OF BIRTH						
Australia (Reference)	42,818	10				
Oceania and Antarctica (excluding Australia)	1,861	1	0.955	0.798	1.142	0.612
N.W. Europe	13,043	1	1.115	1.051	1.184	<0.001
S. & E. Europe	1,073	1	0.877	0.725	1.062	0.179
N. Africa & M.E	1,741	1	1.125	0.952	1.329	0.166
S.E. Asia	1,155	1	0.713	0.56	0.907	0.006
N.E. Asia	788	1	1.131	0.923	1.387	0.235
S. & C. Asia	566	1	0.716	0.519	0.987	0.042
Americas	368	1	0.781	0.517	1.181	0.241
Africa (Excluding N. Africa)	1,196	1	0.915	0.729	1.148	0.441
Not Available	5,965	1	1.133	0.973	1.32	0.109
GENDER						
Male (Reference)	27,660					
Female	42,914	1	0.538	0.511	0.566	<0.001

A second regression analysis including 5 increments of ANA was also performed. Data are shown in Table 5-16. This model demonstrated that ANA level predicted mortality.

The ANA ranges >20 – 30IU/ml and >30IU/ml had ORs of 1.23 (95%CI 1.072-1.412) and 1.687 (95%CI 1.350-2.107) respectively.

TABLE 5-16 MULTIVARIATE LR ANALYISIS OF MORTALITY ADJUSTED FOR GENDER, AGE AT TIME OF ANA TESTING, ANA LEVEL, INDIGENOUS STATUS AND COUNTRY OF BIRTH.

Variables in the Equation	N	df	OR	OR (95% C.I)		
				Lower	Upper	P value
AGE **(at time of ANA testing)**		1	1.087	1.085	1.089	<0.001
GENDER						
Male (Reference)	27,660					
Female	42,914	1	0.536	0.509	0.564	<0.001
ANA		4				
<7IU/ml (Reference)	57,986					
7-10IU/ml	6,604	1	1.038	0.955	1.13	0.381
>10 - 20IU/ml	3,128	1	1.033	0.916	1.165	0.592
>20 -30IU/ml	2,157	1	1.23	1.072	1.412	0.003
>30IU/ml	699	1	1.687	1.35	2.107	<0.001
INDIGENOUS STATUS						
Non Indigenous (Reference)	62,835	2				
Indigenous	3,514	1	4.149	3.714	4.636	<0.001
Not Grouped	4,225	1	0.101	0.069	0.147	<0.001
COUNTRY OF BIRTH						
AUSTRALIA **(Reference)**	42,818	10				
Oceania and Antarctica (excluding Australia)	1,861	1	0.954	0.798	1.142	0.608
N.W. Europe	13,043	1	1.116	1.052	1.184	0
S. & E. Europe	1,073	1	0.879	0.726	1.063	0.184
N. Africa & M.E	1,741	1	1.122	0.95	1.325	0.176
S.E. Asia	1,155	1	0.713	0.561	0.907	0.006
N.E. Asia	788	1	1.132	0.923	1.388	0.233
S. & C. Asia	566	1	0.711	0.516	0.981	0.038
Americas	368	1	0.781	0.517	1.18	0.241
Africa (Excluding N. Africa)	1,196	1	0.913	0.728	1.146	0.433
Not Available	5,965	1	1.139	0.977	1.327	0.095

5.3.13 KAPLAN MEIER SURVIVAL ANALYSIS

The Kaplan Meier estimation was used to determine the influence of ANA on survival (data shown in Figure 5.5). The log rank test showed there was a statistically significant difference in the survival rate for a positive and negative incident ANA ($\chi 2 = 36.139$, p =<

0.001). The proportion of deceased males and females with a positive ANA was higher than the proportions in the ANA negative individuals 21.4% v 16.4%, and 11.4% v 9.6% respectively (Table 5-17).

TABLE 5-17 FREQUENCY OF DEATHS ASSOCIATED WITH ANA IN MALES AND FEMALES.

Gender	ANA	Deceased N	Censored N	Deceased %	Total N	P Value
Male	< 7IU/ml	3952	20137	16.4%	24089	
	≥ 7IU/ml	764	2807	21.4%	3571	< 0.001
	Overall	4716	22944	17.0%	27660	
Female	< 7IU/ml	3256	30641	9.6%	33899	
	≥ 7IU/ml	1030	7987	11.4%	9018	<0.001
	Overall	4286	38628	10.0%	42,914	

When adjusted for gender, survival was significantly different for ANA positive and negative individuals ($\chi 2 = 86.188$, p =< 0.001). See Figure 5.5.

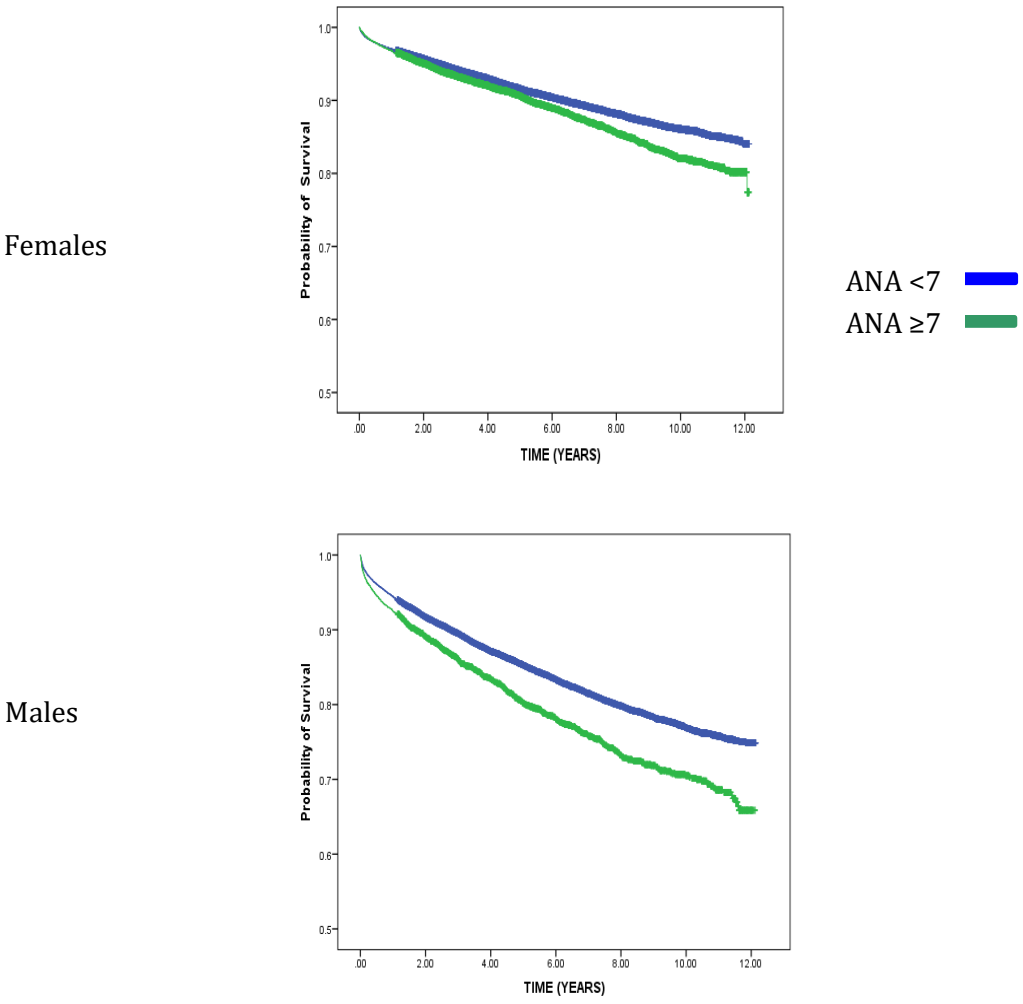


FIGURE 5.5 KAPLAN MEIER PLOT SHOWING SURVIVAL RATES FOR ANA <7 AND ≥7IU/ML.

Kaplan Meier analysis was also conducted to compare survival when individuals were further sub-grouped into ANA <7, 7-20 and >20IU/ml; males and females; and age groups: 0 – 30, >30 – 60 and >60 years. The categories used in this analysis were selected to reflect ANA groupings and stages of life shown to be significant in Section 5.3. (Data shown in Figure 5.6.)

Males and females in the >30 to 60 year age group differed in their overall survival (91.0 v 95.8% respectively). However in the female group a pairwise comparison using the log rank test did not show a significant difference in survival between ANA levels. Survival in females aged 0 – 30 years and >60 years differed significantly between ANA >20 and both ANA <7 ($p \leq 0.001$) and 7-20IU/ml ($p<0.001$). Percent survival data are shown in Table 5-18.

There were no significant differences in survival between ANA levels in males ≤30 years, however in the >30 – 60 year age group, the ANA negative group survival (91.2%) was significantly higher than the rate in both the ANA 7–20IU/ml (89.4% $p = 0.017$) and ANA >20IU/ml, (87.2%, $p =0.005$). Similarly in males >60 years of age a statistically significant difference in survival was shown for ANA negative males compared with the >20IU/ml group ($p=0.034$).

TABLE 5-18 COMPARISON OF KAPLAN MEIER SURVIVAL MODEL OF DEATH ASSOCIATED WITH ANA FOR INDIVIDUALS WITHIN GENDER AND AGE GROUPS.

Gender	Age (years)	Grouped ANA IU/ml	Censored N	Deceased N	% Deceased	Total N	
Males	0 - 30	<7	3590	97	2.6%	3687	
		7 - 20	334	10	2.9%	344	
		>20	79	3	3.7%	82	
		Overall	4003	110	2.7%	4113	
	>30 - 60	<7	12032	1156	8.8%	13188	a,b
		7 - 20	1350	160	10.6%	1510	a
		>20	251	37	12.8%	288	b
		Overall	13633	1353	9.0%	14986	
	>60	<7	4515	2699	37.4%	7214	c
		7 - 20	650	440	40.4%	1090	
		>20	143	114	44.4%	257	c
		Overall	5308	3253	38.0%	8561	
Females	0 - 30	<7	6014	74	1.2%	6088	d
		7 - 20	1141	8	0.7%	1149	e
		>20	365	11	2.9%	376	d, e
		Overall	7520	93	1.2%	7613	
	>30 - 60	<7	18792	828	4.2%	19620	
		7 - 20	3521	159	4.3%	3680	
		>20	1118	49	4.2%	1167	
		Overall	23431	1036	4.2%	24467	
	>60	<7	5835	2354	28.7%	8189	f
		7 - 20	1402	557	28.4%	1959	g
		>20	440	246	35.9%	686	f,g
		Overall	7677	3157	29.1%	10834	

Paired characters a-g denote significant differences within gender and age groups

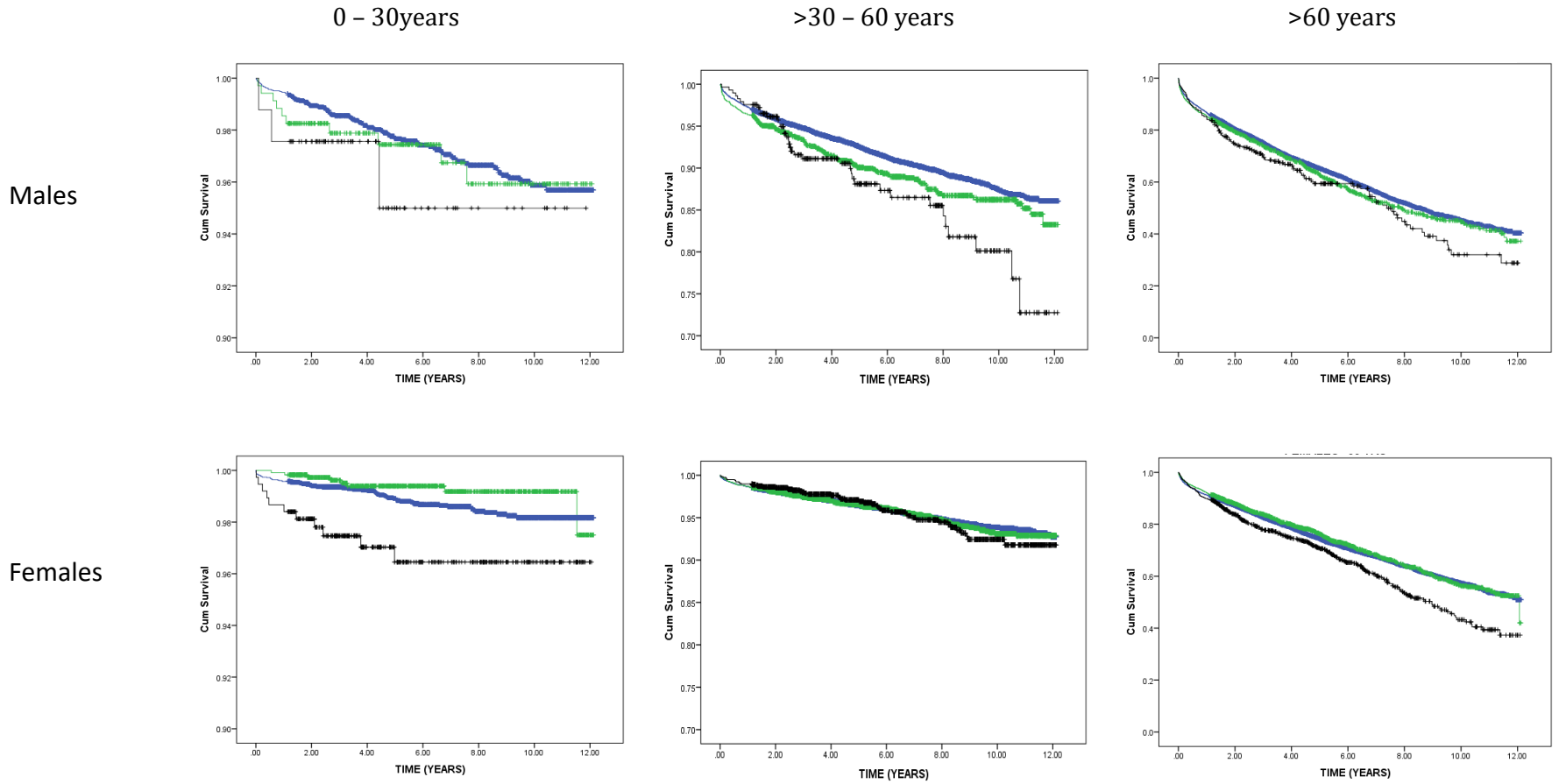


FIGURE 5.6 KAPLAN MEIER SURVIVAL CURVES FOR MALES AND FEMALES IN 3 AGE GROUPS 0 – 30 YEARS, >30 – 60 YEARS AND >60 YEARS FOR ANA AT THREE LEVELS 0-<7IU/ML (BLUE), 7-20IU/ML (GREEN) AND >20IU/ML (BLACK).

A comparison of survival rate for Indigenous and non-Indigenous individuals with an ANA <7IU/ml showed a significant difference (84.6 and 87.0% respectively; log rank test p=0.006), however a statistically significant difference was not seen between the two groups for an ANA ≥7IU/ml (81.6 and 85.0% respectively; log rank test p>0.05). Data are shown in Figure 5.7 and Table 5-19.

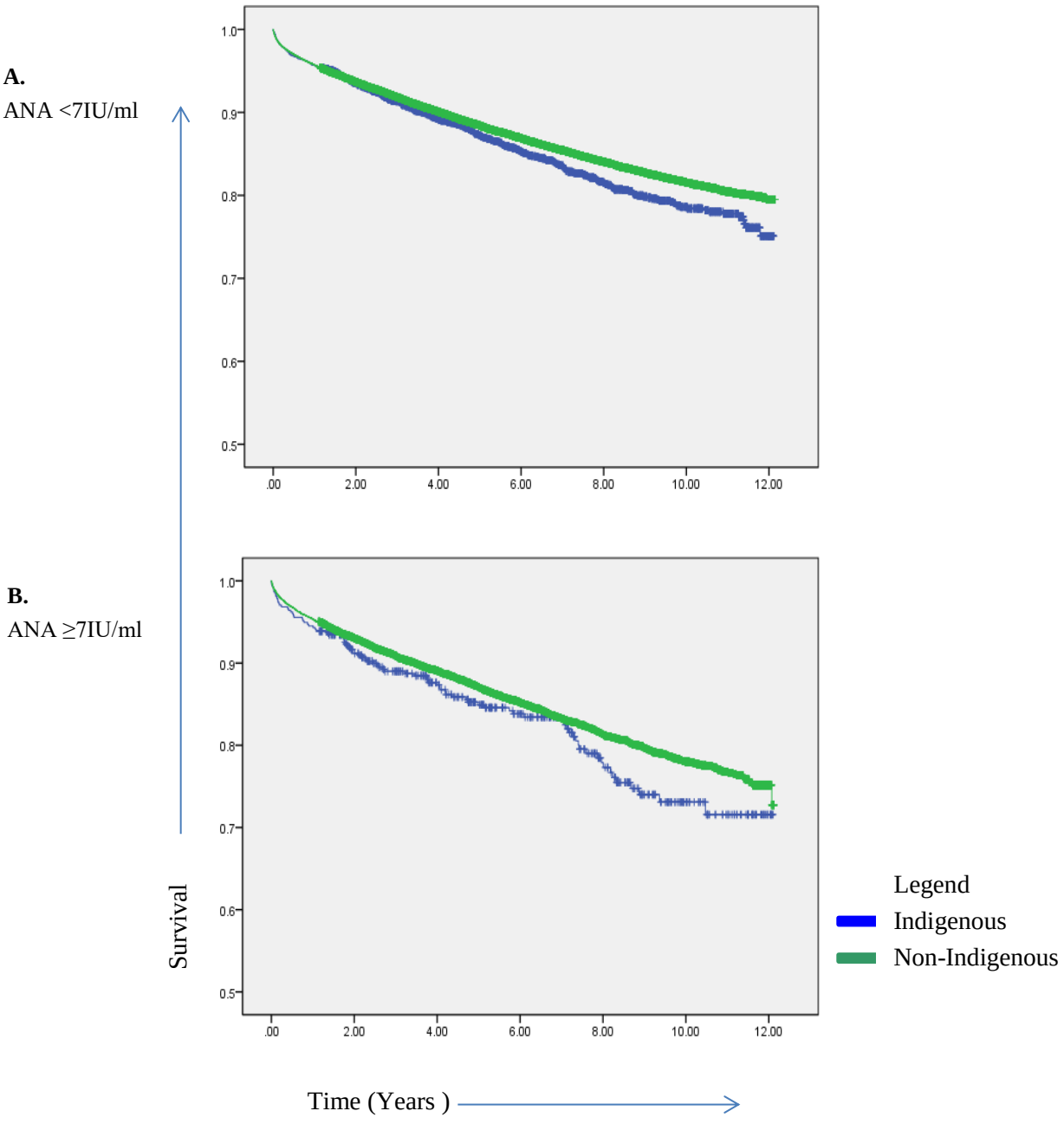


FIGURE 5.7 KAPLAN MEIER SURVIVAL CURVES FOR **A.** ANA <7IU/ML AND **B.** ANA ≥7IU/ML IN INDIGENOUS AND NON-INDIGENOUS INDIVIDUALS.

TABLE 5-19 INDIGENOUS STATUS SURVIVAL DATA FOR ANA <7IU/ML AND ≥7IU/ML.

Indigenous Grouping	ANA	Deceased	Censored	Total N	% Survival (Censored)
Indigenous	< 7IU/ml	467	2,573	3,040	84.6%
	≥ 7IU/ml	87	387	474	81.6%
	Total	554	2,960	3,514	84.2%
Non Indigenous	< 7IU/ml	6,713	44,746	51,459	87.0%
	≥ 7IU/ml	1,702	9,674	11,376	85.0%
	Total	8,415	54,420	62,835	86.6%
Overall	< 7IU/ml	7,180	47,319	54,499	86.8%
	≥ 7IU/ml	1,789	10,061	11,850	84.9%
	Total	8,969	57,380	66,349	86.5%

5.3.14 ANA AND CAUSE OF DEATH

The association between incident ANA and cause of death was investigated for the ANA cohort with linked mortality data (n = 9,002). ICD-10 codes were available for 87.1% of individuals who died.

To investigate the incident ANA associated with Cause of Death; ICD codes were grouped according to the first tier categories of the ICD Classification system and sub-grouped to include autoimmune related diseases. Malignant neoplasms (ICD-10 coding of C00 – C97) as a first tier category of the ICD-10 system were investigated and accounted for 23.4% of deaths, of which 79.4% were ANA<7IU/ml compared to 20.6% ANA ≥7IU/ml.

A subgrouping of Systemic Connective Tissue autoimmune diseases (including Rheumatoid Arthritis, SLE, SSc and Wegner granulomatosis) were the listed Cause of Death in 135 individuals of which 47% had an ANA ≥7IU/ml on their incident ANA test. The deaths included 25 individuals (9 males and 16 females) with SSc (ICD-10 M34) with <5 individuals being ANA <7IU/ml on the first reported result.

SLE was the recorded cause of death in 15 cases (<5 males; all ANA<7IU/ml and 12 females of which 8 were ANA ≥7IU/ml and 4 ANA <7IU/ml at the first ANA test). A further 34 deaths were listed as RA (23 females, 11 males, 71% (n=24/34) were ANA <7IU/ml), and 11 deaths were reported as Wegener granulomatosis (4 female, 7 males, 90.1% ANA <7IU/ml).

Deaths associated with the circulatory system (including heart disease) accounted for 24.0% of all deaths in the study, 20.7% of these deaths were incident ANA ≥7IU/ml.

Multivariate LR analysis adjusted for gender, age at time of ANA testing, Indigenous Status, country of birth and ANA outcome as either i. <7IU/ml and ≥ 7IU/ml or ii.as a grouped variable (3 groups) was performed with ICD-AM code grouped cause of death (detailed in Table 5-20) as the outcome variable. The ANA variable expressed in either way was a significant predictor ($p < 0.001$) of mortality for the musculoskeletal disease (ICD-10 M00 – 39.9). Compared with ANA<7IU/ml as a reference the OR for ANA ≥7IU/ml, 7–20IU/ml and >20IU/ml, were 3.441 (95%CI 2.43–4.87) 1.984 (95%CI 1.266 – 3.109) and 7.938 (95%CI 5.197-12.125) respectively.

The distribution of ANA <7IU/ml and ≥7IU/ml for morbidity data grouped by ICD-10 1st tier codes is shown in Table 5-20. Demographics of the deceased cohort are described in Appendix Table 10-2 and 10-3.

TABLE 5-20 ANA RELATED TO THE MOST FREQUENT ICD-10 CODED CAUSES OF DEATH IN THE INCIDENT ANA CLINICAL COHORT.

Disease association	ICD-10	ANA		Total (% of all deaths)
		Negative N (%)	Positive N (%)	
Endocrine	E00.0–35.9	295 (83.3)	59 (16.7)	354 (4.5)
Circulatory (including heart)	I01.8-I99.9	1712 (79.3)	448 (20.8)	2,160 (27.6)
Kidney	N0-39.9	221(84.0)	42 (16.0)	263 (3.4)
Respiratory	J40.0–99.9	485 (78.0)	137 (22.0)	622 (7.9)
Liver	K70.0–77.9	355 (84.8)	64 (15.3)	419 (5.3)
Musculoskeletal/connective tissue (MSD)	M00–39.9	72 (53.3)	63 (46.7)	135 (1.7)
Cancer	C00–C97.9	1670 (79.4)	433 (20.6)	2,103 (26.8)
Total Deceased		6,254	1,583	7,837

5.3.15 ANA PATTERN AND CAUSE OF DEATH

The ability of ANA pattern and level to predict an outcome of death described by the first tier ICD-10 code was investigated in the incident ANA clinical cohort (data are shown in Figure 5.8 and Table 5-23). The highest frequency of deaths for each pattern described in Table 5-21 was recorded for ICD code C (neoplasms) and I (Circulatory Disease). Deaths recorded for each of the common patterns are discussed below. The data shown in this section does not include deaths with a text description of cause of death (n=1,165), however the statistical significance of the relationships in a χ^2 analysis did not change when these cases were included in the total number of deaths (n=9,002) (data not shown).

Speckled Pattern: The highest frequency of deaths associated with the speckled ANA pattern was noted in the broad ICD-10 group Cancer (3.6%) followed by diseases of the circulatory system (3.0%), however in these cases the majority of deaths (40-71%) were ANA 7-10IU/ml. Of the deaths associated with musculoskeletal disease (n = 11), 54.5% were ANA >30IU/ml. A Chi-square analysis of speckled ANA pattern and ANA level in patients with and without MSD as cause of death showed a significant difference in the frequency of death across the levels of ANA ($\chi^2 = 49.639$, $p < 0.001$).

Homogeneous Pattern: The total number of deaths associated with a homogeneous ANA pattern and an ICD-AM code of MSD was 25. In this group the frequency of deaths in each ANA level ranged from 0.6% (ANA >10-20IU/ml) to 0.9% (ANA >20-30IU/ml). A Chi-square analysis of MSD as a cause of death in individuals at each level of homogeneous ANA showed a significant difference in the frequency of death across the levels of ANA ($\chi^2 = 75.165$, $p < 0.001$). Respiratory disease was associated with 1.2% of individuals with a homogeneous ANA (Table 5-21) and showed a trend of increased frequency of death with increasing level of ANA however this was not significant in a Chi-square analysis.

In each cause of death category >58% of deaths had a homogeneous ANA of ≤20IU/ml (Figure 5.8).

Homogeneous Plus Speckled Pattern: In each "cause of death" category >85 % of the deaths were associated with an ANA ≤20IU/ml. One percent of individuals with a homogeneous plus speckled ANA pattern had a recorded cause of death (ICD code) associated with a respiratory disease. The frequency of death decreased with an increase in ANA level in each cause of death except for the kidney and musculoskeletal groups.

Mixed Nucleolar Patterns: This group includes nucleolar only and nucleolar in the presence of other ANA patterns i.e. homogeneous or speckled. In this ANA pattern group a statistically significant difference was recorded in a χ^2 analysis of ANA level and frequency of death in both the musculoskeletal and cancer as a cause of death ((χ^2 = 49.165 $p<0.001$ and χ^2 = 15.658, $p=0.005$, respectively). The frequency of death was highest in the ANA≥30IU/ml for both the musculoskeletal (4.35%) and cancer (9.8%) groups.

Nucleolar: This is a subset of the mixed nucleolar pattern group. χ^2 analysis of ANA level and death in individuals with a nucleolar pattern indicated a significant association between ANA level and an ICD coded musculoskeletal death (χ^2 = 78.792, $p<0.001$). Of the >5 deaths recorded 75% were recorded in the ANA >30IU/ml group.

Centromere: Statistically significant differences were determined by χ^2 analysis for mortality associated with circulatory and musculoskeletal disease and ANA levels, (χ^2 = 26.650 $p<0.001$ and χ^2 = 233.384, $p<0.001$). The frequency of death associated with circulatory disease increased from ANA >20 – 30 to ANA >30IU/ml. The frequency of deaths associated with the musculoskeletal disease were highest at the ANA >20 – 30IU/ml level. Interestingly a centromere pattern was not recorded as a cause of death associated with kidney disease.

Cytoplasmic Pattern: Antibody binding to the elements of the cytoplasm were reported in 4,531 individuals of which deaths were recorded in 17.1% (n = 777). χ^2 analysis of a reported cytoplasmic pattern and cause of death showed a statistically significant increased proportion of cytoplasmic antibody positive individual in the deceased cohorts for each of the cause of death groups describe in Table 5-21 and 5-22 with the exception of diseases of the kidney.

5.3.16 ANA PATTERN AND SURVIVAL

Kaplan Meier survival estimates and Cox proportional regression were performed for the three most frequently reported ANA patterns stratified by age <50 and ≥50 years (i.e. homogeneous, speckled and homogeneous plus speckled). Kaplan Meier survival analysis of individuals with an incident ANA pattern of homogeneous plus speckled pattern in the <50 age group had an improved survival compared to homogeneous plus speckled pattern not reported (Figure 5.9).

Cox Proportional Hazard Regression analysis of an outcome of death including the variables ANA pattern, age (grouped as ≤ 50 and >50 years of age at the time of ANA testing) and gender showed age and gender to be statistically significant in the model. Nucleolar (including mixed), centromere and cytoplasmic pattern were also significant predictors of death. In the Cox multivariate regression analysis for each pattern age was highly predictive of death, female gender had an OR < 1 indicating a reduced likelihood (i.e. homogeneous pattern OR for age ≥50 years = 0.593 (95%CI 0.569-0.618). Neither the speckled or homogeneous plus speckled patterns were significant predictors of death in this model. (Data are shown in Table 5-22).

TABLE 5-21 PERCENT FREQUENCY OF DEATH DESCRIBED BY 1ST TIER ICD CODE RELATED TO ANA PATTERN.

	Cause of Death						
Pattern (ANA ≥7IU/ml)	ENDOCRINE N = 354	CIRCULATORY N = 2,160	KIDNEY N = 263	RESPIRATORY N = 622	LIVER N = 419	MUSCULOSKELETAL N = 135	CANCER N = 2,103
Speckled N = 2,630	0.5%	3.0%	0.2%	0.8%	0.4%	0.4%	3.6%
Homogeneous N = 3,433	0.6%	3.9%	0.3%	1.2%	0.5%	0.7%	3.1%
Homogeneous plus Speckled N = 2,674	0.3%	3.3%	0.5%	1.0%	0.3%	0.1%	2.9%
Mixed Nucleolar N=2,380	0.4%	4.1%	0.5%	1.6%	1.0%	0.4%	5.2%
Nucleolar N=1,461	0.2%	2.7%	0.4%	1.3%	1.0%	0.3%	4.0%
Centromere N=486	1.2%	6.6%	0.0%	1.0%	0.6%	2.7%	4.1%
ANA < 7U/ml N = 57,032	0.5%	3.0%	0.4%	0.9%	0.6%	0.1%	2.9%
Cytoplasmic N=4,531	0.8%	4.8%	0.4%	1.4%	1.4%	0.4%	4.3%
All N = 69,509	0.5%	3.1%	0.4%	0.9%	0.6%	0.2%	3.0%

Note Individuals with a text description of cause of death but no recorded ICD 10 code have been excluded from the analysis.
Where ANA level is <7IU/ml a nuclear pattern is not assigned.

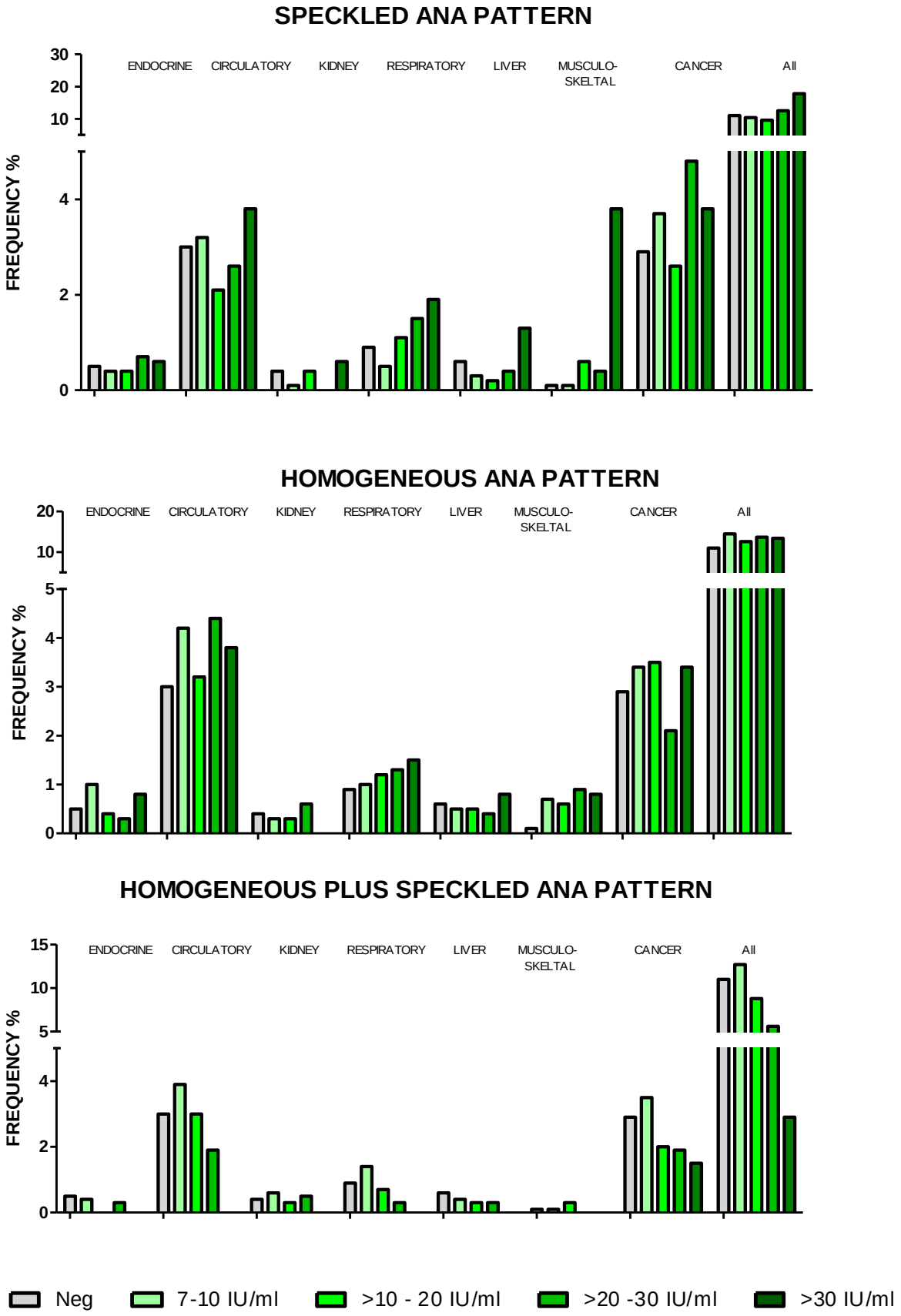


FIGURE 5.8 PERCENT OF INDIVIDUALS WITH A SPECIFIED ANA PATTERN AND LEVEL WHO HAD A CAUSE OF DEATH IN THE DEFINED CATEGORIES.

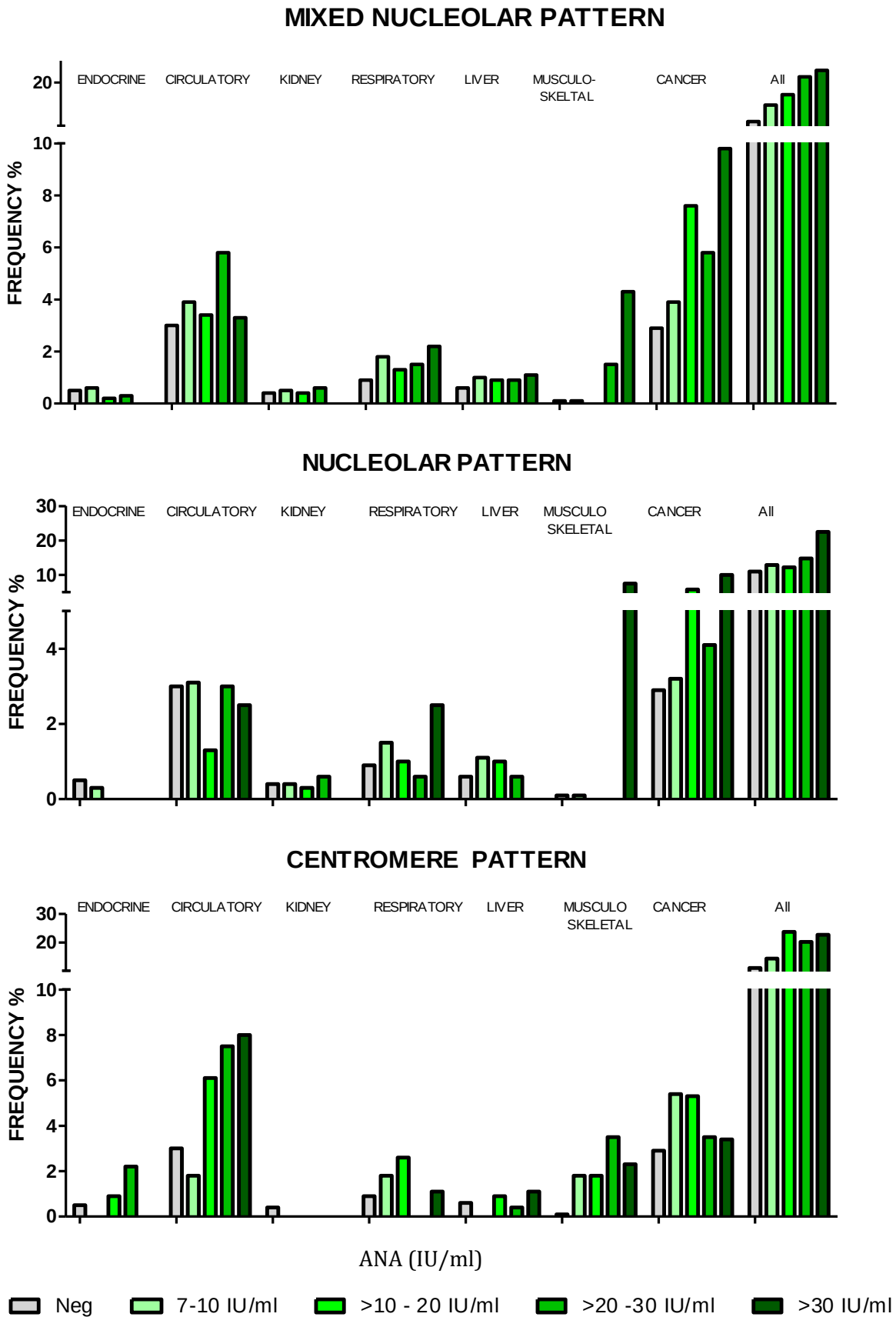


FIGURE 5.8 CONTINUED: PERCENT OF INDIVIDUALS WITH A SPECIFIED ANA PATTERN AND LEVEL WHO HAD A CAUSE OF DEATH IN THE DEFINED CATEGORIES.

TABLE 5-22 COX PROPORTIONAL HAZARD MULTIVARIATE ANALYSIS OF THE ASSOCIATION BETWEEN MORTALITY AND ANA PATTERN, AGE AND GENDER.

	OR (95%CI)			p Value		
	Pattern	Age	Gender	Pattern	Age	Gender
Homogeneous	1.099 (1.007-1.200)	7.405 (6.99-7.844)	0.593 (0.569-0.618)	0.035	< 0.001	< 0.001
Homogeneous plus speckled	0.945 (0.845-1.058)	7.425 (7.009-7.866)	0.595 (0.571-0.620)	0.327	< 0.001	< 0.001
Speckled	1.083 (0.97 – 1.209)	7.421 (7.006-7.862)	0.594(0.570 – 0.619)	0.154	< 0.001	< 0.001
Nucleolar	1.171 (1.022-1.341)	7.417 (7.002-7.857)	0.594 (0.570-.620)	0.023	< 0.001	< 0.001
Nucleolar including mixed patterns	1.384 (1.225-1.484)	7.407 (6.992-7.846)	0.594 (0.570-0.619)	< 0.001	< 0.001	< 0.001
Cytoplasmic	1.375 (1.283-1.473)	7.304 (6.894-7.739)	0.588 (0.564-0.613)	< 0.001	< 0.001	< 0.001
Centromere	1.448 (1.206-1.739)	7.397 (6.983-7.836)	0.592 (0.568-0.617)	< 0.001	< 0.001	< 0.001

Reference variable: Gender = Male, Pattern: not detected and Age 0 – 50 years, survival calculated from the time of ANA testing.

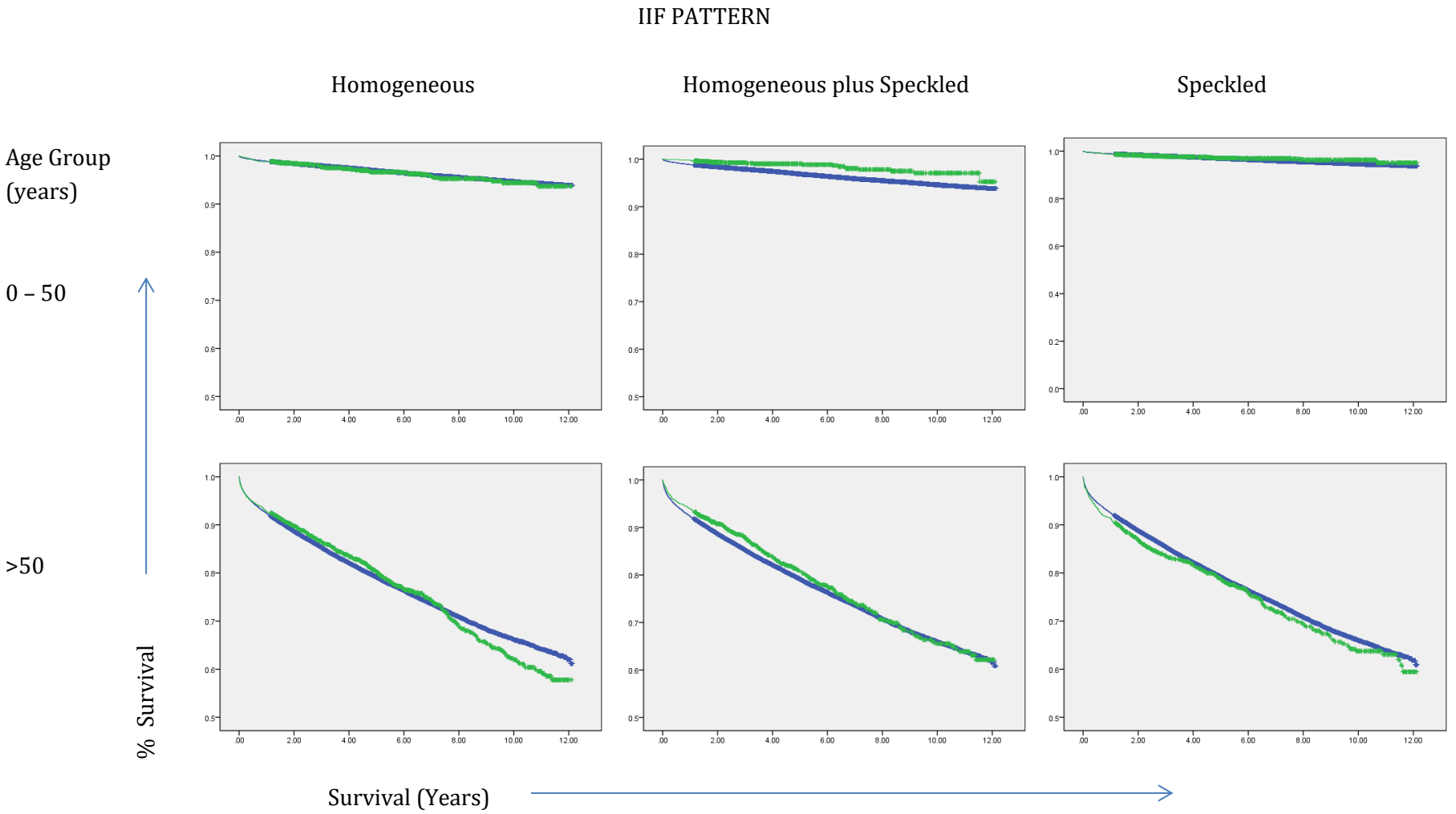
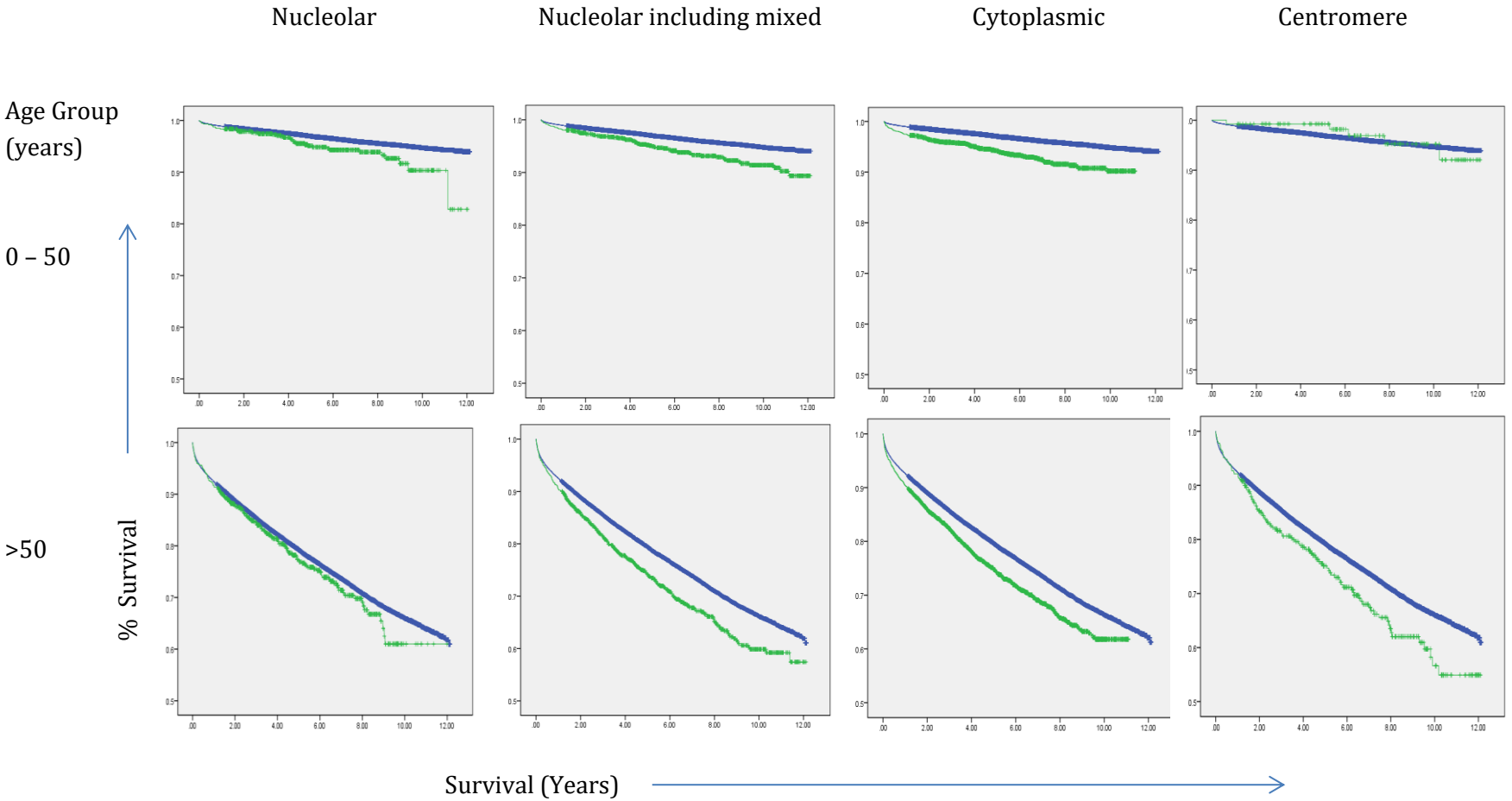


FIGURE 5.9 SURVIVAL RATES FOR INDIVIDUALS WITH IIF PATTERNS ASSOCIATED WITH ANA ADJUSTED FOR AGE GROUPED AS 0-50 AND >50 YEARS OF AGE. THE LOG RANK STATISTIC AND P VALUE FOR EACH COMPARISON IS INCLUDED (GREEN = PATTERN REPORTED, BLUE = PATTERN NOT REPORTED).

IIF PATTERN



	Nucleolar	Nucleolar including mixed	Cytoplasmic	Centromere
Log Rank χ^2 (p value)	5.012 (0.025)	34.07 (<0.001)	57.490 (<0.001)	5.932 (0.015)

FIGURE 5.10 SURVIVAL RATES FOR INDIVIDUALS WITH ANA PATTERNS ADJUSTED FOR AGE GROUPED AS 0-50 AND >50 YEARS. THE LOG RANK STATISTIC AND P VALUE FOR EACH COMPARISON IS INCLUDED. (GREEN = PATTERN REPORTED, BLUE = PATTERN NOT REPORTED).

5.4 DISCUSSION

This chapter summarised diagnostic ANA data collected from a clinical cohort over an 11-year period presenting the incident ANA test result for an individual recorded in the testing laboratory. The pathology requests have come from; specialists and general practitioners, major tertiary hospitals, smaller metropolitan and regional medical centres and includes residents in both rural and metropolitan locations.

This study is one of the largest conducted in this area, comprising over 85,000 individuals. We found the female to male ratio was 1.5:1 indicating that females were more likely to be tested for an ANA. Notably, the ratio of females to males with a positive ANA result was 2.5:1. This increase compared to the ratio in tested samples suggests that the higher incidence noted in women is not simply due to the higher frequency of testing in females or better proactive health care seeking behaviour as reported elsewhere (128). Rather, it reflects the reported increased likelihood of autoimmune disease in women compared to men as described by Whitacre (119). The mechanisms for the observed female gender bias in a number of autoimmune diseases (i.e. SLE, SjS and thyroiditis have a female predominance of 85%) is poorly understood, however factors thought to contribute to this observed predominance include: genetics, hormones and an observed dimorphism in the basic immune responses to infection and inflammation in males and females (51, 119, 129).

An early study by Hooper *et al*. (25) reported an ANA prevalence of 2.4% in a general rural Western Australian population, with an increased prevalence in women and a progressive increase in prevalence in individuals up to 75years of age. The rate of ANA positivity described in this retrospective clinical cohort study (17.5%) is notably higher than the level reported by Hooper *et al* and the 13.8% reported in the USA study by Satoh *et al* (127) however it is less than the 28.3% and 23.5% reported in clinical cohorts in a regional Australian study in 2003 (48) of 20,205 patients and a Belgian study (97) of 10,550 patients in 2001 respectively.

ANA testing is not standardised and consequently the designated reference value varies between testing centres (46, 87), thereby complicating comparisons across the literature. The difference between the prevalence in a general population and the clinical cohort is

not surprising. However the varied approaches may contribute to the differences reported in this study compared to the clinical cohorts discussed (8, 53). ANA results are typically reported as the highest dilution at which ANA can be clearly visualised by microscopy. ANA results from this clinical cohort study have been reported in IU/ml; a semi-quantitative measure of antibody which has been shown to reduce inter-assay and inter-laboratory variation (10, 11).

This study is consistent with gender and age trends in ANA testing described in the literature, i.e. a predominance of females in a number of autoimmune diseases associated with a detectable ANA (e.g. ≥80% in SLE, autoimmune thyroid disease and SjS) and an increased incidence with increasing age (14, 25, 30, 46, 51, 119).

Importantly, this study showed a bimodal distribution for the prevalence of ANA which peaked between the 2nd and 3rd decade of life and a trend of increasing prevalence from the 6th decade onwards. Jake *et al* (2012) published a systematic review of the epidemiology of SLE (in the ASIA-Pacific region) (130) for which ANA is a preliminary screening assay. The early peak (2nd to 3rd decade of life) of ANA ≥7IU/ml results is consistent with the age of diagnosis and incidence in Australia for females reported by Jakes *et al*. i.e. mean age of onset of 32.9 years, mean age of diagnosis 31.1 years and a predominance of females 83 – 95% .

A review of ANA-associated rheumatic disease and ANA titre by Abeles *et al* (2013) showed an increasing positive predictive value with increased ANA titre (17). However they also noted that a positive ANA result was clinically meaningful in < 10% of cases (i.e. ANA positive individuals with evidence of associated rheumatic disease). In a study of a 125 healthy individuals 31.7% were positive at the minimum dilution of 1:40 and 3.3% after 4 serial two fold dilutions (1:640) (7). While this clinical cohort study is reported in IU/ml a similar trend was noted with a declining frequency in the upper range of the reportable ANA range. Mortality data linked to the ANA data showed a significant association at all levels of ANA ≥7IU/ml with a mortality categorised in the ICD-10 group of musculoskeletal disease (131).

Females with an ANA >20IU/ml represented 24.7% of all positive females (ANA≥ 7IU/ml), the same comparison in males showed of all ANA positive males 17.5% had an ANA >20IU/ml. Wiik (2005) reports that naturally occurring ANAs are low affinity and

have weak reactivity (5) in contrast to predominantly IgG antibodies present in higher levels in patients with connective tissue disease.

There was a significantly higher proportion of Indigenous females (1.5%) with an ANA level of >30IU/ml compared to the non-Indigenous female group (1.2 %). However, although significant this difference is relatively small and may not be clinically meaningful.

The ABS estimated the Indigenous population in WA during the time frame of the study ranged from 3.1-3.8% of the total state population (132, 133). However, Indigenous individuals comprised 4.1% of this clinical cohort thereby signifying a minor over-representation. Data from the ABS also indicate that males and females represented a similar proportion (50%) of the WA Indigenous and non-Indigenous population in 2006, with a median age of 20.6 years in the Indigenous population compared to 35.4 years in the non-Indigenous population in the same period (134). Consistent with this information the mean age of the Indigenous cohort in this study was lower in both the ANA negative (38.6 years) and positive groups (40.4 years) compared to the non-Indigenous group (ANA negative = 49.9 and ANA positive = 51.9 years). Multivariate analysis of ANA outcome including Indigenous Status as an independent variable did not show the Indigenous group to be at greater risk of an ANA ≥7IU/ml in this clinical cohort, furthermore in an analysis of survival which included ANA and Indigenous Status as independent variables, an increased risk of mortality was not identified in the Indigenous group compared to the non-Indigenous group.

Socioeconomic status and remoteness have previously been reported as significant variables in health outcomes for both Indigenous and non-Indigenous Australians (47, 135). An increased prevalence in ANA positivity was not demonstrated in any quartile of social disadvantage and while residential remoteness was shown to have a significant association with a positive ANA result, when compared to the least remote category a reduced likelihood rather than increased likelihood of a positive ANA in areas of increasing remoteness was evident. An important point to consider here is whether the level of servicing of remote areas is equal to that of the more accessible areas and, if not, does this reduced risk represent under servicing and under diagnosis of ANA related diseases in increasingly remote areas of WA (136, 137). An important consideration is

that this is a clinical cohort study and not referenced against a healthy population in which case the reported effects of IRDS and ARIA may not be as evident.

The ANA patterns detected in two clinical cohorts studies; the Roberts Thompson study (48) and the Belgian Study (97), and the study described here show the same rank of frequency of homogeneous, speckled, nucleolar and centromere patterns. The homogeneous plus speckled ANA pattern, which was not included in the Belgian study, includes a subgroup referred to as dense fine speckled (DFS) with a refined description of dense fine speckles and staining of the chromatin in mitotic cells. Further characterisation of DFS positive sera has identified the target as DFS70which is also known as lens epithelial growth factor (138). The presence of antibodies to DFS70 does not appear to correlate with rheumatic disease and is found in otherwise healthy individuals (126). Watanabe *et al* (2004) investigated the sera of 597 healthy individuals and showed 55% of ANA positive samples were DFS70 positive. In our study the mixed homogeneous plus speckled pattern was the most frequently reported (21.4%) after the homogeneous and speckled patterns detected in isolation. As this is a retrospective analysis of results the specific features of DFS70 cannot be confirmed, however it is likely that DFS70 based on the IIF pattern will represent a proportion of the mixed homogeneous plus speckled ANA group.

A systematic review of studies investigating the epidemiology and progression of SLE identified an increased risk of disease in African American women in the United States of America (14). Interestingly the Americas as a country of birth was identified in this study as a significant risk factor for a positive ANA in the multivariate analysis (n = 368), OR = 1.4 (95%CI 1.1-1.8).

Linked mortality and ANA data showed that 10.5% of individuals in the incident ANA cohort were deceased at the end of the censor period. The WHO ICD-AM 10 codes assigned as cause of death in 86% of cases were wide ranging and were therefore grouped to organ system and cancer for this study. An increased risk of mortality was associated with ANA groups >20-30IU/ml and >30IU/ml. The group with the highest frequency of an ANA ≥7IU/ml included the autoimmune diseases SLE, Sjogren's Syndrome and RA (Musculoskeletal and connective tissue diseases). Further analysis in Chapter 7 will investigate ENA detection and mortality associated with a positive ANA.

Survival was compared for each of the most commonly reported patterns adjusted for age (grouped as <50 and ≥50 years) by a Kaplan Meier survival analysis and Cox proportional regression analysis including gender as an additional predictor. These analyses identified the nucleolar, centromere and cytoplasmic patterns as significant predictors for determining mortality. Although significant in the Kaplan Meier survival analysis the homogeneous plus speckled pattern was associated with a reduced risk of death in the <50 year age group. Interestingly the frequency of death associated with the homogeneous plus speckled pattern decreased with increasing ANA level which raises the question: does this pattern include a high proportion of DFS antibody positive individuals, as this antibody is frequently reported in healthy individuals and considered non-pathogenic when detected in isolation (6).

The findings of this study confirm the higher prevalence of ANA in females (14), and in individuals in the older age groupings. The data also showed a biphasic peak in the frequency of ANA ≥7IU/ml in the 2nd and 8th decades of life. In addition it shows that high level ANA are detected at a marginally increased rate in Australian Indigenous females. The homogeneous, nucleolar, centromere and cytoplasmic patterns were significant variables in survival outcome analysis. Gender, Indigenous Status, age at time of ANA testing, country of birth and ANA levels of ≥20IU/ml were significant predictors of death.

6 dsDNA ANTIBODY

6.1 BACKGROUND

SLE usually affects people between the ages of 15 and 45 years and is predominantly found in females, however it can also occur in childhood and later life (139). Furthermore, the prevalence of SLE differs between ethnic groups, with individuals of African or Asian ancestry reported to be at greater risk of developing the disease(139). The prevalence of SLE is also reported to be higher in Indigenous Australians compared to Caucasians (140). Infection is a major cause of mortality in SLE patients (141).

Autoantibodies to dsDNA are one of the diagnostic markers of SLE, reported in 40 to 90% of SLE patients and associated with renal exacerbations (142, 143). While the mechanism leading to the production of autoantibodies to dsDNA antibody is not clearly elucidated, defects in the clearance of apoptotic and/or necrotic cells have been implicated. DsDNA antibody complexed with free dsDNA or dsDNA combined with histone proteins is deposited in renal tissue leading to an acute inflammatory response with glomerular damage (144, 145).

The antibodies can be detected in patient serum several years prior to the onset of symptoms (19). Importantly, they are also useful in monitoring disease activity with elevated levels associated with a disease flare (110). While rarely found in healthy individuals they have also been reported in patients with chronic liver disease using an ELISA detection method (146).

The gold standard assay for dsDNA antibody determination is a radioimmunoassay using isotope (I^{125}) labelled dsDNA incubated with patient serum producing antibody antigen complexes which are then precipitated using ammonium sulphate (FARR Assay). Antibody bound dsDNA labelled with I^{125} is quantitated by measuring gamma radiation in the precipitate calibrated against a standard curve. Results are reported in IU/ml. The assay measures high avidity dsDNA antibody, without distinguishing antibody isotype (147).

The ANA test is used as a screening assay for the presence of dsDNA antibody. In this clinical incident cohort the threshold ANA level to prompt further dsDNA antibody testing is ≥5 IU/ml. This level is based on a study by Hollingsworth *et al* (1996) which demonstrated that ANA values below 5 IU/ml were unlikely to be detected in SLE

patients and were not predictive of elevated anti-dsDNA (148). The homogeneous ANA pattern is suggestive of antibodies binding dsDNA. However other target antigens for a homogeneous pattern can include single stranded DNA, histones, nucleosomes and chromatin.

In this chapter the association between ANA and dsDNA antibody levels is investigated in the WA Incident ANA Clinical Cohort. Correlations between age, gender, country of birth, Indigenous Status, IRDS and ARIA indexes will also be investigated in relation to dsDNA antibody and ANA levels. In addition mortality in dsDNA antibody positive and negative individuals will be compared.

6.2 METHODS

6.2.1 DSDNA ANTIBODY AND ANA TESTING

The PathWest laboratory protocol for dsDNA antibody testing recommends testing should be carried out on any sample with an ANA ≥ 5IU/ml. This is lower than the reference value for ANA reporting. To accommodate this change in decision point an additional grouping (5-<7IU/ml group) was included in the analysis of ANA and dsDNA antibody data.

DsDNA antibody results were grouped into five levels as described earlier (Section 3.5) for comparison of incident ANA and associated dsDNA antibody results.

6.2.2 STUDY POPULATION

ANA and dsDNA antibody data collected over the 2000 – 2010 period were merged using the unique identifier data linkage key. Linked ANA and dsDNA antibody data were available for 16,999 individuals.

Time separation between the first ANA test and any dsDNA antibody test was calculated from the difference in collection dates of the samples and varied from 9 years preceding the first ANA test to 10 years post the first test. The comparison of ANA and dsDNA antibody correlation was restricted to individuals for whom ANA and dsDNA antibody testing was done on the same sample or a sample collected on the same day.

Merged ANA and dsDNA antibody cases were linked with DoHWA Death registry data as described in section 3.3.2. Individuals without DoHWA Linkage data (n = 14,962) were excluded from the analysis of mortality as the outcome variable data were not available. An additional four cases were removed from the analysis as the date of death preceded the date of sample collection; indicating these samples had come from mortuary requests.

ANA and dsDNA antibody results were available on 12,498 individuals who also had a health record that could be linked to the state mortality database up to the 20th February 2012. Deaths were recorded for 1,697 (13.6%) individuals in this cohort.

6.2.3 ANALYSIS

Descriptive statistics were calculated for age, gender, Indigenous Status and country of birth for i. the incident ANA Cohort with linked dsDNA antibody results and ii. the incident ANA cohort limited to cases where ANA and dsDNA testing were performed on the same sample or a sample collected on the same day as the ANA test sample.

Chi-square analyses was initially performed to compare ANA and dsDNA antibody groups as categorical data and independent-sample t-tests were used for age as a continuous variable. The single individual without a defined sex was excluded from all gender related analysis in this chapter (n = 16,998).

Multivariate LR analysis adjusted for ANA, age, gender, country of birth, Indigenous Status, ARIA and IRDS was performed for dsDNA antibody outcome.

Significant differences in survival rates for dsDNA antibody positive and negative outcomes were determined by the log rank test and/or Cox regression analysis. Survival times were calculated from the time of collection of the incident ANA to the time of death or the censor date of 20th February 2012.

6.3 RESULTS

The dsDNA antibody data were derived only from the subset of the Incident Clinical ANA cohort who went on to anti dsDNA testing according to the algorithm shown in Figure 3.1. These comprised 14.2% (4,911 of 34,571) males and 23.7% (12,087 of 50,954) females.

Descriptive statistics for gender and age with respect to dsDNA antibody status are shown in Table 6-1.

6.3.1 AGE, GENDER AND DSDNA ANTIBODY

Consistent with the frequencies reported in Chapter 5, the number of females tested for dsDNA antibody and ANA was 2.5 times greater than the number of males; notably the ratio of dsDNA antibody positive females to males was 3.2, which is greater than the ratio of females to males tested which again indicates that the increased number of positive females was not just a reflection of the frequency of testing.

Males were older than females, irrespective of dsDNA antibody result (51.8 v 48.4 years). An independent sample t-test showed that the mean age of males with a positive vs negative dsDNA antibody result did not differ significantly. Statistically significant differences ($p < 0.001$) were seen in comparisons of:

i. the mean age of females with a positive or negative result (46.5 v 49.1 years),
ii. males and females with a positive dsDNA antibody result (52.9 v 46.5 years),
iii. males and females with a dsDNA antibody negative result (52.3 v 49.1 years).

Data are shown in Table 6-1

A comparison of the ratios of males and females grouped by decade of life and reported with a positive dsDNA antibody showed a clear increased ratio of females to males in the first and third decade of life (1^{st} decade : ratio 3.9:1 n = 154, M = 1/68, F = 5/86 3^{rd} decade: ratio 2.9:1 n = 1,795, M = 13/402, F = 131/1,393), the 2^{nd} and 4^{th} decades also had an increased ratio (~ 1.3) compared to the remaining groupings. Data are shown in Figure 6.1 and Table 6-2.

TABLE 6-1 **A**. RESULTS OF FIRST DSDNA ANTIBODY TEST **B.** MEAN AGE OF THOSE TESTED FOR DSDNA ANTIBODY.

A.				**% Positive**	
Gender	ANA Cohort	Total Tested	Positive	ANA Cohort	Tested Cohort
		N	N		
Males	34,571	4,911	289	0.8	5.9
Female	50,954	12,087	927	1.8	7.7
All Individuals	85,525	16,998	1,216	1.4	7.2

B.	**Mean Age (years ± SD)**		
	All	**Negative**	**Positive**
Males	52.4 (18.3)	52.3 (18.4)*	52.9 (17.1)§
Female	48. 9 (18.2)	49.1 (18.2) #*	46.5 (18.4)#§
All Patients	49.9 (18.3)	50..0 (18.3)	48.0 (18.3)

#*§ P<0.001

TABLE 6-2 NUMBER OF MALES AND FEMALES WITH A POSITIVE DSDNA ANTIBODY IN EACH DECADE OF LIFE IN THE SUBSET OF INDIVIDUALS WHO HAD ANTI DSDNA ANTIBODY TESTING.

	MALES			**FEMALES**		
Age Group	Positive	Total	% Positive	Positive	Total	% Positive
0-10	1	68	1.5%	5	86	5.8%
>10-20	12	202	5.9%	62	704	8.8%
>20-30	13	402	3.2%	131	1393	9.4%
>30-40	46	629	7.3%	205	2074	9.9%
>40-50	63	954	6.6%	160	2376	6.7%
>50-60	56	980	5.7%	156	2288	6.8%
>60-70	47	807	5.8%	97	1574	6.2%
>70-80	40	664	6.0%	78	1099	7.1%
>80	11	205	5.4%	33	493	6.7%

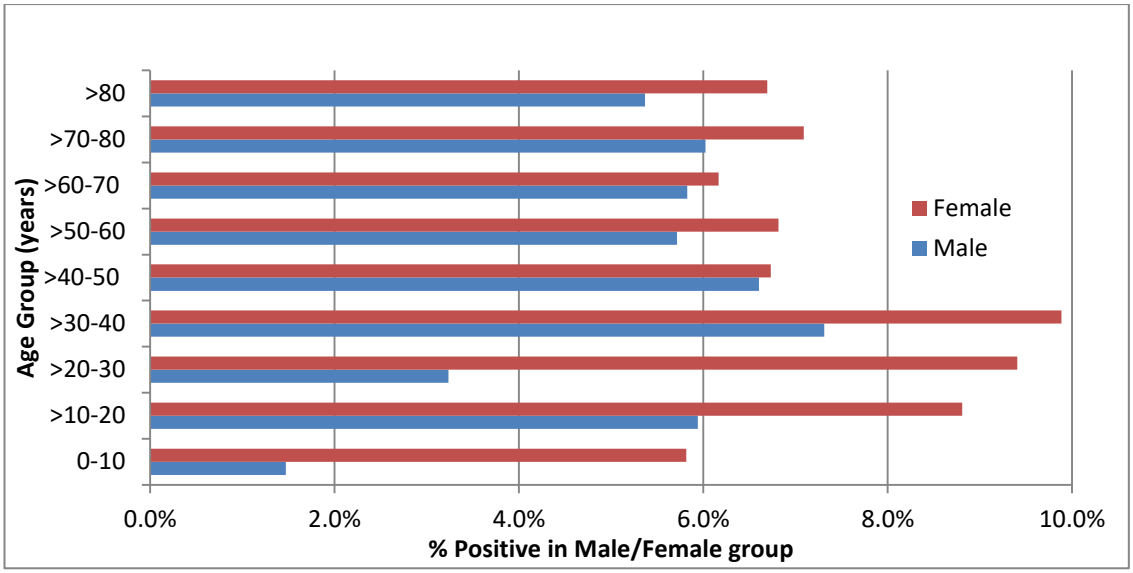


FIGURE 6.1 PERCENT OF TESTED MALES AND FEMALES WITH A POSITVE DSDNA ANTIBODY RESULT GROUPED ACCORDING TO DECADE OF AGE.

Positive dsDNA results were further subdivided into four groups (>reference value-10IU/ml, >10-30IU/ml, >30–70IU/ml and >70IU/ml). The age distribution of individuals at the time of the incident dsDNA antibody test was normally distributed, however the age distribution in sub-groups (separated according to antibody level) as described above did not meet the assumption of a normal distribution; age at the highest level of dsDNA antibody was skewed (Skewness = 0.99 Standard Error = 0.271). Homogeneity of variances as assessed by Levene's test for equality of variances for age in a comparison of positive and negative dsDNA results did not show a significant difference in the variance ($p = 0.69$), a single outlier was identified however it has been included in the analysis (age = 102years).

Both mean and median age differed significantly between dsDNA antibody groups. Mean and median age of individuals with the highest level of dsDNA antibody were lower than the age in all other groups (mean = 35.3, median = 27.6 year). Data are shown in Table 6-3.

TABLE 6-3 DESCRIPTIVE STATISITCS OF DSDNA ANTIBODY LEVEL AND AGE AT TIME OF TESTING.

dsDNA Antibody Level IU/ml	N	Age (years)						
		Median	Mean Age	SD	95% Confidence Interval for Mean		Min	Max
					Lower Bound	Upper Bound		
<Reference Value	15,783	50.0	50.0	18.3	49.7	50.3	0	97
Reference value - 10	758	49.7	51.0	17.8	49.7	52.3	9	95
>10- 30	271	43.4	45.0	17.3	43.0	47.1	0	102
>30 - 70	108	39.7	43.9	17.4	40.6	47.2	14	88
>70	79	27.6	35.3	19.5	30.9	39.6	9	83

6.3.2 INDIGENOUS STATUS AND DSDNA ANTIBODY

Indigenous individuals represented 3.9% (n=669) of the study group with an ANA and dsDNA result (n = 16,999) and 7.4% of all dsDNA antibody positive individuals at the time of their first test (n = 1,216). Chi-square analysis of Indigenous group and dsDNA outcome showed the difference between groups to be significantly (p <0.001). The proportion of males within the Indigenous and non-Indigenous groups with a positive or negative result did not differ significantly (p = 0.089), this is despite a higher than expected number of dsDNA antibody positive males in the Indigenous group (9.7%) compared to the Ungrouped (5.6%) and non-Indigenous groups (5.8%). The proportion of females with a positive dsDNA antibody result was higher than the expected number in the Indigenous female group (14.8%) compared to the Non Indigenous and Ungrouped females (7.4 and 7.3% respectively). This difference was statically significant (p <001).

There was also a statistically significant difference in age between males and females in the Indigenous group (mean = 42.7 years (SD = 16.4) v 39.4 years (SD = 15.2) p = 0.016) and in the Non Indigenous group (mean = 55.1 years, SD = 17.9 v 50.8 years, SD= 18.2, p< 0.001 respectively).

There was a significant difference in age (mean ±SD years) between:

- Non-Indigenous individuals with negative and positive dsDNA antibody (52.1 ±18.2 v 50.1 ±18.7),

- Ungrouped males with a negative and positive dsDNA antibody (43.0 ±16.5 v 49.8±15.1).
- Non-Indigenous females with a negative and positive dsDNA antibody (50.9±18.1v 48.6±19.0)
- Indigenous and non-Indigenous males with a negative and positive dsDNA antibody (mean 43.1 ±16.4 v 55.1 ±18.0 and 39.4 ±5 v 54.7 ±17.1 respectively)
- Indigenous and non-Indigenous females with a negative and positive dsDNA antibody (mean 39.5 ±15.4 v 50.9 ±18.1 and 38.7 ±14.5 v 48.6 ±19.0 respectively).

Data are shown in Table 6-4.

TABLE 6-4 DESCRIPTIVE STATISTICS FOR INDIGENOUS GROUPING, GENDER AND OUTCOME OF DSDNA ANTIBODY TESTING.

		dsDNA Antibody Negative		dsDNA Antibody Positive		Total	
Indigenous Status	Gender	N	Mean Age (SD)	N	Mean Age (SD)	N (% of group)	Mean Age (SD)
Ungrouped	Male	908	43.0 (16.5)	54	49.8 (15.1)	962 (30.2%)	43.4 (16.5)
	Female	2064	43.1 (17.1)	162	40.8 (15.3)	2,226 (69.8%)	42.9 (16.9)
Indigenous	Male	158	43.1 (16.4)	17	39.4 (16.5)	175 (26.2%)	42.7 (16.4)
	Female	421	39.5 (15.4)	73	38.7 (14.5)	494 (73.8%)	39.4 (15.2)
Non Indigenous	Male	3,556	55.1 (18.0)	218	54.7 (17.1)	3,774 (28.7%)	55.1 (17.9)
	Female	8,675	50.9 (18.1)$^{\alpha}$	692	48.6 (19.0)$^{\alpha}$	9,367 (71.3%)	50.8 (18.2)

6.3.3 dsDNA ANTIBODY LEVEL AND COUNTRY OF BIRTH

The highest frequency of dsDNA antibody positive individuals (12%) was recorded in the broad country of birth categories North Africa and the Middle East followed by the Americas (11%). Data are shown in Table 6-5.

TABLE 6-5 FREQUENCY OF INDIVIDUALS TESTED FOR dsDNA ANTIBODY ACCORDING TO COUNTRY OF BIRTH AND PERCENT WITH A POSITIVE dsDNA ANTIBODY.

		Positive dsDNA Antibody	
Country of Birth	N	N*	%
Australia	8756	639	7.3
S. & E. Europe	244	13	5.3
N.W. Europe	2806	170	6.1
S. & C. Asia	125	8	6.4
Not Available	3545	244	6.9
Oceania and Antarctica (excluding Australia)	389	28	7.2
N.E. Asia	176	14	8.0
Africa (Excluding N. Africa)	237	20	8.4
S.E. Asia	254	26	10.2
Americas	98	11	11.2
N. Africa & M.E	369	43	11.7
Total	16,999	1,216	7.2

*dsDNA antibody result above the reference value as described in Section 3.4

6.3.4 MULTIVARIATE LOGISTIC REGRESSION ANALYSIS

Gender, Indigenous Status, country of birth, and age at time of testing were analysed as independent variables in a multivariate LR to predict dsDNA antibody outcome without reference to separation between time of ANA and dsDNA testing. IRDS and ARIA variables were not included in these analyses as they could potentially change with time and the time delay between dsDNA antibody and ANA testing was variable. Each of the variables had a statistically significant effect on the prediction of dsDNA antibody outcome when considered in univariate analysis. In the multivariate LR model all variables remained significant; however increasing age was negatively associated. The OR and p values for each of the significant variables is shown in Table 6-6.

The country of birth category Americas was a statistically significant predictor of a positive ANA outcome (Section 5.3.9), however in the dsDNA antibody regression analysis it did not reach significance. In this analysis the two country of birth categories which significantly associated with a dsDNA antibody positive test were N. Africa &M.E. and S.E. Asia. Individuals with an Indigenous background also had an OR of 2.05 (95%CI 1.612 – 2.607) in the multivariate analysis indicating an increased risk of a positive dsDNA antibody outcome. Furthermore, the likelihood of a positive ANA increased with age; however this trend was reversed with the dsDNA antibody results and showed a marginally higher likelihood in younger patients (OR=0.996. 95%CI 0.992 - 0.999).

TABLE 6-6 SIGNIFICANT VARIABLES IN A MULTIVARIATE LR ANALYSIS OF 1ST DSDNA ANTIBODY RESULT ADJUSTED FOR SEX, AGE, COUNTRY OF BIRTH AND INDIGENOUS STATUS.

		Variables in LR Equation			
				95%CI for OR	
Variables	N 16,998	P value	OR	Lower	Upper
Age*		0.014	0.996	0.992	0.999
Gender§		<0.001	1.291	1.125	1.481
Country of birth					
N. Africa &Middle East	369	0.000	1.808	1.300	2.515
S.E. Asia	254	0.043	1.536	1.014	2.327
Indigenous Grouping					
Aboriginal or TSI	669	<0.001	2.050	1.613	2.608

§undefined gender case excluded, *age calculated at time of dsDNA antibody testing

6.3.5 ANA LEVEL AND PATTERN

The dsDNA antibody results described to this point have considered an individual's first dsDNA antibody result without reference to the ANA result; i.e. the separation in time between ANA and dsDNA antibody testing has not been considered. However, the separation between the two tests was calculated to be up to 10 years from time of collection of the ANA and dsDNA samples in 2,247 cases.

Analysis of the association between ANA and dsDNA antibody results was therefore restricted to data generated on the same sample or a sample collected on the same day (n=14,752 individuals). In this cohort 7.1% of samples were reported as dsDNA antibody positive (n= 1,044).

As mentioned previously, secondary testing for a dsDNA antibody request is recommended at PathWest on samples with an ANA ≥5IU/ml. To separate out the relationship between ANA and dsDNA antibody at the lower level of ANA an additional ANA group was included in the analysis to identify results within the range 5-<7IU/ml. The IIF pattern had not been assigned in this ANA group.

A comparison of incident ANA level and positive dsDNA antibody showed that 2.7% of samples with an ANA<5IU/ml had a positive dsDNA antibody, 5.0% of samples with an ANA level of >5 and <7IU/ml, 4.8% of ANA 7-10IU/ml, 6.9% of ANA>10-20IU/ml and 15.9% of ANA >20IU/ml were dsDNA antibody positive. The relationship between ANA level and dsDNA antibody is shown in Table 6-7.

The correlation between ANA pattern and dsDNA antibody result was also analysed in this cohort. Typically the homogeneous ANA pattern is associated with a dsDNA antibody positive result; in this study of incident ANA and dsDNA antibody data the ANA IIF patterns most frequently associated with a positive dsDNA antibody result were homogeneous (12.4%), homogeneous plus speckled (6.6%) and speckled (5.7%). Furthermore, as the ANA level increased the percent of samples reported with a homogeneous pattern and a dsDNA antibody positive result increased to 19.3% and 39.3% for ANA > 20 and >30IU/ml respectively. A similar trend was observed for the speckled (10.0% and 13.5%) and homogeneous plus speckled (8.6% and 24.0%) pattern for an ANA >20-30IU/ml and >30IU/ml respectively however in each case the frequency of a positive dsDNA antibody was lower compared to the homogeneous pattern.

In summary a detectable dsDNA antibody relates to both ANA level and pattern as shown in Table 6-7 and 6-8.

TABLE 6-7 FREQUENCY OF DSDNA ANTIBODY RELATED TO ANA GROUPED BY LEVEL (N =14,752).

ANA Level	**DSDNA Antibody Result**		
IU/ml	Negative (N)	Positive (N)	% Positive
0 - <5	2,085	57	2.7%
5 - <7	950	50	5.0%
7 - 10	5,403	273	4.8%
>10 - 20	2,898	214	6.9%
>20 - 30	1,849	283	13.3%
> 30	523	167	24.2%

TABLE 6-8 FREQUENCY OF DSDNA ANTIBODY RELATED TO ANA LEVEL AND HOMOGENEOUS, SPECKLED AND HOMOGENEOUS PLUS SPECKLED PATTERN.

	% Positive DSDNA antibody with an IIF pattern (N)		
ANA Group IU/ml	Homogeneous plus Speckled	Speckled	Homogeneous
0 - <5	#	#	#
5 - <7	#	#	#
7 - 10	5.6% (92)	4% (63)	5% (70)
>10 - 20	6.0% (41)	5% (25)	8% (105)
>20 - 30	8.6% (36)	10% (28)	19% (175)
> 30	24.0% (18)	14% (23)	39% (120)

a pattern is not reported for samples < 7IU/ml,

The relationship between ANA level and dsDNA antibody, age and gender was further investigated with the data shown in Table 6-9 and Figures 6.2-6.3. The proportion of dsDNA antibody positive results in all age groups was consistently higher in females; with

the greatest difference between the two groups seen in the >20-40 year age group; 5.7% (males) compared to 9.7% (females). The highest frequency of dsDNA antibody positive results was seen in the >40-60 year age group (6.0%) and >20-40 year age group (9.7%) for males and females respectively.

Eighty six percent (n=72) of dsDNA antibody tests with a result of >70IU/ml were associated with an ANA >20IU/ml, conversely 48% of the low level dsDNA antibody results (≤10IU/ml) were associated with the low level positive ANA (≤10IU/ml).

When these data was further stratified by ANA and dsDNA antibody level, low level positive dsDNA antibody (<10U/ml) was detected in up to 10.6% of individuals tested across all ages and in most ANA levels (10.6% in males with an ANA of >20–30IU/ml and age of >40–60 years) see Figure 6.2 and 6.3. The frequency in females was slightly higher; 12.0% of women in the age range >80 years with an ANA >30IU/ml had a dsDNA antibody level of <10 U/ml (see figure 6.3). Levels above 10U/ml were detected more frequently in the 0–40 year age group for both males and females and tended to be individuals with an ANA >20IU/ml. The highest levels of dsDNA antibody were most frequently reported in both males and females with an ANA >30IU/ml (Figures 6.4 and 6.5).

TABLE 6-9 FREQUENCY OF DSDNA ANTIBODY POSITIVE RESULT ACCORDING TO AGE AND GENDER.

	Individuals Tested (N)		**DSDNA Antibody positive N(%)**	
Age Grouping (years)	Males	Females	Males	Females
0 – 20	241	697	11 (4.6)	57 (8.2)
>20 – 40	908	3031	52 (5.7)	294 (9.7)
>40-60	1675	3934	100 (6.0)	259 (6.6)
>60–80	1275	2360	74 (5.8)	167 (6.7)
>80	190	441	10 (5.3)	30 (6.8)

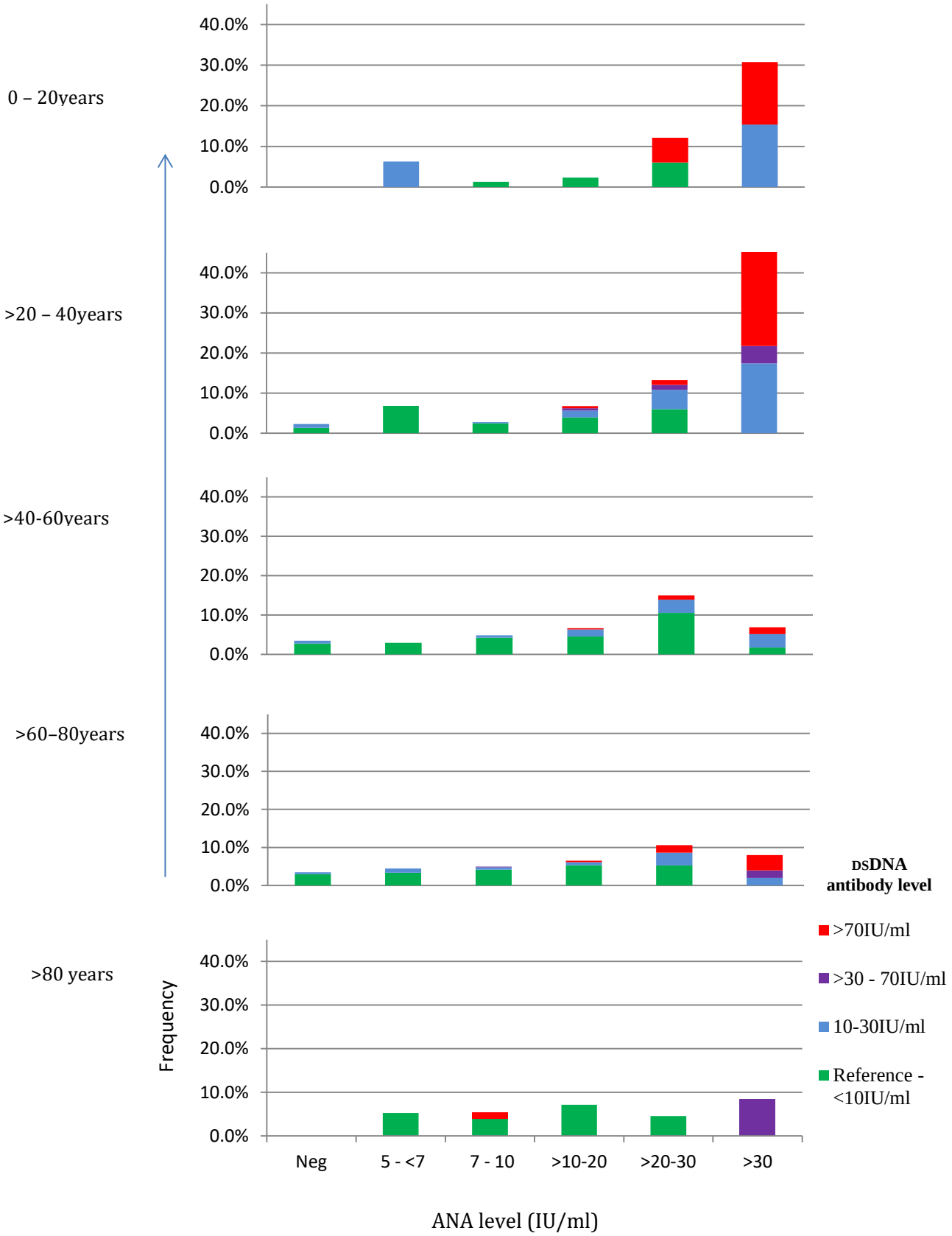


FIGURE 6.2 PERCENT FREQUENCY DISTRIBUTION OF DSDNA ANTIBODY IN MALES BY AGE AND ANA LEVEL.

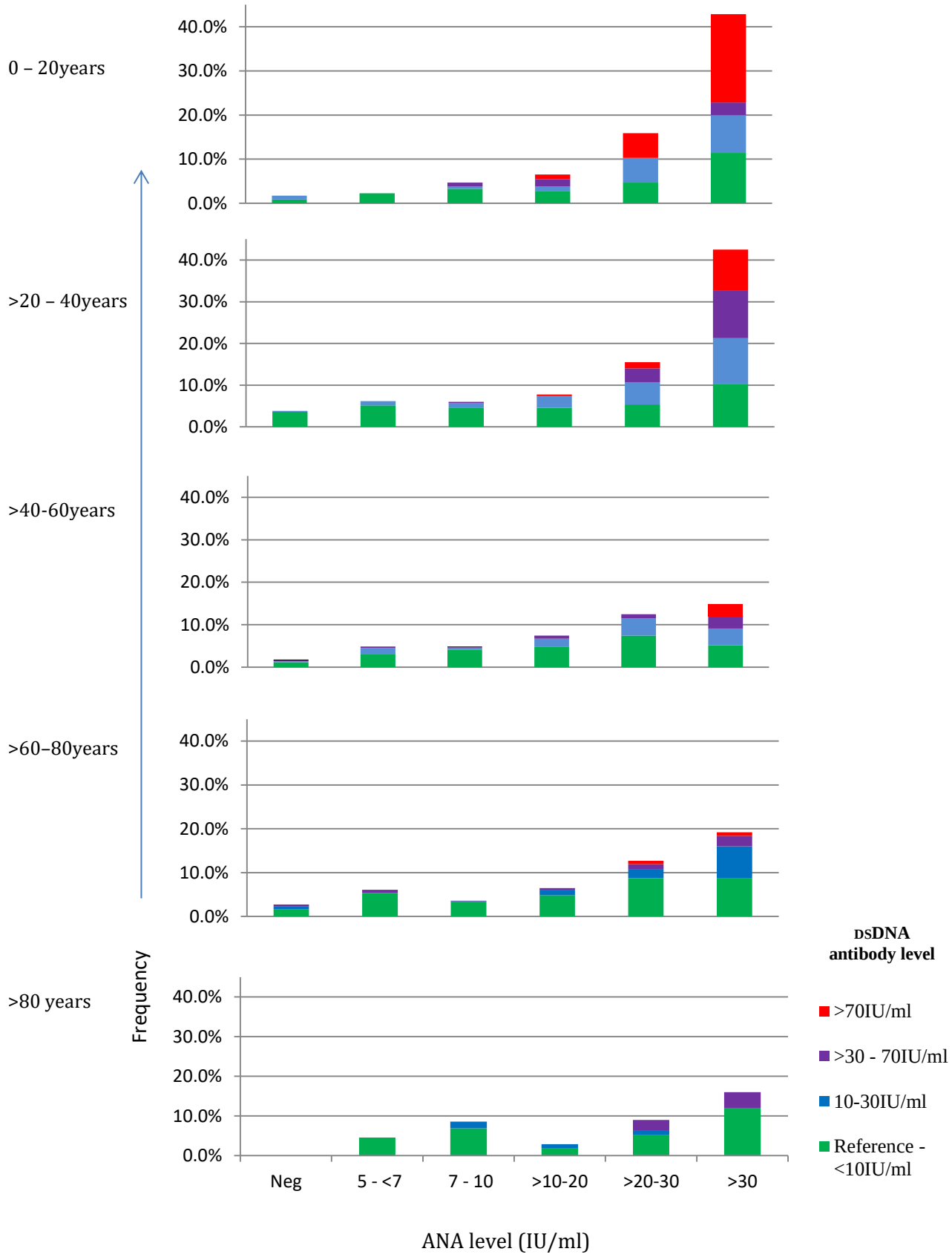


FIGURE 6.3 PERCENT FREQUENCY DISTRIBUTION OF DSDNA ANTIBODY IN FEMALES BY AGE AND ANA LEVEL.

6.3.6 MULTIVARIATE LOGISTIC REGRESSION ANALYSIS FOR DSDNA ANTIBODY OUTCOME

Multivariate LR analysis was also performed on the subset of the cohort where ANA and dsDNA antibody results were obtained on concurrent samples. This allowed additional variables ANA level, ARIA and IRDS to be included to assess their significance in the model to predict a positive dsDNA antibody result. The effect measures for each of the variables when included in the multivariate analysis are shown in Table 6-10.

The multivariate LR model with variables: age, gender, Indigenous Status, country of birth, IRDS, ARIA and ANA level showed the likelihood of developing a positive dsDNA antibody increased with an increased level of ANA ($p<0.001$) i.e. the OR increased from 2.056 (95%CI 1.393 – 3.035) to 12.099 (95%CI 8.794 – 16.647) with an increase in ANA from 5-<7IU/ml to >30IU/ml. Other variables predictive of a positive dsDNA antibody were being Indigenous ($p <0.001$, OR 2.301, 95%CI 1.697 – 3.119), being born in North Africa and Middle East ($p=0.001$, OR 1.853, 95%CI 1.288–2.666) and the quartile of the IRDS ranking.

As previously indicated the complete details for country of birth, IRDS, ARIA and Indigenous Status were not available on all individuals. Consequently multivariate LR analysis was performed on the subset of the cohort with complete details. This model showed that ANA level, Indigenous Status, and North African and Middle Eastern as a country of birth remained significant predictors of a positive dsDNA antibody result (Table 6-10 and 6-11).

Age was also a significant variable in both analyses (complete and incomplete variable data-sets), however the $OR_{Incomplete}$ of 0.995 (95%CI 0.991-0.998) and $OR_{Complete}$ of 0.993 (95%CI 0.989 – 0.998) suggest there was a marginally reduced likelihood (<1% reduction in risk/year of age) of a positive dsDNA antibody result with increasing age. Similarly ARIA classification “moderately accessible” was statistically significant ($p = 0.022$ and 0.047) in incomplete and complete data cohorts, however the ORs $OR_{Incomplete} = 0.753$ (95%CI 0.591 - 0.961) and $OR_{Complete} = 0.745$ (95%CI 0.558 - 0.996) also indicated a reduced likelihood of a dsDNA antibody positive outcome in individuals with a residential location considered to be moderately accessible.

Gender became a significant predictor in the complete analysis with a 20% increase in odds of a dsDNA antibody in females compared to males with an OR of 1.204 (95%CI 1.012 – 1.431; p = 0.036).

IRDS ranked in the 1st quartile of the Incident ANA Clinical cohort was no longer significantly associated with an increased likelihood of a positive dsDNA result when only individuals with complete details were analysed.

TABLE 6-10 MULTIVARIATE LR ANALYSIS OF DSDNA ANTIBODY OUTCOME ADJUSTED FOR SEX, AGE, COUNTRY OF BIRTH ARIA, IRDS AND INDIGENOUS STATUS FOR ALL INDIVIDUALS WITH INCOMPLETE AND COMPLETE INFORMATION AND A MATCHED INCIDENT ANA AND DSDNA ANTIBODY TEST.

	Incomplete Variables Included In the Equation					**Complete Variables In the Equation**				
	N	p Value	OR	95%CI for OR Lower	Upper	N	p Value	OR	95%CI for OR Lower	Upper
AGE (years)		0.004	0.995	0.991	0.998		.002	.993	.989	.998
GENDER										
Male (Reference)	4,289					3,274				
Female	10,463	0.054	1.161	0.997	1.351	8,142	0.036	1.204	1.012	1.431
INDIGENOUS STATUS										
Non Indigenous	11,221	<0.001				521				
Ungrouped	3,008	0.880	0.963	0.591	1.569					
Indigenous	523	0.000	2.301	1.697	3.119	10,895	<0.001	2.558	1.866	3.507
COUNTRY OF BIRTH										
AUSTRALIA (Reference)	7,415					7,398				
Oceania & Antarctica (excl. Australia)	332	0.606	1.122	0.726	1.734	332	0.642	1.109	0.717	1.713
N.W. Europe	2,406	0.817	1.024	0.838	1.25	2,402	0.806	1.025	0.839	1.254
S. & E. Europe	205	0.68	0.877	0.469	1.638	204	0.668	0.872	0.467	1.63
N. Africa & M.E	310	0.001	1.853	1.288	2.666	310	0.001	1.827	1.268	2.631
S.E. Asia	223	0.135	1.418	0.897	2.241	223	0.161	1.387	0.878	2.192
N.E. Asia	152	0.953	1.019	0.54	1.923	152	0.998	1.001	0.53	1.889
S. & C. Asia	110	0.868	0.939	0.446	1.976	110	0.852	0.932	0.442	1.962
Americas	88	0.077	1.809	0.938	3.488	88	0.086	1.778	0.922	3.428
Africa (Excluding N. Africa)	197	0.288	1.336	0.783	2.282	197	0.333	1.302	0.763	2.223
Not Available	3,314	0.807	1.061	0.661	1.701					

TABLE 6-10 CONTINUED MULTIVARIATE LR ANALYSIS OF DSDNA ANTIBODY OUTCOME ADJUSTED FOR SEX, AGE, COUNTRY OF BIRTH ARIA IRDS AND INDIGENOUS STATUS FOR ALL INDIVIDUALS WITH COMPLETE INFORMATION AND A MATCHED INCIDENT ANA AND DSDNA ANTIBODY TEST.

	Incomplete Variables Included In the Equation					Complete Variables In the Equation				
	N	p Value	OR	95%CI for OR Lower	Upper	N	p Value	OR	95%CI for OR Lower	Upper
ARIA										
Highly Accessible (Reference)	9,500					7,898				
Accessible	1,756	0.634	0.948	0.762	1.18	1,235	0.562	0.927	0.716	1.199
Moderately Accessible	1,521	0.022	0.753	0.591	0.961	1,022	0.047	0.745	0.557	0.996
Remote	415	0.865	1.035	0.696	1.54	288	0.415	0.812	0.492	1.34
Very Remote	1,550	0.31	0.883	0.695	1.122	973	0.063	0.75	0.554	1.016
Undefined	10	0.999	<0.001	<0.001	.					
IRDS										
Undefined	33	0.495	0.491	0.064	3.787	2,510	0.366	1.105	0.89	1.372
1st Quartile	3,358	0.015	1.270	1.048	1.539	2,835	0.982	0.998	0.805	1.237
2nd Quartile	3,696	0.543	1.063	0.874	1.292	2,836	0.496	0.93	0.754	1.147
3rd Quartile	3,651	0.522	1.064	0.880	1.285	3,235				
4th Quartile (Reference)	4,014									
ANA (IU/ml)										
0 - < 5 (Reference)	2,142					1,667				
5 - <7	1,000	<0.001.	2.056	1.393	3.035	798	0.004	1.878	1.223	2.884
7-10	5,676	<0.001	1.993	1.488	2.67	4,394	<0.001	1.92	1.397	2.637
>10 - 20	3,112	<0.001	2.865	2.123	3.867	2,366	<0.001	2.681	1.932	3.722
>20 - 30	2,161	<0.001	5.962	4.44	8.007	1,654	<0.001	5.152	3.729	7.118
> 30	661	<0.001	12.099	8.794	16.647	537	<0.001	11.25	7.931	15.958

6.3.7 MORTALITY ASSOCIATED WITH A DSDNA ANTIBODY

Deaths were reported for 1,697 (13.6%) individuals in the incident ANA and dsDNA antibody cohort. ICD-10 coded cause of death was assigned to 87.2% of cases (n=1,485). A negative incident dsDNA antibody result was reported in 1,536 (90.5%) of all deaths and 90.3% (n=1,347) of ICD-10 coded deaths. The highest frequency of all dsDNA antibody positive deaths were recorded in individuals with an ANA level of 7-10IU/ml (n=46) followed by an ANA of >20–30IU/ml (n=43). Ten percent of all dsDNA antibody positive deaths were recorded in individuals with an ANA level >20IU/ml and a dsDNA antibody level >30IU/ml (n=16/161). Data are shown in Table 6-11.

A comparison of death at each level of ANA showed a trend of increasing frequency of death with increasing level of ANA; however a similar comparison of the frequency of death at each level of dsDNA antibody did not show a similar trend. The highest proportion of deaths was recorded in the Reference value<10IU/ml.

TABLE 6-11 FREQUENCY OF DEATHS REPORTED IN INDIVIDUALS WITH A CONCURRENT ANA AND DSDNA ANTIBODY RESULT.

ANA Group IU/ml	dsDNA Antibody Level (IU/ml)					Total Deceased (n)	Total ANA/ dsDNA antibody (n)	% Death
	Negative	Reference <10	10-30	>30-70	>70			
0-<5	10.5%	10.3%	22.2%	0.0%	0.0%	193	1824	10.6%
5-<7	11.6%	14.3%	28.6%	50.0%	0.0%	104	874	11.9%
7-10	13.3%	19.8%	14.3%	11.1%	33.3%	654	4797	13.6%
>10-20	13.1%	23.0%	17.8%	9.1%	0.0%	357	2621	13.6%
>20-30	14.9%	25.6%	7.9%	10.0%	26.3%	278	1804	15.4%
>30	20.5%	21.4%	11.1%	11.5%	14.7%	111	578	19.2%
Total	13.2%	20.8%	13.3%	11.3%	17.5%	1697	12498	13.6%
Deceased (n)	1536	116	25	9	11	1697		
Total (n)	11,610	557	188	80	63	12,498		

Due to the small number of deaths in the dsDNA antibody groups >10IU/ml (n=45), dsDNA antibody levels were grouped as negative, reference value<10 and >10IU/ml. Cox regression analysis of death associated with dsDNA antibody (at 3 levels), age, gender and

Indigenous Status was performed. Age, an Indigenous background and a positive dsDNA result were associated with an increased risk of death. Data are shown in table 6.12.

TABLE 6-12 COX REGRESSION ANALYSIS OF SURVIVAL FOR THE INCIDENT ANA AND DSDNA ANTIBODY COHORT.

Variable	N	df	pValue.	OR	95%CI for OR Lower	Upper
AGE (years)		1	<0.001	1.080	1.076	1.084
GENDER						
Male (Reference)	3,602					
Female	8,896	1	<0.001	0.573	0.520	0.631
INDIGENOUS STATUS						
Non Aboriginal or TSI (Reference)	11,220					
Aboriginal or TSI	523	1	<0.001	2.823	2.272	3.508
Ungrouped	755	1	<0.001	0.092	0.035	0.246
DSDNA ANTIBODY						
Negative (<reference)	11,610	2	0.001			
Reference (Cut Off) - <10IU/ml	557	1	0.004	1.323	1.095	1.601
>10U/ml	331	1	0.014	1.448	1.076	1.949

6.3.8 DSDNA ANTIBODY AND CAUSE OF DEATH

The distribution of dsDNA positive and negative results for the most frequently reported cause of death is shown in Table 6-14, additional data are shown in Appendix Table 9-6. A positive incident ANA result (>30IU/ml) was recorded for 1.1% of cases in the cohort with an ICD-10 cause of death code.

The most frequently reported broad classifications for cause of death in the incident dsDNA antibody and ANA cohort were Diseases of the Circulatory system (28.6%) followed by Malignant Neoplasms (26.6%).

Within the ICD-10 category of Diseases of the Circulatory System three subgroups accounted for >90% of recorded cause of death:

i. Cerebrovascular disease (including stroke; I60 - 69)
ii. Ischemic Heart Disease (I20-25)
iii. And other forms of heart disease including atrial fibrillation and flutter and carditis (I30 – I52).

State wide cause of death data collected by the ABS over the period 2000 to 2010 indicate these subgroups also represented 89.1 % of this category of deaths in WA (149).

DsDNA antibody positive individuals accounted for 14.1% of the deaths in the ICD I30 – I52 group (Other forms of heart disease). Chi-square analysis of dsDNA antibody outcome and cause of death ICD Codes I30–I52 group showed a significant difference in the frequency of death in the dsDNA antibody negative and dsDNA antibody > Reference value outcome ($\chi^2 = 4.754$, $p < 0.029$) with a higher than expected number of deaths in dsDNA positive cases.

TABLE 6-13 RECORDED DEATHS IN THE INCIDENT ANA/DSDNA ANTIBODY COHORT GROUPED ACCORDING TO BROAD CATEGORY ICD-10 CODES.

Disease Group	**DSDNA Antibody**	
	Negative	Positive
	n (%)	n (%)
Ischemic Heart Disease (I20 – 25)	225 (93.8)	15 (6.3)
Cerebrovascular Disease (I60 – I69)	68 (93.2)	5 (6.8)
Other forms of heart disease (I30 – I52)	61(85.9)	10 (14.1)
All Deaths in ICD Category I	392 (92.2)	33 (7.8)
Diseases of liver (K70-K77)	51 (79.7)	13 (20.3)
All Deaths in ICD Category K	89 (83.2)	18 (16.8)
Systemic connective tissue disorders (M30-M36)	28 (77.8)	8 (22.2)
All Deaths in ICD-10 Category M	48 (82.8)	10 (17.2)
Total Deaths	1,347 (90.7)	138 (9.3)

Deaths from malignant neoplasms in individuals with an ANA and dsDNA antibody result totalled 395; however 92.2% were dsDNA antibody negative. Twenty six dsDNA antibody positive cases (83.9%) had a dsDNA antibody level of <10IU/ml. An incident dsDNA antibody level >70IU/ml was not reported in any of the deceased individuals in this group.

Liver disease as a cause of death also showed an increased incidence in the dsDNA antibody positive individuals compared to the overall rate of deaths with a positive dsDNA antibody (20.3% v 9.3%), however there was only one case of a positive for

dsDNA antibody >30IU/ml, the majority of dsDNA antibody positive (n=9 of 13) cases were <10IU/ml.

Deaths in the Musculoskeletal and Systemic Connective Tissue disorders ICD-10 AM M30-M36 also had a higher percent of positive dsDNA antibody results (22.2%) compared to the overall positive dsDNA death rate (9.3%). Data are shown in Table 6-13.

6.3.9 KAPLAN MEIER ANALYSIS OF DSDNA ANTIBODY OUTCOME AND SURVIVAL

Kaplan Meier survival analysis compared dsDNA antibody outcome (by level) across gender and two age groups <50 and ≥50 years. The ratios of males to females in the two age groups were 1:2.4 and 1:1.3 respectively. The number of censored events differed across the dsDNA levels however it was similar between the males and females. The survival curves are shown in Figure 6.4.

A log rank test showed a significant difference in survival outcome for the different levels of dsDNA antibody when the data were adjusted for gender in the <50 year age group (χ^2= 14.13, p=0.007). This difference was not seen for the ≥50 years age group (χ^2=9.019, p=0.061). A pairwise comparison using the log rank test showed a statistically difference between the negative dsDNA antibody and the low level positive <10IU/ml in both age groups (< 50 years χ^2 =11.224, p=0.001 and ≥50 years χ^2=5.338, p=0.021).

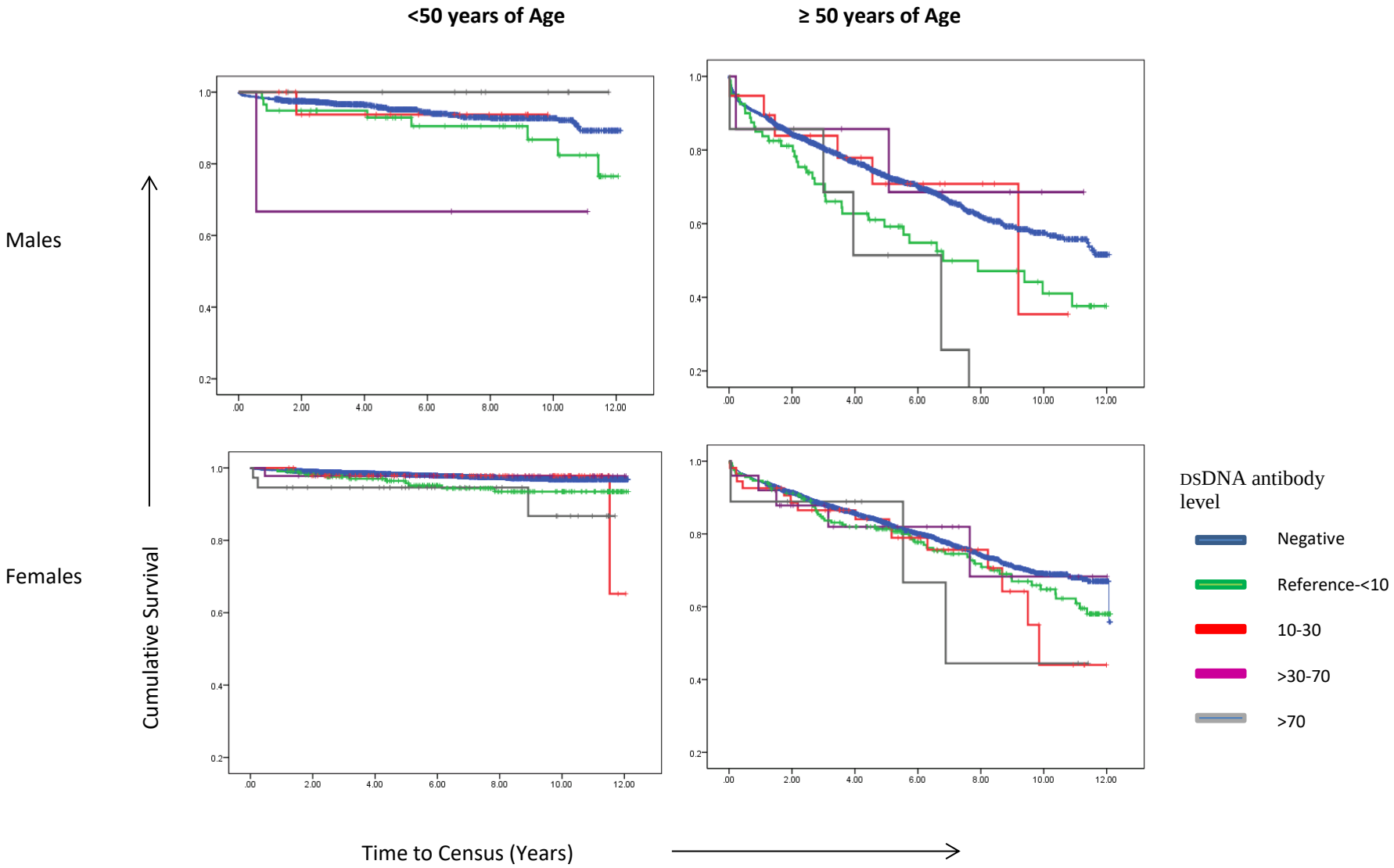


FIGURE 6.4 KAPLAN MEIER SURVIVAL CURVES FOR DSDNA ANTIBODY LEVELS GROUPED BY GENDER AND AGE.

6.4 DISCUSSION

Eleven classification criteria for Lupus are included in the 1982 American College of Rheumatology (ACR) criteria including "antibody to native DNA in abnormal titre". In 2012 the Systemic Lupus International Collaborating Clinics group revised the criteria to "anti-dsDNA antibody level above the laboratory reference range" (150). However, transient dsDNA antibodies have also been described in some cases of infection (151, 152). Age and gender along with a positive dsDNA result are important factors that are taken into account in the diagnosis of Lupus. Antibodies to dsDNA are highly specific for SLE which predominantly affects females (120, 143). The demographics of a Brazilian study of Lupus patients showed the mean age of their Lupus cohort was 29 ± 9.5 years, with a female to male ratio reported in the study of 11.3:1; approximately one third of the Lupus patients had a positive dsDNA antibody(153).

The mean age of the first reported dsDNA antibody result in the clinical cohort described in this study was 46.5 years with a female to male ratio of 3.2:1. The LR analysis identify age as a significant predictor of a dsDNA antibody (OR = 0.996, 95%CI 0.991–0.998) however it suggested that the risk of a positive dsDNA antibody was mildly reduced with increasing age.

Age specific incidence rates showed that a high positive ANA and dsDNA antibody result is more likely to be seen in a male or female under 40 years of age and that low level dsDNA antibody is detected in up to 10% of individuals tested (Figures 6.2 – 6.3). While fewer males are tested for dsDNA antibody the analysis showed an increased proportion of males compared to females with a high positive dsDNA antibody (>30U/ml) and ANA>30IU/ml result in the 20–40 year age groupings. (n=7/23 (30.4%) vs n=37/173 (21.4%)) respectively. Similarly Ferreira *at al* (2005) observed a negative association between dsDNA antibody level and age (154), although their observations were based on a limited number of male participants.

The frequency of dsDNA antibody positive outcomes increased with increasing ANA, the OR shifting from 1 to 11.6 with an increase in ANA from 5-<7IU/ml to >30IU/ml. There was an increase in frequency of a positive dsDNA with increased ANA, particularly in individuals with a homogeneous pattern. Pattern as an additional predictor remains to be

included in a multiple LR analysis. The additional analysis could potentially strengthen the predictive value of ANA in the determination of dsDNA antibodies; however this remains an aspect for further studies.

Gender was statistically significant as an independent variable associated with a positive dsDNA antibody, however in the multivariate analysis of all individuals with matching ANA and dsDNA antibody data adjusted for gender, country of birth, ANA, IRDS and ARIA gender was not a significant predictor of a dsDNA antibody positive result. In a subset analysis of the cohort with only complete variable information for country of birth, ARIA and IRDS, gender reached significance identifying a higher risk of a positive anti DNA antibody in females (OR of 1.2 and $p<0.001$).

Country of Birth analysis identified the broad Country of Birth grouping of N. Africa and Middle East as a significant variable in the determination of a positive dsDNA antibody result. This finding is consistent with the reported increased risk of developing Lupus in individuals of African descent (139, 143). Similarly an increase incidence of Lupus reported in the Australian Indigenous population is consistent with an OR of 2.3 indicating an increased risk of a positive dsDNA antibody level in the Indigenous population.

ANA <5IU/ml dsDNA antibody positive individuals accounted for 5.5% of the positive results. Clinically relevant ANA are generally IgG antibodies which are detected by both the ANA IIF method and the FARR method (8). The ANA testing described in this study uses an anti-human IgG (heavy and light chain) detection antibody which has some cross reactivity between the FC portion of the IgG, A and M isotypes. As previously indicated the FARR method will precipitate IgG, A and M antibodies directed against dsDNA, a negative ANA does not exclude the presence of dsDNA antibodies (96) rather it suggests that it may be IgM or IgA (8) or a low affinity antibody. IgM antibodies to dsDNA are more prevalent in the early stages of SLE (155) although they have been shown to persist in 30% of cases and are negatively associated with lupus nephritis.

DsDNA antibody has been detected prior to the onset of clinical features of SLE (19), and antibody levels often correlate with disease (144); importantly this analysis has been restricted to the first dsDNA and ANA test. Morbidity associated with ANA testing is investigated in Chapter 8, however dsDNA antibody outcomes are not considered in that analysis.

The most frequently reported broad cause of death in the incident dsDNA antibody and ANA cohort was diseases of the circulatory system (28.6%) followed by malignant neoplasms (26.6%). These cause of death data groups also ranked 1st and 2nd in the WA cause of death statistics for the period 2001 to 2010 (32.4 and 30.4% respectively) (156). Similar frequencies for Ischemic Heart disease and Cerebrovascular disease in the WA population and incident ANA/dsDNA antibody Clinical Cohort described here suggest ANA/dsDNA antibodies do not lead to an increased mortality in these disease groups.

A comparison of dsDNA antibody positive and negative outcomes associated with mortality data showed an increased frequency of dsDNA positive cases in the Musculoskeletal and Connective Tissue and Liver disease groups. Survival rates differed significantly for increasing levels of dsDNA antibody in the under 50 year age group however this did not apply to the ≥50 year age group.

In conclusion a positive dsDNA antibody outcome relates to both ANA level and pattern, with the highest frequency of a positive dsDNA antibody outcome recorded in individuals with an ANA >30IU/ml with a homogeneous pattern. The frequency of high level dsDNA antibody is highest in the >20-40 age group in females. High level ANA and dsDNA individuals are associated with younger males and females (0-40 years). Age, Indigenous status and dsDNA antibody level are significant predictors of survival in the incident ANA and dsDNA antibody cohort. ANA at high level are associated with an increased risk of morbidity.

7 ENA ANTIBODY DETECTION

7.1 BACKGROUND

The characterisation of ANA detected by IIF is important in the diagnosis and management of patients with autoimmune diseases. In routine practice samples submitted for ENA antibody detection were screened with a pool of antigens with known rheumatic disease association with (Ro60, Ro52, La, Scl70, Sm, RNP, Jo1, ribosomal P centromere B). Samples positive in the screening assay were then tested against a panel of individual antigens to identify the ENA antibody.

Characterisation by immunodiffusion and CIEP were the methods initially used in diagnostic testing (including this study: see Section 2.10.3), however these methods were labour intensive and required experienced personnel to interpret the precipitation bands which indicate a likely ENA antibody.

In 2004 an immunoblot method was adopted, this allowed a number of autoantibodies to be measured in a semi-quantitative manner on a single substrate; interpretation could be automated and assessed by quantifying the intensity of the reactive band relative to a reference band on a control sample. It also removed the subjectivity that was inherent in interpreting the results of the former methods. The use of purified or recombinant antigens rather than cell lysates also ensured a higher degree of uniformity in the antigen preparations.

An important consideration for the autoantibody characterisation method is that a detectable band by CIEP was considered a clinically significant level of antibody. Evolving technologies are more sensitive in the detection of the autoantibodies and increased frequency of detection may reflect background levels in the general population (6).

In addition, the number of clinically relevant ENA autoantibody targets is increasing and newer ENA antibodies have been incorporated into the screening and detection methods over time. In this study the main change over time for the screening method was the inclusion of Ro52 antigen in the screening ELISA. Detection of autoantibodies targeting CenpB, ribosomal P, histones and Ro52 were included in the characterisation profile with the introduction of the immunoblot detection method.

The aim of this study was to investigate the predictive value of ANA pattern and level for clinically relevant systemic autoantibodies.

7.2 METHODS

The merging of incident ANA and ENA screening and detection data as described in Section 3.5 resulted in combined data for 42,761 cases, which represented 34,912 individuals.

ENA antibody screening results were reported qualitatively as positive or negative for each of the methods reported over the 11 year observation period as described in Section 3.5.1. The majority of individuals had a single screening test for ENA antibodies (85.9%) with <5 individuals having the maximum number of 23tests.

CIEP and ELISA screening overlapped for a 4 year period (2006-2010), during which time samples were generally only tested by CIEP if they were positive on the immunoblot ENA antibody detection assay. The frequency of a positive result was therefore calculated for the CIEP method up to the time of the introduction of the ELISA screening method.

Samples identified by the screening test as likely ENA antibody positive were further investigated to identify the autoantibody target antigen. Three characterisation methods were adopted over the 11 year period of the study. The immunoblot method was in use for the longest period (2002 – 2010). Results entered into the LIS as text comments rather than positive, negative or equivocal were not included in the analysis of ENA detection by immunoblot (n = 65).

Results were routinely reported qualitatively as: positive, negative, detected, not detected or equivocal for each ENA antibody included. The equivocal result recommended by the immunoblot assay manufacturer accounts for background levels of autoantibodies present in the normal individual. The level of antibody detected to be considered positive is set to achieve a 98% specificity for the assay (88). Immunoblot testing was carried out on 5,368 individuals with repeat testing carried out on 248 patients (4.6%).

ENA detection by immunoblot included the most comprehensive range of ENA antibodies and was therefore used to determine the frequency of the various ENA antibodies. It was

also used for the comparison of ANA level and pattern on samples with ANA and ENA antibody testing on concurrent samples.

Results at the time of the first test for both ANA and concurrent ENA antibody assays were available for 4,216 individuals.

7.2.1.1 STATISTICAL ANALYSES

Descriptive statistics were determined for the ENA antibody patient cohort. The frequency of the screening outcome and ENA characterisation were determined.

Multivariate LR analysis to predict a positive ENA antibody outcome was performed on the subset of data where ANA and immunoblot ENA antibody detection results were obtained on concurrent samples. The multivariate LR analysis was adjusted for: gender, age, Indigenous Status, ANA level, ARIA, IRDS, and country of birth. The reference category for each variable was: male, Non Indigenous, ANA<7IU/ml, highly accessible, the 4th quartile of the IRDS and Australia.

Significant differences in survival rates for ANA, ENA and cytoplasmic antibody positive and negative outcomes were determined by the log rank test and/or Cox regression analysis. Survival rates were calculated from the time of collection of the incident ANA to the time of death or the censor date of 20th February 2012.

7.3 RESULTS

The frequencies and mean age at the time of first ENA screening assay for individuals grouped by gender or Indigenous Status are shown in Table 7-1. The higher proportion of females compared to males screened for an ENA antibody (M:F ratio = 1:1.76) is consistent with the higher number of females tested for ANA (M:F ratio = 1:1.47).

TABLE 7-1 GENDER, INDIGENOUS STATUS AND MEAN AGE OF INDIVIDUALS SCREENED FOR ENA ANTIBODIES.

	N (% of Total)	Mean Age (SD)
Gender		
Male	12,625 (36.3)	51.3 (17.8)
Female	22,196 (63.7)	48.6(18.0)
Total	34,829	49.6(18.0)
Indigenous Status		
Ungrouped	6,351 (18.2)	42.9 (16.7)
Indigenous	1,326 (3.8)	40.1 (15.4)
Non Indigenous	27,152 (78.0)	51.6 (17.9)

7.3.1 SCREENING METHODS

As described in Section 3.5.1, three ENA antibody screening methods were adopted over the duration of the study period. ENA screening by immunodiffusion identified a smaller proportion of samples that were potentially ENA antibody positive (7.2%) compared to 16.7% and 16.5% for the ELISA and CIEP methods respectively.

A comparison of ENA screening result for the 1st test compared to a follow-up (2nd) test for an individual showed a higher frequency of a positive result in the second test (data shown in Table 7-2) and likely represents repeat testing of the initial positive test result for confirmation.

TABLE 7-2 PERCENT OF SAMPLES WITH A POSITVE ENA ANTIBODY SCREENING RESULT ASCERTAINED BY ANY OF THE 3 METHODS IMPLEMENTED OVER THE 11 YEAR PERIOD.

Screening Method	All Tests		First Test		Second Test	
	n	% Positive	n	% Positive	n	% Positive
CIEP	20,742	16.5	16,962	14.3	2,342	23.0
ELISA	20,953	16.7	17,028	14.5	2,539	21.6
IMMUNODIFFUSION	893	7.2	839	6.3	46	15.2

7.3.2 DETECTION METHODS

SSA (SSA/Ro60) was detected in 3.5–33.6% of samples sent for ENA antibody characterisation depending on the method of detection at the time (Table 7-3). SSA/Ro52 was the most frequently detected ENA antibody (15.4%) by immunoblot. The frequencies of incident ENA antibodies detected by each characterisation method are shown in Table 7-3.

Sixty eight percent of first time characterisation results by immunoblot were negative for all ENA antibodies included in the characterisation. Detection of a single ENA antibody was reported in 60.0% of incident ENA antibody results detected by immunoblot. In these individuals isolated SSA/Ro52 was the most frequently detected ENA antibody (27.7%) followed by SSA/Ro60 (11.3%) and histones (16.8 %) in these cases. Data are shown in Table 7-4.

TABLE 7-3 FREQUENCY OF INCIDENT ENA ANTIBODIES DETECTED BY EACH CHARACTERISATION METHOD.

	METHOD OF DETECTION		
ENA Antibody	**CIEP** n= 622	**Immunodiffusion** n = 847	**Immunoblot** n = **5,209**
SSA/Ro52			15.4%
SSA*	33.6%	3.5%	11.4%
SSB/La	6.3%	0.6%	7.4%
Sm	6.4%	0.2%	2.9%
Scl70	0.2%	0.0%	2.8%
RNP	11.4%	0.7%	2.4%
Ribosomal P	-	-	0.8%
CenpB	-	-	2.8%
Jo-1	0.6%	0.0%	1.4%
Histones	-	-	6.3%

*Specifically designated Ro60 on the immunblot

TABLE 7-4 RELATIVE FREQUENCY OF SINGLE (LONE) ENA ANTIBODIES DETECTED BY IMMUNOBLOT.

ENA antibody	N	% of Single positive Individuals
SSA/Ro52	278	27.7%
SSA/Ro60	113	11.3%
SSB/La	59	5.9%
SmD	77	7.7%
Scl70	108	10.8%
RNP	52	5.2%
Ribosomal P	8	0.8%
CenpB	83	8.3%
Jo1	56	5.6%
Histones	169	16.8%
Total	1003	100.00%

7.3.3 MULTIPLE ENA ANTIBODIES DETECTED

There was a reverse trend in the number of ENA antibody detected at the time of the first test versus age with fewer ENA antibody detected with an increase in age as shown in Figure 7.1 and Table 7-5. Seven ENA antibodies were detected in <5 individuals all of which were <20 years of age. The autoantibodies detected in these individuals were: SSA/Ro52, SSA/Ro60, SSB/La/ RNP, SmD, histones and either ribosomal P or CenpB.

A subset analysis of the ENA antibodies detected by immunoblot showed the most frequent combinations of ENA antibodies detected were:

i. SSA/Ro52+SSARo60+SSB/La 218/668 (32.6%)
ii. SSA/Ro52+SSARo60 134/668 (20.1%)
iii. SSA/Ro60+SSB/La 22/666 (3.3%)
iv. CenpB and histones 21/668 (3.1%)

Other combinations were detected in fewer than 3% of individuals with multiple autoantibody specificities.

TABLE 7-5 FREQUENCY OF NUMBER OF ENA ANTIBODIES RELATED TO AGE.

ENA Autoantibodies detected (First ENA Test)	Count	AGE (Years)			
		Mean	Standard Deviation	Minimum	Maximum
0	3538	51.3	17.9	1.0	94.0
1	1003	53.5	17.7	5.0	98.0
2	320	52.4	17.3	14.0	86.0
3	281	51.5	17.2	15.0	87.0
4	48	42.5	17.6	16.0	78.0
5	9	38.7	21.1	16.0	81.0
6	8	28.6	12.1	15.0	51.0
7	<5	13.0	5.7	9.0	17.0
All	5209	51.7	17.8	1.0	98.0

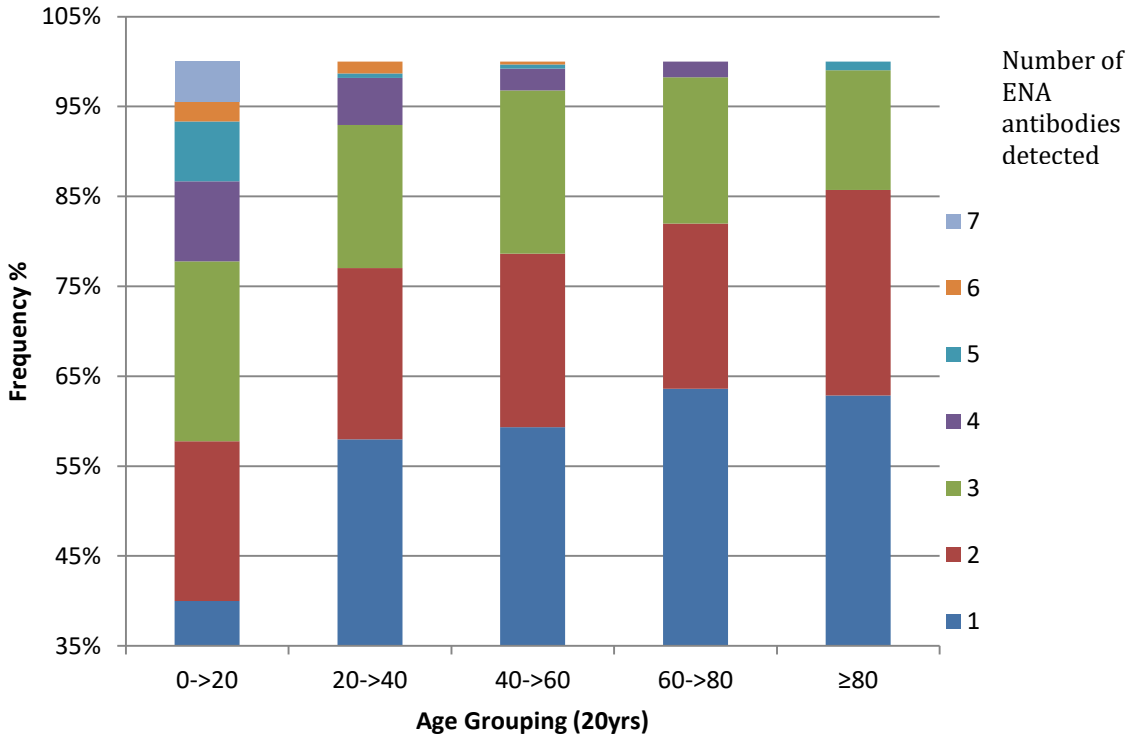


FIGURE 7.1 PROPORTIONS OF MULTIPLE ENA ANTIBODIES RELATED TO AGE.

7.3.4 CHANGE IN ENA ANTIBODY PROFILE

Repeat ENA antibody detection was performed on 484 individuals, a change in ENA antibody profile was noted in 180 (37.2%) of these individuals, however as method changes were implemented over the duration of the study, these changes in profile would

have included testing across the three ENA testing methods which included the addition of ENA antibody such as Ro52 and histone antibodies.

A comparison of the number of ENA antibodies detected by immunoblot on the 1st and 2nd test showed a change in 22% of individuals (n=55), with a gain or loss of a single ENA antibody in 43 cases (78.2%). The maximum change of 5 ENA antibodies was seen in <5 individuals under the age of 20 years at the time of the first report; the gap between ENA antibody reports was 196 days. The shortest time difference between two results was a single day, with a change in Jo-1 antibody from "equivocal" to "detected". As ENA antibody results were reported as "not detected", "equivocal' or "detected" it is not possible to comment on the level of positivity of the antibody and whether the change was small and close to the reference value for the ENA antibody or a substantial change in level.

TABLE 7-6 CHANGE IN THE NUMBER OF ENA ANTIBODIES DETECTED ON REPEAT TESTING BY IMMUNOBLOT.

		Number of ENA Antibodies Detected in the 2nd Test								
		0	**1**	**2**	**3**	**4**	**5**	**6**	**7**	Total
Number of ENA Antibodies detected in 1st Test	**0**	99	6	1	0	0	0	0	0	106
	1	7	55	3	1	0	0	0	0	66
	2	0	8	22	5	0	0	0	0	35
	3	1	5	3	14	4	1	0	0	28
	4	0	0	1	4	3	1	0	0	9
	5	1	0	0	0	0	0	0	0	1
	6	0	0	0	0	1	1	0	0	2
	7	0	0	0	0	0	0	1	0	1
	Total	108	74	30	24	8	3	1	0	248

7.3.5 ANA AND ENA ANTIBODIES

Concurrent ANA and ENA antibody detection by immunoblot results at the time of the first test for both assays (n = 4,216) were analysed to determine the frequency of ENA detected at each ANA level.

The frequency of Sm, RNP and histone autoantibodies increased with increasing level of ANA (data shown in Figure 7.2). In the subset analysis of immunoblot characterisation 46.9% of SSA/Ro52 and 47.9% of SSA/Ro60 antibody positive samples were detected in individuals with an ANA intensity <7IU/ml.

The cytoplasmic IIF pattern is commonly associated with Jo-1 antibodies, however of the Jo-1 antibody positive individuals identified in this study only 12.2% were reported with a cytoplasmic pattern and 75.7% of results were reported as ANA negative.

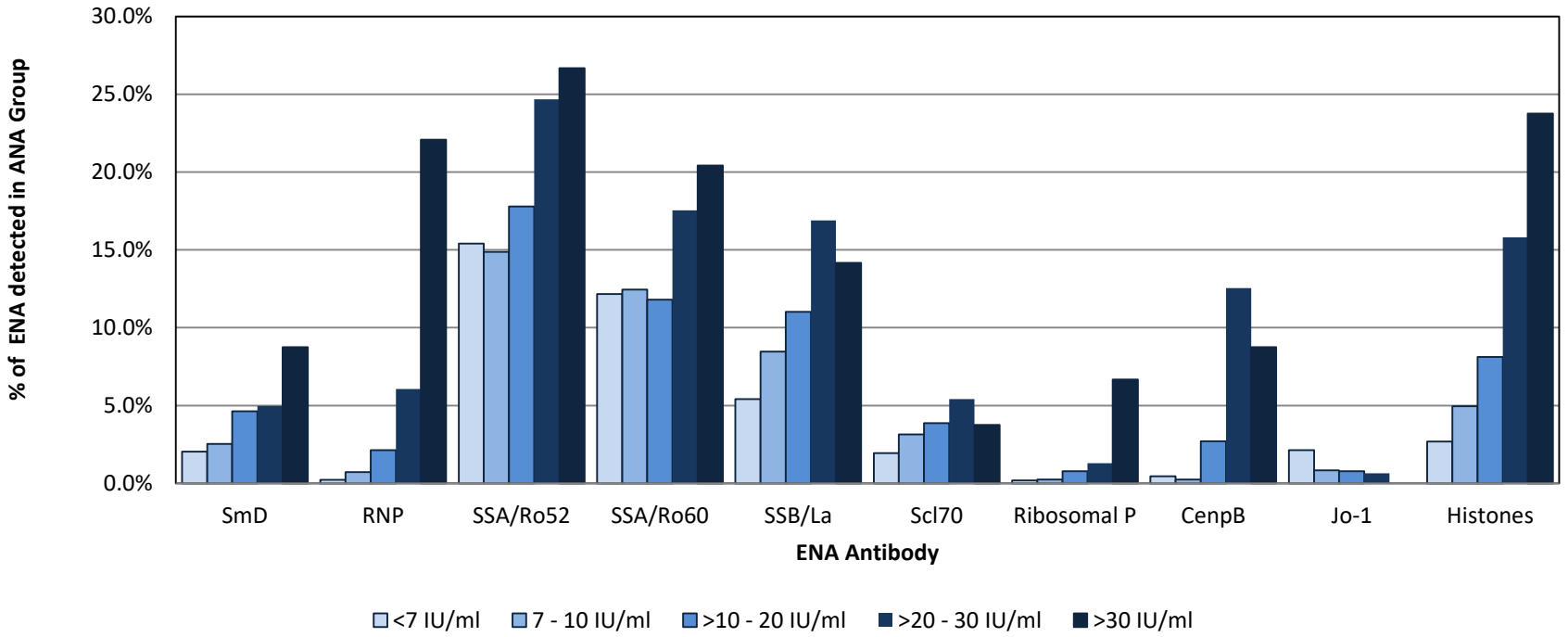


FIGURE 7.2 PERCENT OF SAMPLES WITH AN ENA ANTIBODY DETECTED BY IMMUNOBLOT AT EACH LEVEL OF ANA (N=4,208).

7.3.6 SSA ANTIBODY DETECTION

Samples are screened for SSA/Ro60 antibody by IIF on the Hep2000 cells which are over expressing SSA/Ro60 transfected cells. The characteristic nuclear staining pattern is predominantly nucleolar which is seen in 10–20% of the interphase cells. Fine speckled staining may also be visualised in the remaining 80-90% of cells. SSA antibody detection was included in each of the 3 characterisation methods used in this study, however the antigen used to detect the SSA/Ro was the recombinant SSA/Ro60 rather than the soluble extracted antigen in the cell lysate prepared for the CIEP and Immunodiffusion antigen preparations (157).

TABLE 7-7 CONCORDANCE OF DETECTION OF SSA/RO60 ANTIBODY DETECED BY IIF **A.** CIEP, **B.** IMMUNODIFFUSION, AND **C.** IMMUNOBLOT.

DETECTION MEHTOD

A. CIEP

IIF Screen	NEG	POS	TOTAL		
NEG	232	22	254	Sensitivity	88.5%
POS	115	170	285	Specificity	66.9%
TOTAL	347	192	539	Concordance	74.6%

B. IMMUNODIFFUSION

IIF Screen	NEG	POS	TOTAL		
NEG	680	6	686	Sensitivity	75.0%
POS	76	18	94	Specificity	89.9%
TOTAL	756	24	780	Concordance	89.5%

C. IMMUNOBLOT

IIF Screen	NEG	POS	TOTAL		
NEG	1989	19	2008	Sensitivity	96.6%
POS	1638	538	2176	Specificity	54.8%
TOTAL	3627	557	4184	Concordance	60.4%

A comparison of Ro60 antibody detected by the screening IIF using a cell line transfected with Ro60 and the characterisation method used at the time shows the concordance of the IIF and characterisation method was variable across the methodologies. The sensitivity of the Hep2000 cell as a substrate for detecting Ro60 ranged from 75.0 – 96.6% depending on the characterisation method used however the specificity ranged from 54.8 – 88.9% (Table 7-7).

7.3.7 ANA PATTERN AND ENA ANTIBODY

The ANA pattern observed by IIF can be an indication of the location of the target antigen for the ENA antibody. The correlation between pattern and ENA antibody in this study was investigated for samples with a concurrent ANA and ENA antibody result detected by immunoblot (Figure 7.3 and 7.4). Pattern association will be confounded in samples in which multiple ENA antibodies were detected (i.e. 36% of the individuals assessed for ENA antibody by immunoblot in this study).

The speckled pattern was associated with the highest percent of RNP, SSB/La, Ro52 and Ro60 antibody positive samples (62.1, 28.7, 21.2 and 20.8% respectively). SmD, Scl70, histone and ribosomal antibody positive samples were detected more frequently in samples reported with a homogeneous pattern (21.1, 15.6, 35.1 and 46.8% respectively). Scl70 antibody positive individuals had an ANA pattern associated with a nucleolar pattern reported with a second pattern such as homogeneous or speckled in 27.0% of cases (data shown in Table 7-8 and Figure 7.4).

Increasing ANA level in conjunction with a speckled or speckled plus homogeneous pattern was predictive of an RNP or SmD antibody. Similarly the frequency of a histones positive antibody rose with increased ANA level as shown in Figure 7.4. The associations between ANA level and ENA antibody were investigated further in the multivariate LR analysis described in Section 7.3.10.

TABLE 7-8 CORRELATION OF EACH ENA ANTIBODY (DETECTED BY IMMUNOBLOT) TO ANA PATTERN.

Antibody (N)	**ANA Pattern Reported (%)**							
	Speckled alone	**Homogeneous**	**Homogeneous plus speckled**	**Nucleolar**	**Nucleolar mixed***	**Centromere**	**Cytoplasmic**	**Anti SSA**
Sm (133)	16.5%	21.1%	16.5%	4.5%	9.0%	2.3%	8.3%	9.8%
RNP (103)	62.1%	13.6%	17.5%	1.0%	1.9%	0.0%	2.9%	4.9%
SSA/Ro52 (726)	21.2%	10.7%	9.2%	3.0%	10.3%	2.2%	12.8%	43.3%
SSA/Ro60 (557)	20.8%	9.3%	9.9%	2.0%	11.3%	1.3%	3.9%	67.5%
SSB/La (356)	28.7%	9.0%	9.3%	2.5%	18.5%	1.4%	4.2%	48.9%
Scl70 (122)	11.5%	15.6%	9.0%	5.7%	27.0%	1.6%	11.5%	4.1%
RIBOSOMAL P (32)	15.6%	46.9%	18.8%	0.0%	6.3%	0.0%	6.3%	18.8%
CenpB (105)	2.9%	3.8%	4.8%	1.0%	3.8%	74.3%	15.2%	5.7%
Jo-1 (60)	13.3%	5.0%	1.7%	1.7%	1.7%	0.0%	11.7%	5.0%
HISTONES (271)	8.9%	35.1%	19.2%	4.4%	6.6%	7.4%	9.2%	11.1%

*Nucleolar mixed includes patients with a reported pattern of Nucleolar including those with a homogeneous, speckled pattern etc.

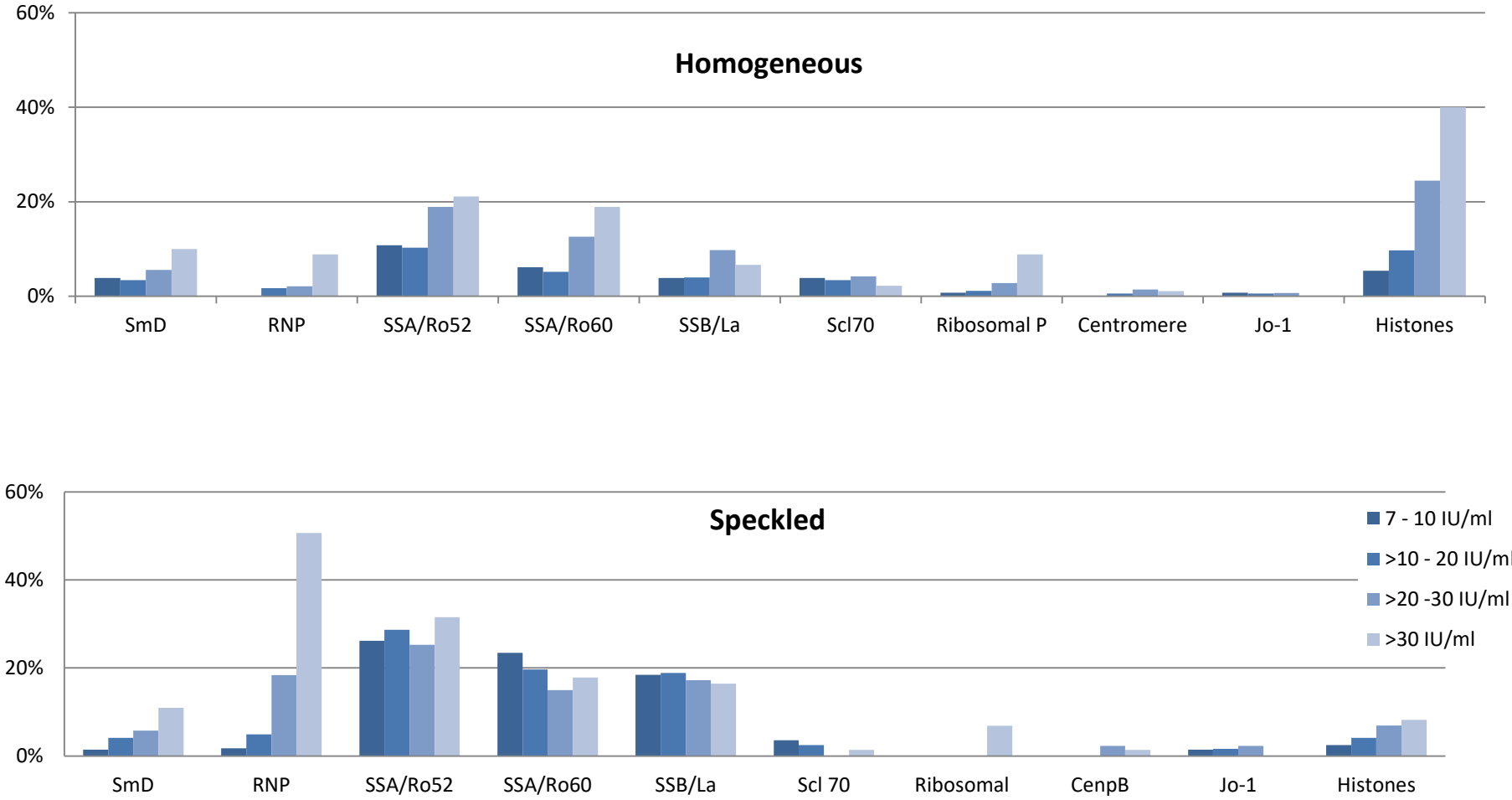


FIGURE 7.3 FREQUENCY OF ENA ANTIBODY (DETECTED BY IMMUNOBLOT ONLY) ACCORDING TO ANA PATTERN (HOMOGENEOUS ALONE AND SPECKLED ALONE) AND TITRE.

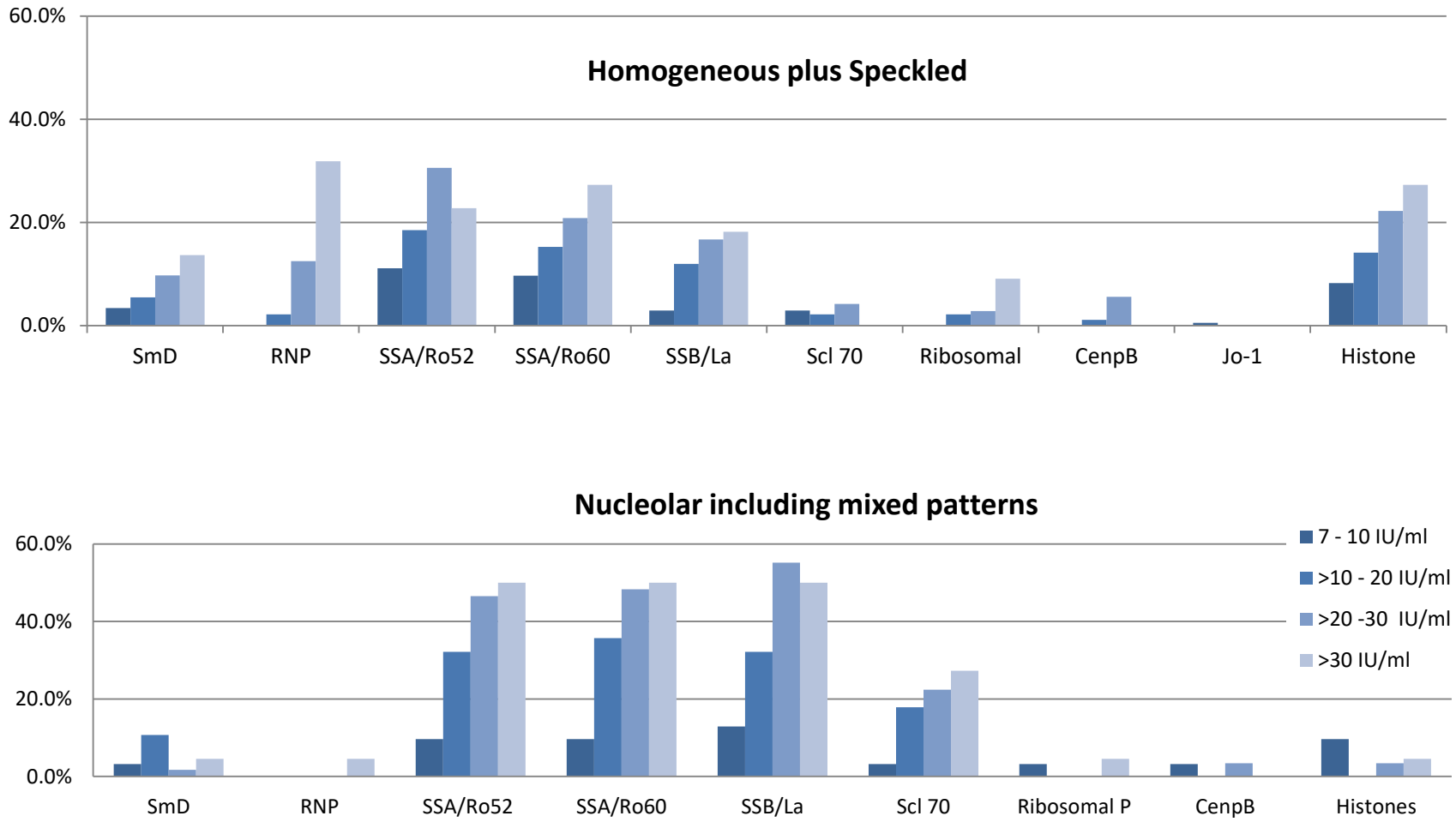


FIGURE 7.4 FREQUENCY OF ENA ANTIBODY (DETECTED BY IMMUNOBLOT ONLY) ACCORDING TO ANA PATTERN (HOMOGENEOUS PLUS SPECKLED AND NUCLEOLAR INCLUDING MIXED PATTERNS) AND TITRE.

7.3.8 LOGISTIC REGRESSION ANALYSIS OF ENA ANTIBODY OUTCOME

ENA antibody characterisation analysis was limited to the immunoblot method as antigen preparation across the methods was not consistent (highly purified, recombinant or crude cell lysate extract) and may have influenced the sensitivity of the assay methods in the detection of ENA antibody. Data were available for up to 4,216 individuals who had a result on their first ANA sample (partial characterisation data were reported for up to 3 individuals).

The multivariate LR analysis showed that Indigenous Status was only predictive of a positive ribosomal antibody. This finding was limited to the multivariate analysis of the dataset in which all variables were complete ($p = 0.043$). With the inclusion of incomplete variable data the p value for Indigenous Status increased to 0.058.

Multivariate analysis of the cohort with complete variable data showed the odds of a positive SSA/Ro60, SSB/La or Ro52 antibody was significantly higher in females compared to males with OR's ranging from 2.199 (95%CI 1.583 – 3.054; SSB/La) to 3.339 (95%CI 2.501-4.457; SSA/Ro60). In the analysis including incomplete variables gender was significant for two additional ENA antibodies i.e. SmD and RNP (OR 1.618 (95%CI 1.036-2.525 and 2.262 (95%CI 1.153-4.434) respectively.

The SSA/Ro52 antibody was the only ENA antibody for which age was a significant variable indicating an increased likelihood. For all other ENA antibodies investigated increasing age was either protective or not significant (Tables 7.9-11).

The three broad country of birth groups which were associated with an increased likelihood of specific ENA antibody were; S. & E. Europe, N. Africa & M.E and S.E. Asia (Tables7.9-11). In a multivariate LR analysis for SmD and ribosomal antibody restricted to the individuals with complete data for all variables there was an increased odds of a positive outcome in individuals with a country of birth of S.E Asia. Similarly North Africa and the Middle East were predictive of RNP antibody (data shown in Tables 7.8–7.10). A country of birth of S. & E. Europe was also predictive of a positive SSA/Ro60 and SSA/La antibody in the analysis of complete data. S. & E. Europe country of birth remained significant when analysis was performed on the SSA/Ro60 incomplete variable dataset.

Accessibility to services classified as remote was a significant factor in the odds of a positive SSA/Ro60 antibody with an OR of 2.094 (95%CI 1.100-3.985). In contrast a classification of very remote was associated with a reduced likelihood (OR=0.600; 95%CI 0.365–0.985). Significant associations were not seen for ARIA and SSA/Ro60 when incomplete variables were included. Accessibility classified as remote was also a predictor for a positive SmD antibody (OR 2.847; 95%CI 1.129-7.177), however this finding was limited to the analysis of the incomplete dataset.

SSA/Ro60 was the only ENA antibody for which IRDS was a significant predictor of a positive test result in the multivariate LR. In the complete dataset the OR for the 3rd Quartile was 1.382 (95%CI 1.039-1.838). This effect was not noted in the incomplete dataset.

Multivariate LR analysis of the complete variable cohort showed an increase in the OR for a positive RNP antibody from 5.225 (95%CI 1.296 – 21.071) to 157.412 (95%CI 47.312-523.722) with an increase in ANA from 7-10IU/ml to >30IU/ml. A similar trend was observed for a positive SmD antibody outcome however the OR's were only significant for an ANA outcome >10IU/ml (>10-20IU/ml OR=2.047 95%CI 1.137–3.686) to ANA >30IU/ml (OR=4.183 95%CI 2.559–7.747). ANA>7IU/ml was also a predictor of SSB/La, and ANA>10IU/ml was a predictor of Scl70 and histones antibody (OR and CI are shown in Table 7-9 and 7-11).

ANA at the highest level i.e. >30IU/ml was significant for SSA/Ro60 antibody, a similar trend was noted in the analysis which included incomplete variables. In fact ANA at the highest level in the complete data set was a predictor for all ENA antibodies except Jo-1, however in the incomplete dataset ANA >30IU/ml remained non-significant for the prediction of Jo-1 antibodies and it was also no longer significant for the prediction of an Scl70 antibody (OR=2.342 95%CI 1.093-5.412 v OR=2.014 95%CI 0.959-4.229 respectively for the complete and incomplete data sets).

TABLE 7-9 MULTIVARIATE LR ANALYSIS FOR THE DETECTION OF ENA ANTIBODIES ADJUSTED FOR AGE, GENDER, COUNTRY OF BIRTH, INDIGENOUS STATUS, ARIA AND IRDS (ALL DATA I.E. INCOMPLETE DATA INCLUDED).

	Sm n= 4,214		**RNP n= 4,215**		**SSA/Ro60 n= 4,214**		**SSB/La n= 4,214**		**Scl70 n= 4,212**		**Ribosomal n= 4,213**	
	p value	**OR 95%CI**	**p value**	**OR 95%CI**	**p value**	**OR 95%CI**	**p value**	**OR 95%CI**	**p value**	**OR 95%CI**	**p value**	**OR 95%CI**
ANA (IU/ml)												
7-10	0.55	1.176 0.691-2.000	0.068	3.038 0.920-10.024	0.734	0.958 0.748-1.227	0.005	1.561 1.145-2.127	0.059	0.875 0.981-2.659	0.762	1.302 0.236-7.176
>10 - 20	0.004	2.122 1.268-3.552	<0.001	8.17 2.799-23.852	0.332	0.862 0.639-1.164	<0.001	2.022 1.447-2.825	0.012	2.013 1.166-3.475	.0093	3.338 0.818-13.615
>20 - 30	0.003	2.201 1.302-3.719	<0.001	25.810 9.803-67.954	0.057	1.31 0.992-1.729	<0.001	3.119 2.286-4.258	<0.001	2.869 1.716-4.797	0.007	5.807 1.600-21.080
>30	<0.001	3.944 2.2700-6.854	<0.001	109.031 42.422-280.229	0.008	1.592 1.131-2.242	<0.001	2.540 1.679-3.843	0.064	2.014 0.959-4.229	<0.001	28.623 9.202-89.032
INDIGENOUS STATUS												
Indigenous	0.093	0.392 0.132-1.167	0.471	1.389 0.568-3.395	0.96	1.011 0.654-1.564	0.244	0.699 0.383-1.276	0.044	0.219 0.050-0.964	0.058	3.88 0.954-15.782
Ungrouped	0.589	1.751 0.229-13.376	0.947	0.929 0.107-8.092	0.049	0.547 (0.300-0.996)	0.193	2.213 0.670-7.313	0.279	0.540 0.177-1.647	0.296	0.303 0.032-2.852
ARIA												
Remote	0.027	2.847 1.129-7.177	0.735	0.765 0.162-3.606	0.072	1.708 0.953-3.058	0.161	1.642 0.821-3.285	0.997	0	0.997	0

Note: Predictors that were non-significant across the ENA antibodies have not been included in the table.

TABLE 7-9 CONTINUED: MULTIPLE LR ANALYSIS FOR THE DETECTION OF ENA ANTIBODIES ADJUSTED FOR AGE, GENDER, COUNTRY OF BIRTH, INDIGENOUS STATUS, ARIA AND IRDS (ALL DATA I.E. INCOMPLETE DATA INCLUDED).

	Sm n= 4,214		**RNP n= 4,215**		**SSA/Ro 60 n= 4,214**		**SSB/La n= 4,214**		**Scl70 n= 4,212**		**Ribosomal n= 4,213**	
	p value	**OR 95%CI l**	**p value**	**OR 95%CI**	**p value**	**OR 95%CI**	**p value**	**OR 95%CI**	**p value**	**OR 95%CI**	**p value**	**OR 95%CI**
AGE	**<0.001**	0.977 0.967-0.988	**<0.001**	0.960 0.947-0.973	0.31	0.997 0.992-1.003	0.088	0.994 0.988-1.001	0.334	1.005- 0.995-1.016	**0.001**	0.958 0.935-0.982
GENDER	**0.034**	1.618 1.036-2.525	**0.0018**	2.262 1.153-4.434	**<0.001**	3.586 2.774-4.638	**<0.001**	2.285 1.705-3.061	0.521	0.875 0.582-1.315	0.053	4.229 0.980-18.254
Country of Birth												
S. & E. Europe	0.813	1.191 0.279-5.079	0.212	2.759 0.561-13.561	**0.015**	**2.129 1.159-3.910**	0.055	2.003 0.985-4.076	0.824	0.849 0.201-3.586	0.997	0
N. Africa & M.E	0.582	1.309 0.502-3.409	**0.010**	3.435 1.330-8.412	0.423	1.239 0.734-2.091	0.486	0.776 0.380-1.538	0.311	0.477 0.114-1.997	0.184	3.088 0.585-16.318
S.E. Asia	**<0.001**	3.396 1.720-6.706	0.072	2.44 0.924-6.443	0.54	1.186 0.687-2.049	0.892	1.047 0.542-2.021	0.729	0.811 0.247-2.658	**0.007**	6.002 1.619-22.249
Americas	0.384	1.943 0.435-8.684	0.106	3.245 0.778-13.542	0.87	1.096 0.367-3.270	0.541	1.412 0.467-4.269	0.313	0.485-9.576	0.223	4.063 0.427-38.685
Other	0.47	0.478 0.064-3.542	0.992	1.010 0.121-8.458	**0.011**	2.101 1.183-3.73	0.211	0.472 0.146-1.531	0.33	1.687 0.589-4.836	0.135	5.337 0.592-48.076

Note: Predictors that were non-significant across the ENA antibodies have not been included in the table.

TABLE 7-10 MULTIVARIATE LR ANALYSIS FOR THE DETECTION OF ENA ANTIBODIES ADJUSTED FOR AGE, GENDER, COUNTRY OF BIRTH, INDIGENOUS STATUS, ARIA AND IRDS (ONLY COMPLETE DATA INCLUDED).

	SmD (n=3,345)		**RNP (n=3,346)**		**SSA/Ro 60 (n=3,345)**		**SSB/La (n=3,345)**		**Scl70 (n=3,344)**		**Ribosomal (n=3,344)**	
	p value	**OR 95%CI**	**p value**	**OR 95%CI**	**p value**	**OR 95%CI**	**p value**	**OR 95%CI**	**p value**	**OR 95%CI**	**p value**	**OR 95%CI**
AGE	**0.001**	**0.98 0.969-0.991**	**<0.001**	**0.955 0.940-0.970**	0.074	0.995 0.989-1.001	**0.013**	**0.991 0.984-0.998**	0.27	1.007 0.995-1.019	**0.004**	**0.960 0.933-0.987**
GENDER	0.122	1.469 0.902-2.392	0.116	1.783 0.867-3.666	**<0.001**	**3.339 2.501-4.457**	**<0.001**	**2.199 1.583-3.054**	0.803	1.06 0.67-1.676	0.072	6.456 0.845-49.346
Country of Birth												
S & E Europe	0.79	1.218 0.285-5.202	0.178	3.012 0.604-15.004	**0.020**	**2.064 1.1237-3.795**	**0.048**	**2.051 1.006-4.182**	0.840	0.862 0.203-3.654	0.997	0.0
N. Africa & M.E	0.539	1.351 0.518-3.525	**0.006**	**3.687 1.445-9.404**	0.528	1.184 0.701-2.002	0.424	0.474 0.365-1.527	0.311	0.477 0.114-2.001	0.222	2.855 0.53-15.395
S.E. Asia	**<0.001**	**3.519 1.777-6.971**	0.064	2.519 0.946-6.706	0.528	1.184 0.701-2.002	0.976	1.01 0.522-1.956	0.760	0.830 0.252-2.732	**0.008**	**5.942 1.600-22.061**
Americas	0.398	1.909 0.426-8.549	0.094	3.398 0.813-14.207	0.624	1.147 0.663-1.984	0.589	1.358 0.447- 4.121	0.312	2.165 0.484-9.687	0.248	3.820 0.394-37.049
ANA (IU/ml)												
7- 10	0.498	1.227 0.679-2.217	**0.020**	**5.225 1.296-21.071**	0.672	1.062 0.804-1.404	**0.036**	**1.463 1.025-2.088**	0.183	1.491 0.829-2.681	0.719	0.667 0.073-6.048
>10 - 20	**0.017**	**2.047 1.137–3.686**	**0.001**	**10.175 2.653-39.023**	0.901	0.979 0.701-1.368	**<0.001**	**2.355 1.634-3.394**	**0.016**	**2.133 1.154-3.942**	0.279	2.321 0.505-10.673
>20 -30	**0.001**	**2.628 1.516–4.623**	**<0.001**	**37.304 11.018-126.302**	0.088	1.315 0.960-1.8	**<0.001**	**2.890 2.030-4.115**	**<0.001**	**3.094 1.747-5.480**	**0.029**	**4.507 1.164-17.445**
>30	**<0.001**	**4.183 2.259-7.747**	**<0.001**	**157.412 47.312-523.722**	**0.003**	**1.786 1.215-2.625**	**<0.001**	**3.182 2.036-4.973**	**0.029**	**2.432 1.093-5.412**	**<0.001**	**20.871 6.252-69.669**

Note: Predictors that were non-significant across the ENA antibodies have not been included in the table.

TABLE 7-10 CONTINUED: MULTIVARIATE LR ANALYSIS FOR THE DETECTION OF ENA ANTIBODIES ADJUSTED FOR AGE, GENDER, COUNTRY OF BIRTH, INDIGENOUS STATUS, ARIA AND IRDS (ONLY COMPLETE DATA INCLUDED).

	SmD n=3,345		**RNP (n=3,346)**		**SSA/Ro 60 (n=3,345)**		**SSB/La (n=3,345)**		**Scl70 (n=3,344)**		**Ribosomal (n=3,344)**	
	p value	OR 95%CI	**p value**	OR 95%CI	**p value**	OR 95%CI	**p value**	OR 95%CI	**p value**	OR 95%CI	**p value**	OR 95%CI
Indigenous Status	0.092	0.383 0.125-1.171	0.634	1.258 0.489-3.234	0.65	1.112 0.703-1.758	0.25	0.691 0.368-1.297	**0.045**	**0.212 0.046-0.967**	**0.043**	**4.914 1.049-23.025**
ARIA												
Remote	0.093	2.589 0.854-7.850	0.933	1.07 0.28-5.264	**0.024**	**2.094 1.1-3.985**	0.287	1.553 0.691-3.493	0.997	0.00	0.997	0.00
Very Remote	0.972	1.016 0.418-2.647	0.345	1.569 0.616-3.997	**0.049**	**0.600 0.365-0.985**	0.236	0.688 0.371-1.277	0.59	1.278 0.523-3.126	0.823	0.834 0.169-4.111
IRDS												
3rd Quartile	0.092	0.383 0.125-1.171	0.498	1.271 0.635-2.543	**0.026**	**1.382 1.039-1.838**	0.250	0.691 0.368-1.297	0.855	1.059 0.575-1.950	0.206	0.455 0.134-1.544

Note: Predictors that were non-significant across the ENA antibodies have not been included in the table.

TABLE 7-11. MULTIPLE LR ANALYSIS FOR THE DETECTION OF ENA ANTIBODIES ADJUSTED FOR AGE, GENDER, COUNTRY OF BIRTH, INDIGENOUS STATUS, ARIA AND IRDS (INCOMPLETE AND COMPLETE DATA).

	Incomplete Variables Included						**All Variables Complete**					
	Ro52 Antibody n=4,214		**Histones Antibody n=4,214**		**Jo-1 Antibody n=4,213**		**Ro52 Antibody n= 3,337**		**Histone Antibody n = 3,345**		**Jo-1 Antibody n=3,344**	
	P	OR 95%CI	P	OR 95%CI	P	OR 95%CI	P	OR 95%CI	P	OR 95%CI	P	OR 95%CI
AGE	**<0.001**	**1.009 1.004-1.014**	0.162	0.995 0.987-1.002	0.86	0.999 0.984-1.014	**0.013**	**1.007 1.001-1.012**	0.252	0.995 0.987-1.003	0.594	0.995 0.979-1.012
GENDER	**<0.001**	**2.319 1.896-2.837**	0.074	1.323 0.973-1.799	0.902	0.967 0.565-1.655	**<0.001**	**2.268 1.81-2.841**	0.156	1.282 0.909-1.808	0.729	1.117 0.596-2.093
ANA (IU/ml)												
7-10	0.468	0.919 0.733-1.154	**0.003**	**1.866 1.239-2.811**	**0.026**	**0.402 0.180-0.898**	0.517	0.919 0.712-1.186	**0.003**	**1.99 1.263-3.135**	**0.046**	**0.382 0.148-0.984**
>10 - 20	0.498	1.093 0.845-1.413	**<0.001**	**3.118 2.065-4.707**	0.054	0.363 0.13-1.019	0.155	1.227 0.926-1.625	**<0.001**	**3.265 2.061-5.172**	0.169	0.479 0.168-1.366
>20 -30	**<0.001**	**1.575 1.231-2.014**	**<0.001**	**6.62 4.589-9.548**	**0.045**	**0.298 0.091-0.966**	**0.004**	**1.493 1.134-1.966**	**<0.001**	**6.325 4.188-9.553**	0.12	0.387 0.117-1.282
>30	**<0.001**	**1.856 1.358-2.536**	**<0.001**	**10.943 7.332-16.332**	0.994	0	**<0.001**	**1.94 1.367-2.754**	**<0.001**	**10.437 6.617-16.462**	0.995	0

Note: Predictors that were non-significant across all ENA have not been included in the table (i.e. Indigenous Status, Country of Birth, ARIA and IRDS).

7.3.9 CENTROMERE ANTIBODY

The centromere associated protein B (CenpB) autoantibody has been associated with the clearly defined IIF pattern of at least 23 discrete speckles in the nucleus of interphase cells and speckling in the chromosome region of mitotic (metaphase) cells. A secondary test is routinely used to confirm the presence of the antibody. In this study cohort of concurrent ENA and ANA testing data (n=4208), 92 individuals had a centromere pattern reported by IIF of which 85% (n=78) were confirmed by immunoblot and a further 3 were recorded as equivocal. An additional 27 were detected and 13 were reported as equivocal by immunoblot without being detected by the IIF screen. The CenpB pattern was most frequently detected at the >20-30IU/ml ANA level. The frequency of CenpB antibody positive individuals (detected by immunoblot) within each ANA (IU/ml) group is shown in Figure 7.1.

Predictive variables for the detection of a CenpB antibody by immunoblot were investigated in a multivariate LR analysis using i. the complete data cohort and ii. all individuals (incomplete variable included). In both analyses gender, increasing age and ANA level were significant predictors for a detectable CenpB antibody. The odds increased from 3.905 (95%CI 1.602–9.522) to 13.552 (95%CI 5.842–31.434) with rising ANA level from >10-20IU/ml to >30IU/ml for the complete variable data, there was a similar increase in OR for the incomplete data set with the OR increasing from 5.542 (CI 2.293–12.421) to 20.472 (CI = 9.389 – 44639).

The OR for females relative to males was 2.185 (95%CI1.164-4.095) and 1.987 (95%CI 1.129-3.497) for the complete and incomplete data sets respectively. This is consistent with the increased female:male ratio of 20:126 for a positive CenpB antibody by immunoblot (data shown in Table 7-12).

TABLE 7-12 MULTIVARIATE LR ANALYSIS OF DETECTION OF CENPB ANTIBODY ADJUSTED FOR AGE, GENDER, COUNTRY OF BIRTH, INDIGENOUS STATUS, ARIA AND IRDS.

	CenpB Antibody (Detected by Immunoblot)			
	Incomplete Variables (n = 4,215)		**Complete Variables (n = 3,346)**	
Variables	p value	OR 95%CI	p value	OR 95%CI
AGE (years)	<0.001	1.034 1.021-1.047	<0.001	1.036 1.021-1.051
GENDER (Reference Male)	0.017	1.987 1.129-3.497	0.015	2.185 1.166-4.095
ANA (IU/ml) (Reference <7)				
7- 10	0.404	0.523 0.114-2.398	0.412	0.528 0.115 – 2.425
>10 - 20	0.003	5.452 2.293-12.421	0.003	3.905 1.602-9.522
>20 -30	<0.001	28.801 14.46-57.367	<0.001	26.102 12.952-52.604
>30	<0.001	20.472 9.389-44.639	<0.001	13.552 5.842-31.434

Note: Predictors non-significant across all ENA antibodies i.e. ARIA, Indigenous Status, country of birth, IRDS and ARIA have not been included in the table.

7.3.10 ENA ANTIBODY AND DEATH

Mortality data were linked through the WADLS to the ENA antibody data. The censor date for the mortality data was 20th February 2012. Deaths were reported for 4,161 individuals with an ENA result; however 536 did not have coded causes of death and were not included in the analysis.

The survival outcome for individuals related to number of ENA antibodies identified was investigated using Kaplan Meier Survival and Log Rank analysis (See Figure 7.7). The differences in survival time did not differ between groups. Survival outcome varied from 67% (number of ENA antibodies=6) to 100% (number of ENA antibodies=5). When the cohort was stratified for gender there was a statistically significant difference in the survival time of females with 5 ENA antibodies (50%, n=4) compared to those with 0–4 ENA antibodies. The difference in survival times between groups for males was not statistically significant.

The CenpB antibody was identified by immunoblot in 105 individuals (89 females and 16 males). Morbidity data were available for 25 individuals (23.8%: 5 males and 20 females) at the end of the study. Individuals greater than 60 years of age accounted for 24 of the 25 deaths. The CenpB antibody was the only ENA antibody for which a statistically significant reduced survival outcome was demonstrated in a Log Rank comparison for a positive antibody.

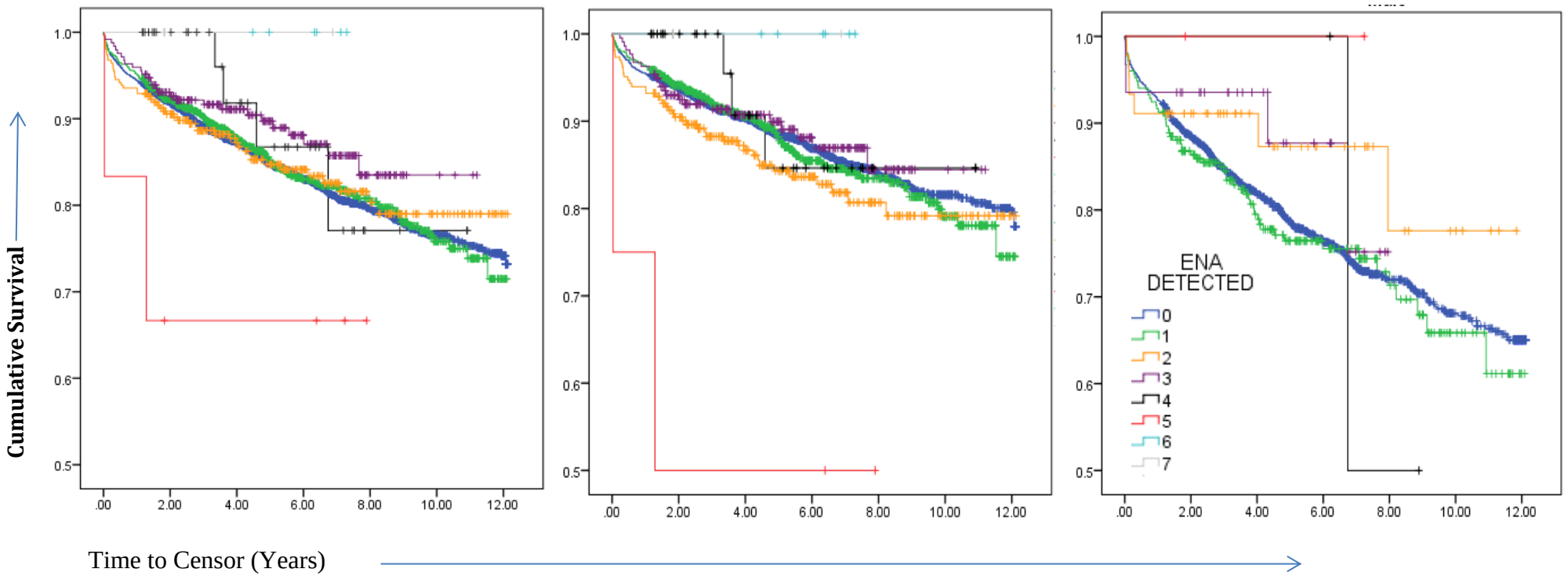


FIGURE 7.5 KAPLAN MEIER SURVIVAL RELATED TO NUMBER OF ENA ANTIBODIES DETECTED AND TO GENDER.

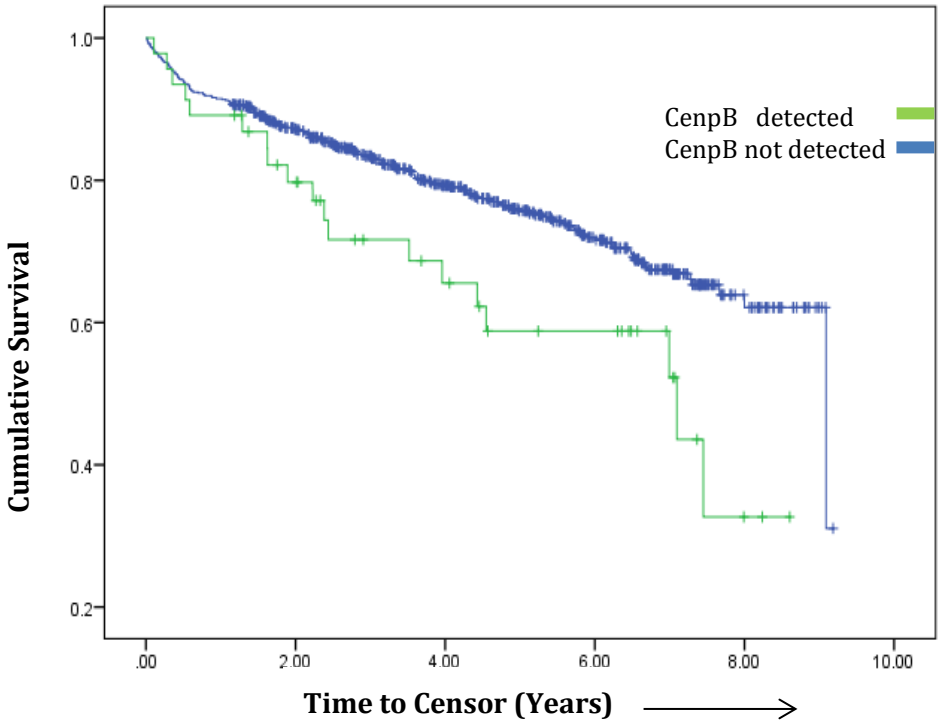


FIGURE 7.6 KAPLAN MEIER SURVIVAL RELATED TO CENPB ANTIBODY IN FEMALES >60 YEARS OF AGE.

7.4 DISCUSSION

ENA antibody screen by the CIEP method has typically been considered the "gold standard" for identifying a clinically significant threshold level of ENA antibody (reviewed in (158)). However the antigens for this method (prepared from the K562 myeloid leukaemia cell line or rabbit thymus extract) were subject to batch variation reliant on consistent cell culture conditions and antigen expression. In contrast, the purified or recombinant antigens used in the screening ELISA can be quantified to ensure a uniform antigen concentration across batches.

Differences in the frequency of the ENA antibody detected over the 11 year study period may also be attributed to the sensitivity of the screening and characterisation/detection assays in use at the time (i.e. the high frequency of RNP antibody detected by CIEP compared to immunoblot).

The change in technology from screen CIEP to ELISA facilitated the introduction of the newly identified ENA SSA/Ro52. Antibodies to SSA/Ro52 have been identified in congenital heart block in infants and are important in sub classifying inflammatory myopathies (159-161). SSA/Ro52 antibodies were not detectable by CIEP.

The data in this chapter demonstrate that ANA level is predictive of a number of ENA antibodies particularly RNP, histone and SSB/La. Furthermore, the percent of samples with SSA/Ro60, histones and Ro52 antibody increased with increasing ANA level and a homogeneous pattern. Similarly the frequency of an RNP antibody increased with increasing ANA in individuals with a speckled or homogeneous plus speckled pattern.

Although Ro52 antibodies are not typically associated with a specific pattern it was the most frequently reported ENA antibody both in isolation and in association with other ENA antibodies detected by the ANA method particularly SSA/Ro60. However, the association with the homogeneous pattern may be due to the concurrent expression of multiple autoantibodies such as dsDNA and histones (162). This study focused on the relationship between ANA and ENA antibodies therefore the association between Ro52 and dsDNA antibodies were not been investigated.

Greater than 50% of the samples sent for ENA antibody characterisation had an ANA <7IU/ml. In these cases it is likely that additional testing had been requested by the treating clinician based on clinical suspicion rather than laboratory findings.

Antibodies which have a cytoplasmic pattern such as ribosomal and Jo-1 are scored on the intensity of the staining in the nucleus. Mahler *et al 2008* (163) reported poor sensitivity for ribosomal P antibody detection by ANA. In the current study the predictive value of increasing ANA level for Jo-1 antibodies indicated a reduced odds of a Jo-1 antibody and in the case of ribosomal P, ANA level was a predictor at >20IU/ml. The finding for ribosomal P antibodies is likely to be due to the presence of additional ANA associated with rheumatic disease i.e. dsDNA, SSA/Ro60 and SSB/La antibodies (164).

Multivariate analysis of concurrent ANA and ENA antibody testing adjusted for age, gender, country of birth, ARIA and IRDS identified being female and older to be associated with Ro52 antibody. The likelihood of a positive test for the remaining ENA was reduced with increasing age. ARIA and IRDS were significant predictors for SSA/Ro60 antibody which is associated with the development of SLE and SjS. Vincent *et al* proposed that socio-economic factors may contribute to the high prevalence of autoimmune disease reported in the Australian Indigenous population (47). However, while Indigenous individuals were at a higher risk of a positive ribosomal antibody associated with SLE, Indigenous Status was not found to be a significant variable in the model for a positive SSA/Ro60 antibody in the current study. In addition the overall effect of socioeconomic disadvantage was not significant in the model for SSA/Ro60 antibody; however the 3rd quartile showed a 38% increased odds of a positive test compared with the highest quartile (representing the highest socioeconomic grouping).

The country of birth in the multivariate analysis for ENA antibody outcome indicated there was an increased likelihood of Sm and ribosomal antibodies typically associated with SLE in individuals from S.E. Asia, (OR=3.519 and 5.942 respectively). This finding is consistent with the increasing number of Asian patients admitted with SLE in a study of Royal Melbourne Hospital patients described by Ong *et al* (165).

Analysis of the number of ENA antibodies detected and age showed that a higher number of ENA antibodies were associated with a lower age at the time of ANA testing. Survival outcome analysis also showed a reduced survival time for females in which 5 ENA

antibodies were detected. A change in the number of ENA antibodies detected between the first two tests was observed in 22% of individuals tested in the period of ENA antibody testing by immunoblot method, in 78% of cases the change was a single ENA antibody. This study has only considered results reported as positive and has categorised equivocal results as negative. Changes in ENA antibody may also be associated with a shift from an equivocal to a positive or negative result rather than complete loss or gain of reactivity.

SSA/Ro52 and SSA/Ro60 antibodies were the most frequently detected ENA antibody which is consistent with previous findings both in PathWest and other laboratories (48).

CenpB antibody detected by immunoblot was the only ENA antibody in this study which was associated with a significantly reduce survival in females, in particular those greater than 60 years of age and gender effects are consistent with reported findings for the frequency and demographics of CenpB antibody (46, 166, 167).

In conclusion the majority of ENA antibodies investigated in this study had a reduced likelihood of a positive antibody with increasing age, however females had a higher likelihood of a detectable ENA antibody, while IRDS and ARIA had minimal effect on the outcome. Indigenous Status as a significant predictor an ENA antibody was limited to ribosomal antibody when the analysis was restricted to individuals for which all variables were complete.

8 ANA AND MORBIDITY

8.1 BACKGROUND

ANA as a first level screening test is used to direct further investigations for likely systemic autoimmune diseases (e.g. SLE, SjS, SSc, RA and inflammatory myopathies). Morbidities associated with autoimmune disease involve various organs and tissues such as heart, lung, kidney, muscle and skin (168-170). Lupus nephritis has a high morbidity associated with the glomerular nephritis, foetal congenital heart block is an acquired autoimmune syndrome in which autoantibodies to SSA/Ro52 are transferred from mother to infant and in severe cases may require the early implantation of a pacemaker (169, 171). Infection is also frequently described as a cause of morbidity in patients with autoimmune disease (141, 172).

Morbidity data linked through the WADLS includes all hospital separations from WA public and private hospitals. In this chapter patient ANA data were matched with WA hospital morbidity data. The most frequent morbidities associated with an incident ANA outcome have been investigated in a multivariate LR model to determine whether ANA level adjusted for age, ARIA, IRDS at time of ANA testing, gender and country of birth is a significant predictor of morbidity.

8.2 METHOD

8.2.1 ANA AND INPATIENT MORBIDITY

Incident ANA cases were linked to morbidity data through the WADLS using probabilistic matching techniques previously described in Chapter 3. ANA often precedes the onset of clinical symptoms (19), however to ensure that the relevant diagnoses were captured, only morbidities that precede the ANA test by more than 1 year were excluded from the analysis. Both the principal and co-diagnoses in the hospital record were included in the determination of morbidity associated with each hospital admission. The number of principal morbidities listed was >1,000 in the cohort of 58,700 individuals.

Morbidities included in the analysis were selected initially according to the following criteria:

i. they represented ≥1% of the principal diagnosis for all ANA positive individuals at the time of their incident morbidity record.

ii. there was a high proportion of ANA positive results in the categories ≥ 10IU/ml.
iii. individual ICD codes (e.g. M36) grouped where appropriate (e.g. any event with ICD-10 M31 – M36: Musculoskeletal and connective tissue disease) were included in a single category of musculoskeletal disease morbidity for the analysis.

In order to accommodate clinical presentation of a patient due to non-specific symptoms prior to a thorough investigation of diagnostic markers of autoimmune disease, hospital admissions up to one year preceding the incident ANA result and any subsequent admissions have been included in the analysis of association between morbidity recorded (ICD-10 codes) and incident ANA outcome. Morbidity records included a principal diagnosis and up to 20 co-morbidities for each inpatient episode.

Multivariate LR analysis with an outcome of a single morbidity or grouped morbidities as indicated above were performed adjusting for ANA level (reference = ANA <7IU/ml), gender (reference = Male), Indigenous Status (reference = Non Indigenous), IRDS (reference = 4th Quartile), ARIA (reference = Highly Accessible) and country of birth (reference = Australia). Age was included as a continuous variable.

The morbidity of infection (defined by ICD-10 code prefix A and B) was determined for the broad ICD groupings B00-B09, B15-B19, B25-B34 and B35-B49. Additional ICD codes for Herpes Zoster Virus (HZV), Cytomegalovirus (CMV) and influenza and pneumonia (J09-J18) were also investigated as they had been referred to in relation to infection and SLE by Feldman *et al* (172). B95 (streptococcus and staphylococcus as the cause of the disease classified) was specifically investigated as it was noted that it was recorded as a morbidity in 5,193 (8.8%) of individuals.

8.2.2 ANA AND CANCER REGISTRY

Cancer registry data were linked to incident ANA data using the WADLS Unique Identifier. Cancer cases were excluded (n= 3,704) from the analysis if the year of diagnosis was more than 365 days before the collection of the incident ANA sample.

The cancer registry was used to define the cancer diagnosis grouped according to the ICD-10 code. Non Hodgkin LB cell lymphoma was grouped according to the WA Cancer Registry definition which includes ICD-10 codes C82.0-C82.2, C82.9, C83.0.C83.2-C83.4, C83.7-C83.8.and C85.1.

The overall frequency of ANA ≥ 7IU/ml in each cancer type reported in the cancer registry was determined. Multivariate LR analysis was performed with diagnosis of specific cancer as the dependant variable. The categorical covariates were; ANA grouped by level, (reference = < 7IU/ml, gender (reference = male, undefined gender (n=1) was excluded), country of birth (reference = Australia), ARIA (reference=highly accessible) and IRDS (reference=4th quartile). Age at time of ANA testing was included as a continuous variable.

8.3 RESULTS

The analysis included 58,700 individuals of which 10,510 (17.9%) had a positive ANA result which is similar to the level reported by Selmi *at al* (173) in a general population study of 2,690 individuals in Italy.

8.3.1 ANA AND A MORBIDITY OF MUSCULOSKELTAL DISEASE ICD-10 CODE CLASSIFICATIONS

Morbidities associated with systemic connective tissue disorders ICD codes M30 – M36 were recorded for 1,520 individuals of which 46% were positive for ANA. Seventy five percent of ANA positive results were ≥ 10IU/ml and the OR for a positive ANA predicting a morbidity outcome of M30 – M36 increased with rising level of ANA OR=1.634 (1.376 – 1.940) – to OR=21.910 (18.068 – 26.571) (data shown in Table 8-1).

The ICD-10 groups M30 – M36 included the major systemic autoimmune diseases SLE, SjS, dermatomyositis (DM) and SSc, all of which are associated with an ANA. Each category M30 through M36 was considered individually as shown in Table 8-1. In this analysis the percent of cases with a positive ANA and the likelihood of the morbidity increased with increasing ANA level (7-<10IU/ml to >30IU/ml) i.e. SLE OR=4.120 to 89.707 (75.3% ANA positive), SSc OR=4.138 – 115.248 (ANA Positive 83.7%), DM OR=3.078 – 9.415 (49.5% ANA positive) and SjS OR=1.515 – 7.059 (ANA positive 36.2%; grouped as shown in Table 8-1).

A positive ANA at any level did not influence the likelihood of an admission with a diagnosis of polyarteritis and related conditions or other necrotising vasculopathies (ICD Group M30 and M31). These conditions are more frequently associated with

autoantibodies directed against antigens expressed on neutrophils (anti neutrophil cytoplasmic antibodies) (174) rather than ANA .

Increasing age was also a significant variable in the multivariate LR model for a morbidity associated with ICD-10 groups M33, M34 and M35, however in the model of M32 (SLE) there was a reduced likelihood of morbidity with increasing age (OR 0.975, 95%CI 0.969-0.981).

Females also had an increased likelihood of hospitalisation with a diagnostic morbidity code of ICD M32, M34 and M35 compared to males. The highest OR for gender was associated with a diagnosis of SLE OR= 2.541(1.880- 3.435; data shown in Table 8-2).

Indigenous individuals had a 2.4 times greater likelihood of an inpatient episode associated with SLE compared to a non-Indigenous individual when controlling for other factors. IRDS did not contribute to the likelihood of an inpatient episode for any of the musculoskeletal conditions described. ARIA scores were also not associated with a risk of hospitalisation. An ARIA of "very remote" was associated with a reduced likelihood of a diagnosis of ICD-10 groups M32 or M35 and "moderately accessible" was associated with a reduced likelihood for ICD group M32 and M34. An ARIA of "Accessible" was associated with a reduced likelihood of an admission of SSc (M34). Country of birth was a significant predictor for a hospitalisation morbidity of DM with country of birth of Americas having an OR of 4.413 (95%CI 1.332-14.3).

Increasing ANA levels were also identified as predictive of an inpatient episode diagnosis or co-morbidity associated with inflammatory polyarthropathies as described in ICD-10 M5-M14 OR=1.285–2.241 ((7-<10IU/ml to >30IU/ml; data shown in Table 8-2). When the groupings M05, M06, M07, M08 and M14 were considered individually increasing ANA from 7IU/ml to >30IU/ml (reference = <7IU/ml) was a predictor of seropositive rheumatoid arthritis OR=2.579 (95%CI 1.851-3.594) to 6.030 (3.311-10.980), juvenile arthritis OR=2.439 (1.117-5.325) to 7.001 (2.032-24.125) and ICD Code M6 "other" rheumatoid arthritis OR=1.660 (95%CI 1.404-1.963) to 3.541 (2.540-4.937). The percent of ANA positive individuals in the polyarthropathies groupings was not as high as the levels seen in the connective tissue disorders M30-36 (i.e. 36.5 – 41.1% compared to 36.2 – 83.7%). Data are shown in Table 8-2.

Age was associated with an increased risk of hospitalisation in the seropositive and "other" rheumatoid arthritis groups. Country of birth (N.W. Europe) had an OR of 1.661 (95%CI 1.253-2.202) in the seropositive rheumatoid arthritis group compared with a country of birth of Australia the reference. Female gender and the first quartile (lowest ranked) of IRDS were associated with an increased likelihood of hospitalisation with a diagnosis of "Other" RA which included seronegative RA, adult onset Still's disease, inflammatory polyarthropathy etc.

ARIA was a significant predictor for both ICD groups M05 and M06, however in all cases it was associated with a reduced risk of hospitalisation with estimates ranging from OR= 0.433 (95%CI 0.203-0.924) for very remote accessibility in M05 to an OR=0.701 (95%CI 0.559-0.877) for moderate accessibility referenced against the category of high accessibility.

TABLE 8-1 DETAILS OF SIGNIFICANT VARIABLES IN A MULTIVARIATE LR ANALYSIS OF MORBIDITIES ASSOCIATED WITH MUSCULOSKELETAL DISEASES IN THE INCIDENT ANA COHORT ADJUSTED FOR AGE, GENDER, COUNTRY OF BIRTH, INDIGENOUS STATUS, ARIA AND IRDS.

ICD-10 Code and Description		**% ANA ≥ 7IU/ml (N)**	**p Value, OR 95%CI For ANA Level***			
			7 - <10IU/ml	10 - <20IU/ml	20 – 30IU/ml	30IU/ml
M30	Polyarteritis nodosa & related Conditions	17.9 (84)	0.969	0.556	0.718	0.993
M31	Other necrotizing vasculopathies	14.6 (363)	0.116	0.567	0.147	0.993
M32	SLE	75.3 (381)	<0.001 4.120 (2.872—5.911)	<0.001 8.979 (6.307-12.784)	<0.001 27.178 (20.282-36.419)	<0.001 89.707 (65.996-121.938)
M33	Dermatopolymyositis	49.5 (107)	<0.001 3.078 (1.836 – 5.138)	<0.001 3.490 (1.815- 6.711)	<0.001 6.574 (3.670-11.775)	<0.001 9.415 (4.238-20.916)
M34	Systemic sclerosis	83.7 (227)	<0.001 4.138 (2.374–7.211)	<0.001 15.317 (9.539– 24.593)	<0.001 56.632 (38.156-84.053)	<0.001 115.248 (74.629–177.975)
M35	Other systemic involvement of connective tissue including SjS	36.2 (469)	0.004 1.515 (1.145 – 2.006)	0.002 1.790 (1.249-2.564)	<0.001 3.297 (2.397-4.534)	<0.001 7.059 (4.771 – 10.445)
M36	Systemic disorders of connective tissue in diseases classified elsewhere	50 (8)	0.352	0.117	0.058	0.006 23.874 (2.451-232.524)
M30 – 36	Systemic Connective tissue disorders	46.1(1520)	<0.001 1.634 (1.376 – 1.940)	<0.001 2.935 (2.433 – 3.539)	<0.001 7.512 (6.416-8.795)	<0.001 21.910 (18.068 – 26.571)

Reference categories: ANA level = <7IU/ml, gender = male, Indigenous Status = non Indigenous, Country of birth = Australia, IRDS = highest (4th quartile) and ARIA = highly accessible.

TABLE 8-1 CONTINUED: DETAILS OF SIGNIFICANT VARIABLES IN A MULTIVARIATE LR AANALYSIS OF MORBIDITIES ASSOCIATED WITH MUSCULOSKELETAL DISEASES IN THE INCIDENT ANA COHORT ADJUSTED FOR AGE, GENDER, COUNTRY OF BIRTH, INDIGENOUS STATUS ARIA AND IRDS.

ICD-10 Code	p value, OR (95%CI) for ANA Level					
	Age	**Gender**	**Indigenous Status**	**Country of Birth**	**IRDS**	**ARIA**
M32	0.001 OR 0.975 (0.969-0.981)	<0.001 OR 2.541 (1.880- 3.435)	<0.001 OR 2.360 (1.531-3.638)	0.107	0.764	MA. 0.006 OR 0.456 (0.279-0.745) VR. 0.008 OR 0.540 (0.343-0.850)
M33	0.001 OR 1.019 (1.008-1.030)	0.683	0.736	S.E. Asia 0.009 OR 3.123(1.329-7.337) Americas 0.013 OR 4.413(1.362-14.300)	0.389	0.700
M34	<0.001 OR 1.016 (1.008 – 1.023)	0.025 OR 1.446 (1.047 – 1.998)	0.068	0.624	0.944	A. 0.033 OR 0.558 (0.326–0.954) MA 0.008 OR 0.429 (0.228-0.804)
M35	<0.001 OR 1.041 (1.035-1.046)	<0.001 OR 1.975 (1.597-2.444)	0.763	0.741	0.905	VR. 0.003 OR 0.392 (0.212-0.725)
M36	0.115	0.076	0.109	0.393	0.173	0.133
M30 – M36	<0.001 OR 1.018 (1.015-1.021)	<0.001 OR 1.356 (1.208-1.521)	0.016 OR 1.417 (1.068-1.881)	0.198	0.933	A. 0.009 OR 0.779 (0.645-0.940) MA <0.001 OR.0.634 (0.511-0.787) VR <0.001 OR .0.433 (0.326-0.576)

M32 SLE, M33 Dermatopolymyositis , M34 Systemic sclerosis, M35 Other systemic involvement of connective tissue including SjS, M36 Systemic disorders of connective tissue in diseases classified elsewhere , M30-36 Systemic Connective tissue Disorders

Reference categories: ANA level = <7IU/ml, gender = male, Indigenous Status = non Indigenous, Country of birth = Australia, IRDS = highest (4th quartile) and ARIA = highly accessible.

Abbreviations A accessible, MA moderately accessible, R = remote and VR = very remote

TABLE 8-2 DETAILS OF SIGNIFICANT VARIABLES IN A MULTIVARIATE LR ANALYSIS OF MORBIDITIES ASSOCIATED WITH MUSCULOSKELETAL DISEASES ICD-10 M05–M14 IN THE INCIDENT ANA COHORT.

ICD-10 Code and Description		% ANA ≥7IU/ml (N)	p value OR and 95%CI For ANA Level			
			7 - <10IU/ml	10 - <20IU/ml	20 – 30IU/ml	30IU/ml
M05	**Seropositive rheumatoid arthritis**	41.1 (248)	<0.001 OR 2.579 (1.851-3.594)	<0.001 OR3.094 (2.043-4.687)	<0.001 OR 2.532 (1.501-4.269)	<0.001 OR 6.030 (3.311-10.980)
M06	**Other rheumatoid arthritis**	34.0 (1260)	<0.001 OR 1.660(1.404-1.963)	<0.001 OR 2.737 (2.264-3.309)	<0.001 OR 2.276 (1.799-2.879)	<0.001 OR 3.541 (2.540-4.937)
M08	**Juvenile arthritis**	36.5 (63)	0.025 OR 2.439 (1.117-5.325)	0.007 OR 3.429 (1.408-8.351)	<0.001 OR 5.065 (2.070-12.391)	0.002 OR 7.001 (2.032-24.125)
M14	**Arthropathies in other diseases classified elsewhere**	23.6 (72)	0.714	0.632	0.033 OR 2.746(1.088-6.934)	0.008 OR 4.869 (1.509-15.713)
M05 – M14	**Inflammatory polyarthropathies**	24.1 (3483)	<0.00 OR 1.285 (1.149-1.437)	<0.00 OR 1.683(1.460-1.941)	<0.00 OR 1.546 (1.297-1.844)	<0.00 OR 2.241(1.731-2.903)

Reference categories: ANA level = <7IU/ml, gender = male, Indigenous Status = non Indigenous, Country of birth = Australia, IRDS = highest (4th quartile) and ARIA = highly accessible

TABLE 8-2 CONTINUED: SIGNIFICANT VARIABLES IN A MULTIVARIATE LR ANALYSIS OF MORBIDITIES ASSOCIATED WITH MUSCULO-SKELETAL DISEASES ICD-10 M05 – M14 IN THE INCIDENT ANA COHORT.

ICD 10 Code	Logistic regression p Value, OR and 95%CI for variables included in the model					
	Age	**Gender**	**Indigenous Status**	**Country of Birth**	**IRDS**	**ARIA**
M05	<0.001 OR 1.028 (1.020-1.035)	0.077	0.755	N.W. Europe <0.001 OR 1.661(1.253-2.202)	0.730	A. 0.584(.354-0.963) VR. 0.433(0.203-0.924)
M06	<0.001 OR 1.027 (1.024-1.031)	<0.001 OR 1.596 (1.409-1.807)	0.248	0.055	*1.* 0.006 OR 1.251(1.066-1.469)	A. <0.0010.688 (0.558-0.849) MA. <.001 0.701(0.559-0.877) VR. <0.001 0.527(0.399-0.723)
M08	<0.001 OR 0.865 (0.844-0.886)	0.368	0.975	0.999	0.997	0.705
M14	0.531	0.040 OR 0.610 (0.381-0.977)	0.003 OR 3.675(1.550-8.711)	1.000	0.309	0.081
M05 –14	<0.001 OR 1.030 (1.028-1.032	<0.001 OR 0.744 (0.694-0.798)	<0.001 OR 2.003 1.709-2.347	Oceania &Antarctic 0.010 OR 1.306 (1.066-1.602) N. Africa & M.E. 0.022 OR 0.742 (0.575-0.957) S.E. Asia 0.003 OR 0.593(0.418-0.839) Americas 0.035 OR 0.488(0.250-0.950) Africa (excl. N Africa) 0.014 OR 0.654(0.467-0.918)	*1.* 1.297(1.170-1.438) *2.* 1.117(1.006-1.240)	A.<0.001 OR0.775(0.683-0.878) MA. 0.048 OR 0.878(0.773-0.999) VR. <0.001 OR 0.761(0.654-0.885)

Abbreviations for ARIA: A accessible, MA moderately accessible, R = remote and VR = very remote, for IRDS: *1.*First quartile *2.* 2nd quartile, *3.* 3rd quartile

M05 Seropositive rheumatoid arthritis, M06 Other rheumatoid arthritis, M07 Psoriatic and enteropathic arthropathies, M08 Juvenile arthritis, M14 Arthropathies diseases classified elsewhere, M05 –14 Inflammatory polyarthropathies

8.3.2 ANA AND OTHER MORBIDITY ASSOCIATIONS

Additional morbidities investigated in the multivariate LR model are detailed in Tables 8.3-8.6. Broad category groups: oral cavity, gastrointestinal, liver and myoneural morbidities had a significant association with a high positive ANA result however the associations were weaker (ORs ranged from 1.354 to 1.789) than seen in the systemic autoimmune groups (ORs ranged from 2.241 to 115.248).

Morbidities were initially considered for broad classifications i.e. K00–K14, K20-K31 and K72-K77. K73–K75 were then analysed at a more specific disease morbidity ICD-10 level. Increasing ANA level predicted diseases of the Liver i.e. ICD-10 K72-K77 with an OR=1.306 95%CI 1.089-1.565 increasing to OR=1.789 95%CI 1.369-2.339 for an increase in ANA level from 20-30IU/ml to >30IU/ml. The disease group Chronic Hepatitis; not classified elsewhere had odds for ANA levels 10-<20IU/ml and 20-30IU/ml which increased from 2.109 (95%CI 1.095-4.064) to 4.579 (95%CI 2.587-8.104), the odds were reduced for an ANA >30IU/ml OR=3.684 (1.344-10.101). ANA was not a significant predictor for fibrosis and cirrhosis of the liver.

For the group of diseases of the Myoneural Junction and Muscle ANA 10-<20IU/ml and >30IU/ml were predictive of disease (OR=1.548 95%CI 1.061-2.257 and OR=2.098 95%CI 1.074–4.098 respectively). When considered individually the ORs increased to 2.945 (1.439-6.030) and 4.149 (95%CI 1.281-13.433) for G70 Myasthenia Gravis and other myoneural disorders. For the group G73 (Diseases of myoneural junction and muscle disease classified elsewhere) the OR at >20-30 and >30IU/ml were predictive with ORs of 17.503 (95%CI 6.887-44.487) and 19.709 (95%CI 5.381-72.196) respectively.

Interestingly increasing ANA ≥10IU/ml was associated with a reduced likelihood of a morbidity grouping of Type II diabetes (ANA 10-<20IU/ml OR=0.833 95%CI 0.729-0.951; ANA≥ 30IU/ml OR=0.566 95%CI 0.417-0.768).

Increasing age was a significant variable in the Multivariate LR model for the broad ICD groups analysed with the exceptions of:

- diseases of the oral cavity, salivary glands and jaw in which increasing age was associated with a reduced likelihood of a morbidity outcome

- LE and the local connective tissue disorders in which age was not a significant variable.

Male was set as the reference value for gender and while being female was associated with an OR >1 in a number of the systemic autoimmune disease groups M06, M32 thru M36, the OR for gender was frequently <1 or a non-significant variable in the other conditions reported here (see Tables 8.2-4).

The reference group for Indigenous Status was Non Indigenous and significant associations were seen for Type II Diabetes and Glomerular disorders with OR=9.33 (95%CI 8.407-10.355) and 7.633 (95%CI 4.662-12.565) respectively (See Tables 8.5)

The only instance in which ARIA had an OR > 1 was for the remote category in the LE ICD grouping OR = 3.476 (95%CI 1.453-8.318).

Relative to the 4th Quartile of the IRDS there was an increased likelihood of a morbidity associated with nutritional anaemias in the LR model including ANA outcome. This also applied to diseases of the liver however when this analysis was refined and sub groups Chronic Hepatitis and Fibrosis and Cirrhosis of the liver were analysed separately IRDS was not significant.

The statistically significant effect of the country of birth was variable (reference Australia) was noted in a number of the ICD codes analysed, the categories of countries which were significant included:

- S.E. Asia (OR <1 or not significant for all groupings)
- N.E. Asia was associated with an increased risk of morbidity for nutritional anaemias and had a reduced likelihood for pulmonary embolism and “other” pulmonary heart disease.
- N. Africa and the Middle East were at reduced risk of diseases of the oral cavity and an increased risk of fibrosis and cirrhosis of the liver.

TABLE 8-3 SIGNIFICANT VARIABLES IN A MULTIVARIATE LR ANALYSIS OF MORBIDITY ASSOCIATED WTH DISEASES OF THE CIRCULATORY SYSTEM OR SKIN ADJUSTED FOR VARIABLES ANA LEVEL, AGE, GENDER, INDIGENOUS STATUS, COUNTRY OF BIRTH, IRDS AND ARIA AT THE TIME OF THE INCIDENT ANA.

ICD-10 Code and Description		**% ANA ≥7IU/ml (N)**	**p value OR (95%CI) For ANA level**			
			7 - <10IU/ml	**10 - <20IU/ml**	**20 – 30IU/ml**	**30IU/ml**
L93	Lupus Erythematosus	49.2 (63)	0.108	0.023 OR2.997 (1.161-7.738)	<0.001 OR 9.770 (4.856-19.657)	<0.001 OR 18.986 (8.590-41.967)
L94	Other Localised Connective Tissue Disorder	44.1 (59)	0.549	0.384	<0.001 OR 7.725(3.645-16.371)	<0.001 OR 23.146 (10.895-49.173)
I26	Pulmonary Embolism	21.1 (1,137)	0.410	0.503	0.090	<0.001 OR 2.248 (1.495-3.381)
I27	Other Pulmonary Heart Disease	24.4 (1,335)	0.858	0.002 OR 1.442 (1.144-1.819)	<0.001 OR 2.094 (1.652-2.653)	<0.001 OR 3.129 (221-4.407)

Reference categories: ANA level = <7IU/ml, gender = male, Indigenous Status = non Indigenous, Country of birth = Australia, IRDS = highest (4th quartile) and ARIA = highly accessible

Abbreviations for ARIA: A accessible, MA moderately accessible, R = remote and VR = very remote, Abbreviations for IRDS: *1*.First quartile *2*. 2nd quartile, *3*. 3rd quartile.

TABLE 8-3 CONTINUED: SIGNIFICANT VARIABLES IN AMULTIVARIATE LR ANALYSIS OF MORBIDITY ASSOCIATED WTH DISEASES OF THE CIRCULATORY SYSTEM OR SKIN ADJUSTED FOR VARIABLES ANA LEVEL, AGE, GENDER, INDIGENOUS STATUS, COUNTRY OF BIRTH, IRDS AND ARIA AT THE TIME OF THE INCIDENT ANA.

ICD-10 Code and Description		p Value, OR and 95%CI					
		Age	**Gender**	**Indigenous Status**	**Country of Birth**	**IRDS**	**ARIA**[§]
L93	Lupus Erythematosus	0.817	0.292	3.409(1.561-7.446)	0.990	0.390ns	R. 0.005 OR 3.476(1.453-8.318)
I26	Pulmonary Embolism	<0.001 OR 1.019 (1.015-1.022)	0.114	0.004 OR 1.522 (1.145-2.025)	N. Africa & M.E. 0.003 OR 0.460 (0.275-0.771) S.E. Asia 0.049 OR 0.561(0.351-0.999) N.E. Asia 0.024 OR 0.445 (0.220-0.898)	0.083	A. <0.001 OR 0.657(0.526-0.819) M.A. 0.001 OR 0.658(0.520-0.833) R. 0.004 OR 0.484(0.296-0.793) V.R. 0.002 OR 0.655(0.505-0.851)
I27	Other Pulmonary Heart Disease	<0.001 OR 1.046 (1.042-1.050)	<0.001 OR 0.775 (0.694-0.866)	<0.001 OR 3.311 (2.594-4.226)	N.E. Asia 0.004 OR 0.376 (0.193-0.732)	0.097	A. <0.001 OR 0.565(0.451-0.707) M.A 0.001 OR. 0.675(0.540-0.843) V.R <0.001 OR. 0.565(0.434-0.737)

[§] Reference categories: ANA level = <7IU/ml, gender = male, Indigenous Status = non Indigenous, Country of birth = Australia, IRDS = highest (4th quartile) and ARIA = highly accessible

Abbreviations for ARIA: A accessible, MA moderately accessible, R = remote and VR = very remote, Abbreviations for IRDS: *1*.First quartile, *2*. 2nd quartile, *3*. 3rd quartile

TABLE 8-4 MULTIVARIATE LR ANALYSIS OF LIVER DISEASE MORBIDITY ADJUSTED FOR ANA, AGE, GENDER, INDIGENOUS STATUS, COUNTRY OF BIRTH, IRDS AND ARIA AT THE TIME OF THE INCIDENT ANA.

ICD-10 Code and Description		% ANA ≥7IU/ml (N)	p Values, OR and 95%CI For ANA Level			
			7 - <10IU/ml	10 - <20IU/ml	20 – 30IU/ml	>30IU/ml
K00 – 14	**Diseases of the oral cavity, salivary glands and jaw**	18.5 (3,945)	0.804	0.245	0.017 OR 1.242(1.040-1.483)	0.038 OR 1.354(1.017-1.804)
K20 –31	**Diseases of the oesophagus, stomach and duodenum**	19.6 (14,576)	0.303	0.634	0.616	0.001 OR1.363 (1.144-1.622)
K72 – 77	**Diseases of the Liver (excluding Alcoholic liver disease Toxic Liver Disease)**	18.5 (3,821)	0.	0.096	0.004 OR 1.306(1.089-1.565)	<0.001 OR 1.789(1.369-2.339)
K73	**Chronic Hepatitis (not classified elsewhere)**	29.9 (137)	0.352	0.026 OR 2.109 (1.095-4.064)	<0.001 OR4.579(2.587 – 8.104)	0.011 OR 3.684(1.344-10.101)
K75	**Other Inflammatory liver disease**	28.7 (654)	0.063	<0.001 OR 1.970(1.462-2.656)	<0.001 OR 2.897(2.144-3.915)	<0.001 OR 4.053(2.639-6.225)

Reference categories: ANA level = <7IU/ml, gender = male, Indigenous status = non Indigenous, Country of birth = Australia, IRDS = highest (4th quartile) and ARIA = highly accessible

TABLE 8-4 CONTINUED: MULTIVARIATE LOGISTIC REGRESSION ANALYSIS ADJUSTED FOR ANA, AGE, GENDER, INDIGENOUS STATUS, COUNTRY OF BIRTH, IRDS AND ARIA AT THE TIME OF THE INCIDENT ANA RESULT.

ICD-10 CODE AND DESCRIPTION		p Value OR and 95%CI					
		Age	**Gender**	**Indigenous Status**	**Country of Birth**	**IRDS (Quartile)**	**ARIA[§]**
K00–14	Diseases of the Oral cavity, salivary glands and jaw	<0.001 OR 0.980 (0.978-0.982)	0.122	<0.001 OR 1.304 (1.131-1.504)	N Africa and M.E. <0.001 OR0.529 (0.407-0.686) S.E. Asia <0.001 OR 0.590(0.441-0.790)	*1.* 0.001 OR 0.839(0.762-0.925) *2.* <0.001 OR 0.782(0.709-0.862) *3.*0.006 OR 0.881(0.804-0.964)	VR. <0.001 OR 0.663 (0.581-0.757) UN.0.027 OR 4.358(1.184-16.049)
K20–31	Diseases of the oesophagus, stomach and duodenum	<0.001 OR 1.014 (1.013-1.015)	0.581ns	<0.001 OR 1.220(1.107-1.344)	Oceania & Antarctic0.035 OR 0.877 (0.777-0.991) N.W. Europe 0.021 OR 1.060 (1.009-1.113) S & C. Asia 0.014 OR 0.707(0.603-0.828)	*2.* 0.002 OR 0.914(0.864-0967)	<0.001* A. OR 0.834 (0.781 – 0.891) MA. OR 0.778(0.724-0.836) R. OR 0.734 (0.645-0.835) VR OR .0.577(0.531-0.627)
K72–77	Diseases of the Liver (excluding Alcoholic liver & Toxic Liver Disease)	<0.001 OR 1.008 (1.006-1.010)	<0.001 OR 0.532 (0.457-0.568)	<0.001 OR 2.430 (2.103-2.807)	N. Africa & M.E <0.001 OR 1.495 (1.247-1.793) S.E. Asia OR 0.012 OR1.333 (1.064-1.671)	*2.* 0.001 OR1.173 (1.065-1.292)	<0.001* A. OR 0.542 (0.474 – 0.621) MA OR 0.632 (0.552-0.724) R. OR 0.646 (0.512-0.814) VR OR .0.457 (0.393-0.533)
K73	Chronic Hepatitis (not classified elsewhere)	0.226	<0.001 OR 0.503 (0.357–0.709)	0.491	0.417	0.907	0.523
K75	Other Inflammatory liver disease	0.636	0.018 OR 0.826 (0.705-0.968)	0.946	0.879	0.319	A .<0.001 OR 0.507 (0.363 – 0.707) MA 0.024 OR. 0.702(0.516-0.955) VR <0.001 OR . 0.489(0.333-0.717)

Abbreviations for ARIA: A accessible, MA moderately accessible, R = remote and VR = very remote, See additional information on Table 8-4

* P value =< 0.001 for each category

TABLE 8-5 SIGNIFICANT VARIABLES IN THE MULTIVARIATE LR ANALYSIS FOR MORBIDITIES GROUPED BY ICD-10 CODE ADJUSTED FOR ANA, AGE, GENDER, INDIGENOUS STATUS, IRDS, ARIA AND COUNTRY OF BIRTH.

ICD-10 Code and Description		% ANA ≥7IU/ml (Total)	p Value, OR (95%CI) For ANA Level			
			7 - <10IU/ml	10 - <20IU/ml	20 – 30IU/ml	>30IU/ml
D50-53	Nutritional Anaemias	20.7 (4,440)	0.037 OR 1.116(1.007-1.236)	0.555	0.002 OR1.296 (1.103-1.523)	0.003 OR1.477 (1.139-1.916)
E11	Type II Diabetes	16.3 (7,138)	0.081	0.007 OR 0.833 (0.729-0.951)	0.001 OR 0.749 (0.636-0.883)	<0.001 OR 0.566 (0.417-0.768)
G70 – 73*	Diseases of myoneural junction and muscle	21.0 (443)	0.314	0.023 OR1.548(1.061-2.257)	0.070	0.030 OR 2.098 (1.074-4.098)
G70	Myasthenia gravis and other myoneural disorders	32.8 (73)	ns	2.945 (1.439-6.030)	ns	4.149 (1.281-13.433)
G73	Diseases of myoneural junction & muscle disease classified elsewhere	56.0 (25)	0.786	0.143	<0.001 OR 17.503 (6.887-44.487)	<0.001 OR 19.709 (5.381-72.196)
N08	Glomerular Disorders	34.8 (155)	0.903s	0.003 OR = 2.468 (1.349-4.517)	<0.001 OR=4.818 (2.770-8.381)	<0.001 OR=15.748 (9.256-26.794)

* Further analysed in subgroups

Reference categories: ANA level = <7IU/ml, gender = male, Indigenous Status = non Indigenous, Country of birth = Australia, IRDS = highest (4th quartile) and ARIA = highly accessible

TABLE 8-5 CONTINUED: SIGNIFICANT VARIABLES IN THE MULTIVARIATE LR ANALYSIS FOR MORBIDITIES GROUPED BY ICD-10 CODE ADJUSTED FOR ANA, AGE, GENDER, INDIGENOUS STATUS, IRDS, ARIA AND COUNTRY OF BIRTH.

*ICD -10 Code	OR and 95%CI					
	Age	**Gender**	**Indigenous Status**	**Country of Birth**	**IRDS (Quartile)**	**ARIA[§]**
D50-53	<0.001 OR1.026 (1.024-1.028)	<0.001 OR 1.149 (1.077-1.225)	<0.001 OR 3.95 (3.48-4.49)	S.E. Asia 0.005 OR 0.653 (0.484-0.879) N.E. Asia <0.001 OR 1.525 (1.213-1.918)	*1.* <0.001 OR 1.38(1.26-1.51) *2.* 0.043 OR 1.102 (1.003-1.212) *3.* <0.001 OR 1.214(1.110-1.328)	A. <0.001 OR 0.754 (0.675-0.843) MA. <0.001 OR 0.683 (0.604-0.771) R. 0.001 OR 0.709 (0.575-0.875) VR. <0.001 OR 0.467 (0.405-0.539)
E11	<0.001 OR 1.048 (1.046-1.049)	<0.001 OR 0.731 (0.694-0.771)	<0.001 OR9.330 (8.407-10.355)	N.W. Europe 0.002 OR 1.11(1.04-1.18) S.E. Europe 0.002 OR 1.336(1.109-1.608) N. Africa & M.E 1.668(1.428-1.949) N.E Asia <0.001 2.005(1.656-2.428)	*1.* <0.001 OR 1.734 (1.601-1.878) *2.* <0.001 OR 1.349 (1.244-.463) *3.* <0.001 OR 1.382 (1.278-1.495)	A. 0.006 OR 0.817 (0.745-0.897) MA.<0.001 OR 0.873 (0.792-0.962) VR <0.001 OR. 0.653 (0.584-0.730)
G70 – 73	<0.001 OR 1.025 (1.019-1.031)	0.002 OR 0.744 (0.616-0.900)	<0.001 2.203 (1.433-3.387)	0.246	0.752	A. 0.030 OR 0.68 (0.48-0.96) MA. 0.006 OR 0.57 (0.38-0.85) VR. 0.014 OR 0.570 (0.36-0.89)
G70	<0.001 OR 1.02(1.01-1.04)	0.119	0.264	0.505	0.499	0.180
N08	0.753	0.001 OR0.587 (0.424-0.812)	<0.001 OR 7.653 (4.662-12.565)	0.889	0.0119	MA. 0.021 OR 0.439 (0.218-0.885) VR. <0.001 0.172 (0.080-0.370)

[§]Abbreviations for ARIA: A= accessible, MA = moderately accessible, R = remote and VR = very remote, UN= undefined. Abbreviations for IRDS: *1.*1st quartile, *2.* 2nd quartile, *3.* 3rd quartile

*D50-53 Nutritional Anaemias, E11 Type II Diabetes, G70-73 Myoneural junction and muscle G70 Myasthenia gravis and other myoneural disorders, G73 Myoneural junction and muscle classified elsewhere, N08 Glomerular Disorders.

8.3.3 ANA AND CANCER INPATIENT MORBIDITY

The influence of an ANA result on hospital admission for cancer morbidities was also investigated. The frequency of malignancies and benign neoplasms described in the ICD-10 Codes (alpha character A and B) were calculated for the first hospital admission and all admissions in the dataset and groups with a proportion of ANA results ≥ 10IU/ml greater than 10% were investigated further.

In the ANA positive group Melanoma and other malignant neoplasms of the skin (n = 541) were reported with the highest frequency of a neoplasm in the primary diagnosis when all admissions were included n = 113,843, however the ANA result was not a significant variable in determining the likelihood of an admission related to the ICD groups C43 – C44 when considered in univariate analysis p = 0.557 or in the multivariate LR model p = 0.163 (OR=0.905, 95%CI 0.787 – 1.041).

Conversely, the ANA levels ranging from ≥7 to 30IU/ml were significant groups in the multivariate analysis of hospital admission for malignant neoplasm of the respiratory and intrathoracic organs. In this broad category 26.4 % of individuals were ANA positive. Other significant variables were age (OR=1.046, 95%CI 1.041-1.051), a country of birth of N.W. Europe (OR=1.331, 95%CI 1.115-1.589) and individuals in the 1st Quartile of the IRDS OR=1.676 (95%CI 1.340-2.096).

Malignant neoplasms of the oesophagus and secondary and unspecified malignant neoplasms of the lymph nodes were only significant at the highest level of ANA. Additional ICD groups which had a statistically significant OR in the LR model analysis are shown in Tables 8.7 and 8.8.

TABLE 8-6 SIGNIFICANT VARIABLES IN A MULTIVARIATE LR ANALYSIS FOR CANCER MORBIDITIES GROUPED BY ICD-10 CODE ADJUSTED FOR ANA, AGE, GENDER, INDIGENOUS STATUS, IRDS, ARIA AND COUNTRY OF BIRTH.

ICD-10 Code and Description		% ANA ≥7IU/ml (N)	p Value, OR (95%CI) For ANA Level			
			7 - <10IU/ml	10 - <20IU/ml	20 – 30IU/ml	>30IU/ml
C15	Malignant neoplasm of the oesophagus	32.1 (78)	0.134	0.069	0.115	<0.001 OR 8.705 (3.419-22.166)
C22.0	Malignant neoplasm Liver cell carcinoma	16.2 (296)	0.027 OR 1.484 (1.045-2.106)	0.055	0.255	0.773ns
C30 – C39	Malignant neoplasms of respiratory and intrathoracic organs	26.4 (652)	0.001 OR 1.484 (1.170-1.882)	<0.001, OR 1.843 (1.361-2.497)	0.003 OR 1.772 (1.222 – 2.571)	0.094
C34	Malignant neoplasm of bronchus and lung	27.3 (586)	<0.001 OR 1.593 (1.249-2.032)	0.001 OR 1.744 (1.257-2.418)	0.002 OR 1.817 (1.236-2.670)	0.045 OR 1.920 (1.013-3.638)
C77	Secondary and unspecified malignant neoplasms of lymph nodes	21.8 (1012)	0.474	0.067	0.193	0.002 OR 2.042 (1.309-3.185)
C79	Secondary of other and unspecified	23.5 (857)	0.005 OR1.355(1.098-1.672)	<0.001 OR 1.645(1.255-2.156)	0.596	0.877
C83.3	Diffuse Large B Cell lymphoma	21.6 (153)	0.182	0.001 OR 2.444 (1.462-4.087)	0.860	0.670

Reference categories: ANA level = <7IU/ml, gender = male, Indigenous Status = non Indigenous, Country of birth = Australia, IRDS = highest (4th quartile) and ARIA = highly accessible

TABLE 8-6 CONTINUED. SIGNIFICANT VARIABLES IN A MULTIVARIATE LR ANALYSIS FOR CANCER MORBIDITIES GROUPED BY ICD-10 CODE ADJUSTED FOR ANA, AGE, GENDER, INDIGENOUS STATUS, IRDS, ARIA AND COUNTRY OF BIRTH.

ICD-10 Code and Description		p Value, OR (95%CI)					
		Age	**Gender**	**Indigenous Status**	**Country of Birth**	**IRDS**	**ARIA**
C15	Malignant neoplasm of the oesophagus	<0.001 OR 1.05 (1.04-1.07)	<0.001 OR 0.285 (0.18-0.46)	0.273	0.879	0.844	0.862
C22.0	Malignant neoplasm Liver cell carcinoma	<0.001 OR 1.029 (1.022-1.037)	<0.001 OR 0.180 (0.135-0.240)	<0.001 OR 2.947 (1.663-5.222)	See below [β]	0.176	MA 0.018 OR 0.488 (0.269-0.885) VR 0.013 OR 0.469 (0.258-0.852)
C30–39	Malignant neoplasms of respiratory and intrathoracic organs	<0.001 OR 1.046 (1.041-1.051)	<0.001 OR 0.495 (0.422-0.581)	0.458	N.W. Europe 0.002 OR 1.331 (1.115-1.589)	*1.* <0.001 OR 1.68 (1.340-2.096)	0.259
C34	Malignant neoplasm of bronchus and lung	<0.001 OR 1.047 (1.04-1.05)	<0.001 OR 0.55 (0.463-0.647)	0.544	N.W. Europe <0.001 OR 1.434 (1.191-1.726)	*1.* <0.001 OR 1.682 (1.331-2.125)	0.398
C77	Malignant neoplasms of lymph nodes	<0.001 OR 1.030 (1.026-1.034)	0.055	0.760	Africa (Excluding N. Africa) 0.017 OR 0.374 (0.167-0.840)	0.764	0.112
C79	Secondary of other and unspecified	,0.001 OR 1.042(1.038-1.047)	<0.001 OR 0.647 (0.564-0.743)	0.822	0.345	*1.* 0.008 OR 1.305 (1.072-1.588)	0.233
C83.3	Diffuse Large B Cell lymphoma	<0.001 1.033 (1.023-1.044)	0.009 OR0.650 (0.471-0. 896)	0.984	S. & .E. Europe 0.001 OR 3.487 (1.720-7.067) Undefined 0.001 OR 2.92 (1.54-5.55)	0.279	0.736

[§]A Accessible, MA Moderately Accessible, R Remote, VR Very Remote, UN undefined

[β]Oceania and Antarctic (excluding. Australia) 0.039, OR 1.987(1.034-3.819), S.& E. Europe <0.001, OR 3.489(2.037-5.976), N. Africa & M.E <0.001, OR 4.291(2.654-6.939), S.E. Asia <0.001, OR 6.335(3.903-10.283), Americas 0.010, OR 3.764 (1.367-10.365), Africa (excl. N Africa) 2.743(1.376-5.465)

8.3.4 ANA AND CANCER REGISTRY DATA

The linking of the ANA incident cohort and cancer registry entries resulted in merged data from both databases for 6,424 individuals. As year of diagnosis was the variable for date of diagnosis in the cancer registry cases were excluded if the cancer diagnosis preceded the ANA result by more than 1 year.

An incident ANA ≥7IU/ml was reported in 18.6% of the individuals identified in the Cancer Registry. The most frequently reported cancers were breast (ICD-10 Code C50; n = 532, %ANA ≥7IU/ml = 21.8%), bronchus and lung (ICD-10 Code C34; n = 518, %ANA ≥7IU/ml = 26.1%), prostate (ICD-10 Code C61; n = 481, % ANA ≥7IU/ml = 15.6 %), colon (ICD-10 C18; n = 323, %ANA ≥ 7IU/ml = 22.0%) and skin (ICD Code C43, n = 301, %ANA ≥ 7IU/ml = 16.6%).

The cancer diagnosis with the highest frequency of ANA ≥7IU/ml was non-Hodgkin mature B cell lymphoma (n = 206, %ANA ≥7IU/ml = 27.7%, followed by lung cancer (ICD-10 Code C34) as described in Section 8.3.3 (n=518, %ANA ≥ 7IU/ml = 26.1%).

An increased risk of Lung and Oesophagus Cancer was observed in both the inpatient morbidity and cancer registry data cancer with either increasing ANA level or at the highest level of ANA (for cancer of the Oesophagus). Data are shown in Tables 8-6 and 8-7.

TABLE 8-7 SIGNIFICANT VARIABLES INVESTIGATED IN A MULTIVARIATE LR ANALYSIS FOR REGISTRY CANCER CASES ADJUSTED FOR ANA, AGE, GENDER INDIGENOUS STATUS, IRDS, ARIA AND COUNTRY OF BIRTH.

Cancer Site Description	**% ANA ≥7IU/ml (Total)**	**p Value. OR (95%CI)**			
		7 - <10IU/ml	**10 - <20IU/ml**	**20 – 30IU/ml**	**>30IU/ml**
Non Hodgkin, mature B cell Lymphoma	27.7% 203	0.367 OR 1.230 (0.784–1.930)	0.008 OR 1.993 (1.200-3.309)	0.092 OR 1.743 (0.912-3.329)	<0.001 OR 4.345 (2.105-8.967)
Diffuse Large B Cell lymphoma C83.3)	29.8% 94	0.480	<0.001 OR 3.487 (1.944– 6.255)	0.189	0.037 OR 3.472 (1.08–11.161)
Lung (C34)	26.1% 518	0.012 OR 1.409 (1.079-1.841)	0.003 OR 1.690 (1.193-2.395)	0.009 OR 1.737 (1.148-2.627)	0.063
Oesophagus (C15)	23.1% 52	0.262	0.994	0.526	0.03 OR4.89 (1.16 – 20.49)

Reference categories: ANA level = <7IU/ml, gender = male, Indigenous Status = non Indigenous, Country of birth = Australia, IRDS = highest (4th quartile) and ARIA = highly accessible

TABLE 8-8 SIGNIFICANT VARIABLES INVESTIGATED IN A MULTIVARIATE LR ANALYSIS FOR CANCER REGISTRY CASES ADJUSTED FOR ANA, AGE, GENDER, INDIGENOUS STATUS, IRDS, ARIA AND COUNTRY OF BIRTH.

ICD-10 Code and Description	p Value, OR and 95%CI					
	Age	**Gender**	**Indigenous Status**	**Country of Birth**	**IRDS**	**ARIA**
Non Hodgkin, mature B cell Lymphoma	<0.001 1.04 (1.03-1.04)	0.006 0.68 (0.51-0.89	0.005 Ungrouped 0.45 (0.01-0.34)	0.017 S. & E. Europe 2.491 (1.242-4.995) Undefined 2.881 (1.632-5.088)	ns	ns
Diffuse Large B Cell lymphoma C83.3)	<0.001 OR1.046 (1.033-1.059)	0.192	0.999	0.164	0.192	0.609
Lung (C34)	<0.001 OR 1.055 (1.049-1.062)	<0.001 OR 0.534 (0.448-0.638)	0.789	0.075	*1* <0.001 OR 1.718 (1.335-2.211) *3.* <0.001 *OR* 1.381 (1.078-1.769)	0.082
Oesophagus (C15)	<0.001 OR 1.053 (1.035-1.072)	<0.001 OR 0.289 (0.159 - 0.524)	0.981	0.997	0.959	0.858

Reference categories: ANA level = <7IU/ml, gender = male, Indigenous Status = non Indigenous, Country of birth = Australia, IRDS = highest (4th quartile) and ARIA = highly accessible

Abbreviations for ARIA: A accessible, MA moderately accessible, R = remote and VR = very remote

Abbreviations for IRDS: *1.*1st quartile *2.* 2nd quartile, *3.* 3rd quartile

8.3.5 ANA AND A MORBIDITY OF INFECTION

Infection is reported as a common cause of hospital admission in a number of autoimmune conditions (175) and as such ICD codes for broad categories of infection were investigated in a Multivariate LR analysis (See Table 8-9). In this analysis the influence of ANA on predicting the likelihood of admission for infection in the incident ANA cohort was investigated in a model adjusted for: Indigenous Status, age at time of ANA testing, gender, IRDS, ARIA, country of birth and ANA grouped into levels.

ANA ≥20IU/ml or ≥30IU/ml was associated with a hospitalisation and diagnosis of infection in each of the broad categories of infection investigated as shown in Table 8-9. An ANA ≥7IU/ml was reported in 22.5, 22.1 and 20.5% of positive cases of HZV, CMV and Viral Infections respectively characterised by skin and mucous membrane lesions (see Table 8-8). The highest predictive values of ANA ≥30IU/ml for infection were associated with an HZV or CMV infection. Low level ANA was significant in the multivariate LR model for the broad group of "Other Viral Infections".

An ANA of 10-<20IU/ml was associated with a reduced likelihood of viral hepatitis (OR=0.739, 95%CI 0.571-0.955) and staphylococcus or streptococcus infection (OR=0.827, 95%CI 0.710-0.963).

Where age was a significant predictor of an outcome of infection, increasing age was associated with a reduced likelihood of a diagnosis of CMV (OR=0.986, 95%CI 0.976-0.997), Viral Hepatitis (OR=0.972, 95%CI 0.970-0.975) and Other Viral Infections (OR=0.979, 95%CI 0.976-0.983). Increased age was associated with viral infections of skin and mucous membrane lesions (OR=1.019, 95%CI 1.015-1.023), HZV (OR=1.046, 95%CI 1.039-1.052), Streptococcus and Staphylococcus (OR=1.023, 95%CI 1.021-1.025), and Mycoses (OR=1.024, 95%CI 1.022-1.026). Data are shown in Table 8-8.

Where gender was a predictor of an inpatient morbidity of infection (Viral Hepatitis, CMV, Streptococcus and Staphylococcus and Influenza and Pneumonia), it was shown to be protective in females in the model adjusted for each of the variables Indigenous Status, age, gender, IRDS, ARIA and country of birth and ANA grouped in levels.

In these models Indigenous individuals were more likely to have a morbidity of viral infections (including HSV and CMV infection) and mycoses and Streptococcus and

Staphylococcus infection compared with non-Indigenous individuals. The strongest association was seen between Indigenous Status and with a Streptococcus and Staphylococcus infection (OR = 4.418, 95%CI 3.954-4.936).

Country of Birth referenced against Australia was significantly associated with a diagnosis of Viral Hepatitis for Oceania & Antarctica (excluding Australia) (OR=1.863; 95%CI 1.507-2.304), S & E Europe (OR=1.690, 95%CI 1.274-2.243), N. Africa & M.E (OR=3.551, 95%CI 2.973-4.241) and S.E. Asia (OR4.434, 95%CI 3.668-5.360). Data are shown in Table 8-9.

The multivariate LR analysis did not identify ARIA (referenced against the most accessible areas) as a variable predictive for an increased likelihood of infection.

Lower IRDS was associated with an increased likelihood of viral infections of skin & mucous membrane lesions, viral hepatitis, other viral illnesses, mycoses and Streptococcus and Staphylococcus. Data are shown in Tables 8.8.

TABLE 8-9 SIGNIFICANT VARIABLES IN THE MULTIVARIATE LR ANALYSIS FOR INFECTION (DEFINED BY ICD-10 CODES) AS A MORBIDITY ADJUSTED FOR ANA LEVEL, INDIGENOUS STATUS, AGE, GENDER, COUNTRY OF BIRTH, IRDS AND ARIA.

ICD-10	Code and Description	% ANA ≥7IU/ml (Total)	p Value, OR 95%CI			
			7 - <10IU/ml	10 - <20IU/ml	20 – 30IU/ml	>30IU/ml
B00 – B09	Viral Infections characterised by skin and mucous membrane lesions	20.5 (837)	0.420	0.964	0.009 OR 1.538(1.111-2.128)	<0.001 OR 2.5781.669-3.981
B15 – B19	Viral Hepatitis	13.1 (2,177)	0.406	0.021 OR 0.739(0.571-0.955)	0.023 OR 0.685(0.494-0.950)	0.206
B25 – B34	Other Viral Illnesses	19.7 (1,184)	0.004 OR 1.308(1.087-1.573)	0.497	0.296	0.032 OR 1.660(1.044-2.638)
B35-B49	Mycoses	19.9 (2,991)	0.732	0.398	0.046 OR1.222(1.004-1.487)	0.010 OR 1.498(1.102-2.034)
B02	Herpes Zoster (HZV)	22.5 (386)	0.259	0.170	0.021 OR1.7(1.084-2.664)	<0.001 OR 3.014(1.674-5.426)
B25	CMV	22.1 (122)	0.517	0.536	0.599	0.002 OR 4.273(1.715-10.649)
B95	Streptococcus and Staphylococcus	17.2 (5,193)	0.253	0.015 OR 0.827(0.710-0.963)	0.432	0.003 OR 1.458(1.136-1.872)
J09 - J18	Influenza and pneumonia	19.2 (1,180)	0.629	0.158	0.004 OR1.249(1.074-1.451)	<0.001 OR1.605(1.272-2.026)

Reference categories: ANA level = <7IU/ml, gender = male, Indigenous Status = Non-Indigenous, Country of birth = Australia, IRDS = highest (4th quartile) and ARIA = highly accessible

TABLE 8-9 CONTINUED: SIGNIFICANT VARIABLES IN THE MULTIVARIATE LR ANALYSIS FOR INFECTION (DEFINED BY ICD-10 CODES) AS A MORBIDITY ADJUSTED FOR ANA LEVEL, INDIGENOUS STATUS, AGE, GENDER, COUNTRY OF BIRTH, IRDS AND ARIA.

ICD-10 Code and Description		**p Value, OR and 95%CI**		
		Age	**Gender**	**Indigenous Status**
B00 – B09	Viral Infections (skin and mucous membrane lesions)	<0.001 1.019 (1.015-1.023)	0.432	0.030 OR 1.440 (1.035-2.004)
B15 – B19	Viral Hepatitis	<0.0010.972 (0.970-0.975)	<0.0010.306 (0.279-0.336)	0.726
B25 – B34	Other Viral Illnesses	<0.001 OR 0.979 (0.976-0.983)	0.082	0.486
B35-B49	Mycoses	<0.001 OR 1.024 (1.022-1.026)	0.531	<0.001 OR 3.063 (2.632-3.565)
B02	Herpes Zoster (HZV)	<0.001 OR 1.046 (1.039-1.052)	0.726	0.030 OR 1.801 (1.059-3.061)
B25	Cytomegalovirus (CMV)	0.009 OR 0.986 (0.976-0.997)	0.003 OR 0.583 (0.406-0.836)	0.046 OR 2.115 (1.014-4.414)
B95	Streptococcus and Staphylococcus	<0.001 OR 1.023 (1.021-1.025)	<0.00 OR 0.740 (0.698-0.785)	<0.001 OR 4.418 (3.954-4.936)
J09-18	Influenza and pneumonia	<0.001 OR 1.036 (1.035-1.038)	<0.001 OR 0.648 (0.613-0.684)	<0.001 OR 5.301 (4.763-5.900)

Reference categories: ANA level = <7IU/ml, gender = male, Indigenous Status = Non-Indigenous, Country of birth = Australia, IRDS = highest (4th quartile) and ARIA = highly accessible

TABLE 8-9 CONTINUED: SIGNIFICANT VARIABLES IN THE MULTIVARIATE LR ANALYSIS FOR INFECTION (DEFINED BY ICD-10 CODES) AS A MORBIDITY ADJUSTED FOR ANA LEVEL, INDIGENOUS STATUS, AGE, GENDER, COUNTRY OF BIRTH, IRDS AND ARIA.

ICD-10 Code and Description		OR and 95%CI		
		Country of Birth	**IRDS**	**ARIA**
B00 – B09	Viral Infections (skin & mucous membrane lesions)	0.785	*1.0.044 OR* 1.236(1.005-1.518)	R.0.017 OR 0.687(0.504-0.936)
B15 – B19	Viral Hepatitis	Oceania & Antarctica§ <0.001 OR 1.863 (1.507-2.304) S & E Europe <0.001 OR 1.690 (1.274-2.243) N. Africa &M.E. `<0.001 OR 3.551(2.973-4.241) S.E. Asia <0.001 OR 4.434(3.668-5.360 Other 0.002 OR 1.468(1.145-1.881)	*1.*<0.001 OR 1.372 (1.210-1.556) *3.*0.002 OR 1.207(1.069-1.362)	A. <0.001 OR 0.430 (0.352-0.526) MA <0.001 OR 0.402 (0.324-0.500) R <0.001 OR 0.556 (0.403-0.766) VR <0.001 OR 0.343(0.276-0.425)
B25 – B34	Other Viral Illnesses	N.W. Europe 0.045 OR 0.840 (0.709-0.996) S.E. Asia 0.008 OR 0.446(0.245-0.813) N.A 0.008 OR 0.517(0.318-0.840)	*2.* 0.039 OR 1.1197(1.009-1.420)	MA 0.040 OR 0.795(0.639-0.990) VR 0.015 OR 0.753(0.599-0.947)
B35-B49	Mycoses	N. Africa &M.E. 0.015 OR 0.716 (0.547-0.937) S.E. Asia 0.031 OR 0.689 (0.491-0.966) N.A 0.004 OR 0.672 (0.513-0.880)	*1.* <0.001 OR 1.226(1.098-1.370) *3.* 0.028 OR 1.127(1.013-1.253)	A . <0.001 OR 0.680(0.593-0.780 MA . <0.001 OR 0.654(0.564-0.758) R . <0.001 OR 0.587 (0.449-0.768) VR . <0.001 OR 0.578(0.491-0.680)
B95	Streptococcus and Staphylococcus	N. Africa & M.E. 0.003 OR 0.610 (0.441-0.845) N.E. Asia <0.001 OR 0.508 (0.354-0.728)	*1.*<0.001 OR 1.409 (1.293-1.536) *3.* 0.001 OR 1.149(1.056-1.251)	A. <0.001 OR 0.693 (0.623-0.771) MA <0.001 OR 0.618 (0.550-0.694) R <0.001 OR 0.684 (0.564-0.829) VR <0.001 OR 0.601 (0.534-0.677)
J09 - 18	Influenza and pneumonia	N. Africa &M.E. 0.042 OR 0.752 (0.571-0.990) S.E. Asia 0.028 OR 0.752 (0.582-0.970) N.E. Asia<0.001 OR 0.491(0.352-0.686)	*1.* <0.001 OR 1.459(1.344-1.585) *2.* <0.001 OR 1.174(1.079-1.277 *3.* <0.001 OR 1.268(1.171-1.374	A. <0.001 OR 0.693 (0.627 – 0.767) MA <0.001 OR.0.768 (0.693-0.852) VR <0.001 OR.0.709(0.634-0.794)

OR's for HZV and CMV variables for country of birth, IRDS and ARIA were not significant. §Excludes Australia Reference categories: ANA level = <7IU/ml, gender = male, Indigenous Status = Non-Indigenous, Country of birth = Australia, IRDS = highest (4^{th} quartile) and ARIA = highly accessible

8.4 DISCUSSION

The broad spectrum of over 100 autoimmune diseases reported to affect 5% of Australians (176) lead to significant disability resulting from immune mediated organ and tissue damage. For example, the risk of cardiovascular events or nephritis is increased in SLE and SSc (139, 177, 178), and haematological morbidities related to coagulation, pulmonary fibrosis or hypertension and skin involvement morbidities are associated with systemic sclerosis (179). The consequences of tissue and/or organ damage may result in hospitalisation for treatment and disease management.

Linked hospital discharge and ANA outcome data were used in this study to determine the spectrum of clinical presentations for which individuals with an ANA outcome were hospitalised and the significance of the ANA result in the specific morbidities recorded during the course of disease.

Morbidity associated with increasing age is an important confounder in this investigation of morbidity associated with ANA. The frequency of a number of morbidities (e.g. Cataract, heart failure, stroke and arterial disease (180-184) are known to increase with age along with the frequency of ANA ≥7IU/ml as shown in Section 5.3.1.

Due to the expansive range of morbidity codes recorded in the morbidity dataset, the analysis of morbidity data was initially performed on broad groupings based on frequency data from the primary diagnosis code at the first presentation for an individual. As a consequence, the morbidities investigated were not refined; however they covered key hospital episode diagnosis in autoimmune disease or diagnoses in which there was a high frequency of ANA ≥7IU/ml.

In a multivariate LR model, adjusted for Indigenous Status, age, gender, country of birth, ARIA, IRDS, age remained a significant predictor for a number of the morbidities investigated; i.e. for: Dermatomyositis, SSc, systemic connective tissue disorders, and RA (with the exception of Juvenile Arthritis).

Increasing ANA levels ≥7IU/ml were statistically significant predictor variables for a morbidity associated with the musculoskeletal diseases SLE, SjS, SSc, local connective tissue disease and glomerular disorders (OR ranged from 1.5 – 115.2).

Low level ANA (7 - <10IU/ml) was not a significant factor in the multivariate analysis of morbidity associated with the circulation, digestive system or myoneural junction and muscle (ICD-10 Code G73). However at the highest level of ANA (>30IU/ml) the majority of the morbidities investigated were significantly associated with hospitalisation for the above conditions. Neuromuscular disorders include myasthenia gravis and involve antibodies targeting the acetyl choline receptor, muscle specific kinases or the voltage gated channel proteins and these antibodies do not typically have an associated ANA (185). Interestingly, autoimmune thyroid disease and rheumatic diseases including SjS and SLE can be associated with neuromuscular disease and may account for the significance of the high ANA in this patient group (186, 187).

Country of birth and ANA were significant variables for a number of morbidities. The broad classification of N.W. Europe was associated with an increased likelihood of seropositive RA, malignancies of the respiratory and intrathoracic organs, bronchus and lung and diseases of the oesophagus, stomach and duodenum. The Americas group was identified as significant for a morbidity of Dermatomyositis (OR=4.4, 95%CI 1.3 – 14.3). North Africa and the Middle East and S.E. Asia were both more likely to have a hospital admission for diseases of the liver OR=1.495 (95%CI 1.247-1.793) and OR=1.333 (95%CI 1.064-1.671) respectively compared with Australian born individuals.

A comparison of the prevalence of SLE in population studies in various countries reviewed by D'Cruz *et al* (139) showed an increased frequency in all races in the USA (52.2/100,000), Spain (91.0/100,000), Afro-Caribbean (159.4/100,000), China (92.9/100,000), the UK, (52.2/100,000) in Italy (71/100,000) and Aboriginal population in Australia (63.1/100.000). A significant OR for an inpatient morbidity associated with dermatomyositis was noted for Country of Birth Americas.

Reports in the literature comment on the influence of various factors such as genetic association, variation across regions and smoking status in the prevalence and severity of inflammatory myopathies (188-190). Rider *et al* (189) describe a higher incidence of myositis associated and specific antibodies detected in racial groups categorised as White verses Black and in a study of adult myositis patients in a USA Cohort.

Although the variables ARIA and country of birth have been included in the model for morbidity these variables will not include additional environmental factors which may

also account for an increased risk of and ANA. Differences such as level of sunlight (vitamin D), infection rate and exposure to other environmental agents such as silica and smoking which may also account for differences in prevalence in place of residence (14, 190-192).

ARIA was not associated with increased odds of morbidity associated with an ANA, more often indicating that less accessible locations were protective relative to the highly accessible locations. This may suggest that those with significant autoimmune disease are living in closer proximity to medical care or those living in remote locations are being managed at regional clinics (192) which are not captured in the morbidity data collection And are not being hospitalised (193)

The predictive value of ANA for a cancer related hospital admission was variable. Unspecified malignant neoplasms of the lymph nodes and oesophagus had a significant OR at the highest level of ANA only (OR=2.0, 95%CI 1.3 - 3.2 and OR=8.7, 95%CI 3.4 – 22.2 respectively). In contrast, the increased likelihood of a hospital admission for liver cell carcinoma was limited to low level positive ANA 7-10IU/ml. The odds of a hospitalisation due to malignant neoplasms of the bronchus, lung, respiratory and intrathoracic organs increased incrementally with ANA level. Selmi *et al* (173) did not demonstrate a similar increased risk of cancer associated with ANA in a general population study in Northern Italy. The authors reviewed 15 year follow up clinical outcome data and ANA for 2,663 randomly selected individuals, however it is important to note that the authors refer to a grouping of "Cancer" without further qualification. The Incident ANA Clinical Cohort used in the current study identified sub groups within cancer with an increased likelihood of hospitalisation with ANA≥7IU/ml.

Autoimmune hepatitis type 1 is a chronic liver disease associated with autoantibodies including ANA (194, 195). The association between chronic liver disease morbidity and ANA was confirmed in this study, the OR for a morbidity related to disease of the liver and more specifically chronic hepatitis ranged from 2.1 to 3.7 in individuals with an ANA greater than 10IU/ml.

Feldman *et al* (172) described a significant burden of infection and an increased risk of viral infections in SLE and Lupus Nephritis patients, however in this study only high level ANA ≥ 20IU/ml was associated with a hospitalisation due to HZV, influenza and

pneumonia, the highest level of ANA was significant in a broad grouping of infections and in CMV infection. In the multivariate LR model the OR's for both Indigenous individuals and a high ANA were significant predictors of hospitalisation due to CMV, HZV or one of the broad group of infections investigate here.

In the multivariate LR model adjusted for the previously defined variables, Indigenous Status along with ANA was a significant variable in a number of morbidity groups including SLE, inflammatory polyarthropathies, pulmonary embolism and various infections (excluding Influenza and Pneumonia). These findings are consistent with reported studies of autoimmune disease in the Indigenous population across Australia which describe a high prevalence of SLE (47, 139, 140, 192) although the organ involvement varies between reports.

9 DISCUSSION

This study has reviewed clinical ANA data collected over 11 years with reference to follow on testing and morbidity and mortality data associated with ANA. The ANA test has been used for the last five decades in diagnostic laboratories and is considered a sensitive screening assay for rheumatic disease. The commonly used IIF assay provides both the pattern of nuclear and/or cytoplasmic staining indicating the presence of autoantibodies typically associated with autoimmune disease; and an estimate of the level of antibody present.

Clinical ANA data reported over 11 years in a diagnostic laboratory using a single ANA method was analysed using a multivariate logistic regression analysis approach to consider the effect of age, gender, country of birth, social disadvantage and residential remoteness on ANA and the predictive value of ANA for hospital morbidity and mortality outcomes.

Typically ANA results are reported as the highest dilution at which a clearly discernible pattern is visible. A novel aspect of this investigation is the method of reporting ANA in IU/ml; measuring fluorescence intensities using a calibration curve standardised against World Health Organisation 66/223 reference serum (10, 11). This has allowed levels to be grouped and analysed in a quantitative manner.

ANA is often reported to be more frequent in older females (173, 196, 197). In addition, ANA in the absence of clinical symptoms is detected in the general population (6, 7, 46, 197). In the current study a higher proportion of females were tested for ANA and a higher proportion were positive for ANA indicating this is not just a reflection of the number of individuals tested but a higher incidence in females compared to males.

In a multivariate analysis age was a significant predictor of an ANA ≥7IU/ml with an OR indicating that the likelihood of a positive ANA increased with age. However, follow-up testing for disease specific markers ENA and dsDNA antibodies did not show the same increased likelihood with age with the exception of antibodies to SSA/Ro52. Notably the highest levels of incident dsDNA antibody were detected in the younger age groups. Individuals with a high ANA were more likely to have a positive dsDNA antibody. Similarly the highest number of coincident ENA antibodies was detected in individuals in the 10 – <20 year age group.

The majority of ANA reported in this clinical cohort were negative or low level <10IU/ml. Indigenous individuals did not have an increased risk of a positive ANA compared to non-Indigenous individuals. However when ANA level, gender and Indigenous Status were compared, a slightly higher but statistically significant proportion of Indigenous females had an ANA >30IU/ml compared to males and non-Indigenous females. In addition a multivariate analysis for dsDNA antibody outcome showed that Indigenous individuals had a significantly higher likelihood of a dsDNA antibody positive result when adjusted for all other variables (140). Indigenous individuals also had an OR >1 for a positive ribosomal antibody which has been associated with liver manifestations in SLE (198, 199).

Analysis of survival outcome from mortality records did not identify ANA outcome or Indigenous Status as significant predictors of survival. Poor survival rates in the Indigenous group were not evident in the current study when ANA level or outcome was included in the survival analysis. This may be attributed to improved diagnostic tools, the introduction of therapeutic agents such as monoclonal antibodies as therapeutic drugs which target inflammatory cytokines and remove the antibody producing B cells for disease management (200-202).

Importantly, and consistent with reports in the literature, dsDNA and ENA antibody are detected in individuals with a negative ANA (203); this may be dependent on a number of factors including the assay antigen preparation (e.g. recombinant v purified antigen) and the result of antibodies binding epitopes exposed on the ENA or dsDNA assay substrate (4) and not exposed on the ANA substrate. It may also be due to the assay method and the immunoglobulin classes detected (i.e. detection of IgM antibodies in the dsDNA antibody FARR assay).

Both ANA level and pattern were found to be important indicators of a detectable ENA antibody. RNP, SSA/Ro60 and SSB/La antibodies were detected more frequently with increasing level of ANA with the homogeneous plus speckled pattern (173, 204). The increased association of ANA with age did not translate to an increased likelihood of a dsDNA antibody positive result or a detectable ENA antibody result. Furthermore, low level dsDNA antibody was detected more frequently in the elderly without a significant

reduction in survival suggesting dsDNA antibody in the elderly is not clinically significant (49, 205).

Multivariate LR analysis of hospital morbidity outcome adjusted for ANA level, age at time of ANA testing, Indigenous Status, ARIA, IRDS and country of birth, confirmed that there was a significantly increased likelihood of an inpatient morbidity of a musculoskeletal condition associated with autoimmune disease in individuals with increasing level of ANA relative to an ANA <7IU/ml outcome. ANA level was also identified as a significant predictor of an inpatient morbidity of glomerular and myoneuronal disorders. Other morbidities that had an increased risk associated with ANA included liver disease and neoplasms related to the respiratory system.

ARIA and SEIFA at the time of the incident ANA report did not show odds ratios in excess of 1.5 for the inpatient morbidities investigated with the exception of dermatomyositis. For this morbidity the country of birth group Americas also had an increased likelihood; with a 4.4-fold greater odds than those born in Australia. ARIA groupings were often associated with reduced likelihood of morbidity when compared to the reference group "highly accessible". The hygiene hypothesis proposes that reduced exposure to pathogens and allergens has resulted in increased levels of allergy and autoimmunity (206). Infection and environmental factors including smoking and exposure to solvents and silica have also been investigated in relation to an increase risk of developing autoimmune disease (206-208). The interaction between residential remoteness and exposure to various environment factors as listed above may need to be considered in the investigation of ANA rather than ARIA as a single factor to account for environmental influences.

The analysis of mortality data did not show a reduced survival rate associated with an ANA ≥7IU/ml with the exception of the specific ANA pattern anti-centromere which is a marker of primary biliary cirrhosis and scleroderma.

The data in this study suggest that the threshold level of ANA relevant to a clinical diagnosis varies between conditions and when used as a screening test, ANA level should be considered in conjunction with clinical presentation before requesting follow up testing. Alternatively, and to aid clinical decision making, additional reference data should be

provided with the ANA laboratory report to indicate the significance of the level for age and gender.

In addition, inpatient morbidity with a description of autoimmune disease related to rheumatic disease was significantly associated with increased levels of ANA. The OR for high level ANA suggests these patients may require more aggressive treatment or more frequent clinical review to minimise the need for hospitalisation.

Overall this study confirms female gender and increasing age are associated with a detectable ANA in a clinical cohort. Background low level ANA is detected in the aging general population (52) which is not associated with a clinical diagnosis of autoimmune disease (119).

High level ANA were detected in both males and females and were associated with clinically significant disease markers dsDNA and ENA antibody, particularly RNP, SmD, SSB and Scl70 antibodies. Interestingly Ro52 antibody detected as a coincidental finding is associated with increasing age and in females, however it is reported disease associations are limited to cardiac block and myositis.

In some cases a clinically significant ANA threshold level of 5 or 7IU/ml for markers such as dsDNA or ENA antibody respectively may be an overestimate and specificity of the assay may be improved if the reference value was raised to 10IU/ml. This could be investigated with the dataset available for this study however it was outside the scope of this study.

9.1.1 LIMITATIONS OF THE STUDY

One of the limitations of this study is that the morbidity details associated with the ANA data were based on inpatient morbidity data and did not include outpatient clinic data. Chronic autoimmune conditions may be managed at the outpatient clinic level; therefore the significance of disease/morbidity outcomes associated with ANA reported here may be an underestimate of the true disease association. However, as it is mandatory to notify a cancer diagnosis to the WA Cancer registry, the analysis of the association between ANA and cancer is likely to be an accurate estimate of association. Interestingly the findings from the ANA and inpatient morbidity analysis and cancer associations were consistent between the two sources.

ENA antibodies are reported at each level of ANA, in the case of RNP, SSB and Scl70 antibody the likelihood of a positive result increases with increasing ANA, however this study does not extend to an investigation of patient morbidity associated with ENA antibody detected at low level ANA compared to high level ANA.

A further limitation of this study is that the morbidity outcomes are based on inpatient diagnostic coding data which was broadly grouped to achieve suitable group sizes. This may have impacted the results by reducing the sensitivity of the analysis to differences between refined disease classifications.

One of the criticisms reported in the literature directed against the country of birth variable is that it is not necessarily an accurate indication of ethnicity due to migration and intermixing of ethnic groups. This may account for differences in prevalence outcomes in population groups reported in the literature compared to significance of the country of birth findings in the current study.

Despite the large dataset that has been investigated in this study only 12.4% of the incident ANA cohort were linked to mortality data for analysis, this is consistent with the fact that autoimmunity is a relatively low prevalence disease and that the ANA test is used as a screening test to exclude some of the autoimmune rheumatic diseases.

9.1.2 CONCLUSIONS

ANA were detected in a higher proportion of females compared to males. The majority of detected ANA were low level. In general an increasing level of ANA was associated with an increased likelihood of a dsDNA antibody and antibodies to RNP, SmD, SSB/la, Scl70, SSA/Ro60, Ro52 and CenpB. ANA was more predictive of these antibodies in younger individuals.

Residential remoteness and socioeconomic disadvantage were not significant predictors of morbidity or mortality adjusted for ANA, age and gender. Country of Birth the Americas was a predictor of a positive ANA, however in the analysis of a morbidity of RA and, diseases of the liver adjusted for ANA level various countries including N.W. Europe, Africa (excluding N. Africa) and S.E. Asia were significant predictors of outcomes.

The current study has demonstrated that ANA pattern and quantitation are significant predictors of morbidity and survival particularly that associated with musculoskeletal and connective tissue diseases.

9.1.3 OUTCOMES

The analysis of the quantitative ANA data has enabled the relationship between ANA level and outcome to be estimated for a number of disease associations. The strength of including the inpatient morbidity data in the analysis is that up to 11 years of follow-up for individuals is included in the study. This is an advantage of the study as autoantibodies can be identified 5 or more years before symptoms of the disease.

9.1.4 FUTURE WORK

The predictive value of ANA level for length of stay or frequency of hospitalisation has not been considered and may add additional benefit to the use of the screening assay in considering patient management options.

Furthermore, this study has demonstrated that in many cases the high level ANA is a predictor of anti dsDNA or ENA antibodies and morbidity. This finding suggests that age and gender adjusted reference values should be investigated for ANA reporting to guide decisions on reflex testing and disease association.

10 APPENDIX

10.1.1.1 LOGISTIC REGRESSION FOR ANY ANA ≥7IU/ML RESULT

TABLE 10-1 MULTIVARIATE LR ANALYSIS OF AN ANA≥7IU/ML RESULT ADJUSTED FOR GENDER, INDIGENOUS STATUS AND COUNTRY OF BIRTH.

Variable	df	p value.	OR	95% C.I. for OR	
				Lower	Upper
COUNTRY OF BIRTH	10	<0.001			
AUSTRALIA (Reference)					
Oceania and Antarctica (excluding Australia)	1	0.190	1.081	.962	1.215
N.W. Europe	1	0.016	1.064	1.012	1.118
S. & E. Europe	1	0.027	1.184	1.019	1.376
N. Africa & M.E	1	0.607	.968	.857	1.094
S.E. Asia	1	0.092	1.132	.980	1.308
N.E. Asia	1	0.102	1.155	.972	1.374
S. & C. Asia	1	0.645	1.050	.852	1.295
Americas	1	0.009	1.377	1.083	1.750
Africa (Excluding N. Africa)	1	0.004	.795	.679	.930
Not Available	1	0.250	1.073	.951	1.211
INDIGENOUS STATUS	2	<0.001			
Non Aboriginal (Reference)					
Ungrouped	1	0.001	0.811	.716	.918
Aboriginal or TSI	1	<0.001	0.733	.666	.807
GENDER	2	<0.001			
Male (Reference)					
Female	1	<0.001	1.877	1.808	1.949
Undefined	1	0.446	.456	.060	3.441

TABLE 10-2 FREQUENCY OF ANA RESULTS AND AGE GROUPED BY ICD-10 DISEASE CODING FOR CAUSE OF DEATH.

ICD-10 Description and Code		**ANA <7IU/ml**					**ANA ≥7IU/ml**				
		N	AGE (years)				N	AGE (years)			
			Mean	Min	Max	SD		Mean	Min	Max	SD
Infection	A	100	69.1	25.0	94.0	15.9	17	75.1	47.0	87.0	11.1
	B	77	50.5	19.0	88.0	16.6	13	60.3	32.0	81.0	17.7
Malignant Neoplasms	C	1,670	66.0	15.0	97.0	13.8	433	67.7	7.0	94.0	13.0
In situ, benign & unknown behaviour neoplasms & Diseases of blood and blood-forming organs & certain disorders involving the immune mechanism	D	141	70.5	24.0	95.0	14.2	42	70.5	25.0	94.0	15.9
Endocrine, nutritional and metabolic diseases	E	371	66.3	18.0	97.0	16.0	80	70.6	36.0	96.0	13.1
Mental and behavioural disorders	F	125	74.4	38.0	95.0	14.9	29	74.6	38.0	92.0	15.3
Diseases of the nervous system	G	198	67.3	16.0	92.0	15.4	61	71.5	30.0	89.0	13.4
Diseases of the eye and adnexa ear and mastoid process	H	2	56.5	43.0	70.0	19.2	0				
Diseases of the circulatory system	I	1,712	72.3	14.0	100.0	14.0	448	73.9	19.0	101.0	12.3
Diseases of the respiratory system	J	584	70.6	2.0	98.0	13.4	163	72.3	26.0	95.0	13.0
Diseases of the digestive system	K	536	60.8	15.0	96.0	15.9	112	65.4	16.0	90.0	15.8
Diseases of the skin and subcutaneous tissue	L	25	73.2	47.0	95.0	13.5	5	76.0	61.0	88.0	13.8
Diseases of the musculoskeletal system and connective tissue	M	106	71.8	20.0	96.0	12.9	66	64.1	19.0	90.0	17.4
Diseases of the genitourinary system	N	225	70.4	21.00	95.0	15.4	44	69.9	17.0	92.0	15.6
Pregnancy, childbirth and the puerperium	O	0					1	24.0	24.0	24.0	
Congenital malformations, deformations and chromosomal abnormalities	Q	17	44.9	0.0	82.0	21.7	4	47.0	25.0	69.0	18.3

TABLE 10-2: CONTINUED: FREQUENCY OF ANA RESULTS AND AGE GROUPED BY ICD-10 DISEASE CODING FOR CAUSE OF DEATH.

ICD-10 Description and Code		ANA <7IU/ml					ANA ≥7IU/ml				
		N	AGE (years)				N	AGE (years)			
			Mean	Min	Max	SD		Mean	Min	Max	SD
Symptoms, signs and abnormal clinical and laboratory findings, not elsewhere classified	R	33	50.9	15.0	85.0	18.6	12	59.0	25.0	77.0	16.7
Accidents/Assault/Events of Undetermined intent	V	46	45.9	16.0	81.0	17.0	7	48.6	31.0	73.0	15.4
	W	71	67.5	26.0	94.0	19.2	10	69.9	51.0	91.0	12.6
	X	178	48.1	16.0	101.0	18.5	29	53.6	18.0	89.0	21.1
	Y	37	59.3	29.0			7	52.1	18.0	81.0	22.2

TABLE 10-3. FREQUENCY OF ANA RESULTS BY ICD-10 DISEASE CODING.

ICD-10 Description and Code		ANA ≥7IU/ml			
	ICD-10 Alpha character	7 – 10 IU/ml	>10 – 20 IU/ml	>20 -30IU/ml	>30IU/ml
Infection	A	7	7	2	1
	B	7	5	1	0
Malignant Neoplasms	C	225	111	67	30
In situ, benign & unknown behaviour neoplasms & Diseases of blood and blood-forming organs & certain disorders involving the immune mechanism	D	16	12	9	5
Endocrine, nutritional and metabolic diseases	E	44	14	17	5
Mental and behavioural disorders	F	16	8	5	0
Diseases of the nervous system	G	32	9	17	3
Diseases of the eye and adnexa ear and mastoid process	H	0	0	0	0
Diseases of the circulatory system	I	239	94	89	26
Diseases of the respiratory system	J	84	43	23	13
Diseases of the digestive system	K	53	29	22	8
Diseases of the skin and subcutaneous tissue	L	3	1	1	0
Diseases of the musculoskeletal system and connective tissue	M	17	13	22	14
Diseases of the genitourinary system	N	25	9	9	1
Pregnancy, childbirth and the puerperium	O	0	0	1	0
Congenital malformations, deformations and chromosomal abnormalities	Q	2	1	0	1
Symptoms, signs and abnormal clinical and laboratory findings, not elsewhere classified	R	6	1	4	1
Accidents/Assault/Events of Undetermined intent	V	5	2	0	0
	W	5	3	2	0
	X	17	11	1	0
	Y	6	0	0	1

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