ENHANCING DIAGNOSIS AND MANAGEMENT OF SYSTEMIC LUPUS ERYTHEMATOSUS

by

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Submitted to the Graduate Faculty of

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Graduate School of Public Health in partial fulfillment

of the requirements for the degree of

Doctor of Philosophy

University of Pittsburgh

2005
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ACKNOWLEDGEMENTS

I would like to thank Dr. Susan Manzi for introducing me to epidemiology of systemic lupus erythematosus, for her knowledge, guidance and support. I would also like to thank the University of Pittsburgh Lupus Center of Excellence for the opportunity to work in their great team, for providing helpful insights, clinical samples, and skilled technical as well as administrative support. I would also like to thank the University of Pittsburgh Graduate School of Public Health for the knowledge I gained there and for the generous financial support.

I would also like to thank Dr. Kim-Sutton Tyrrell for her guidance and support throughout my graduate studies. I would also like to thank Dr. Songer for introducing me to cost-effectiveness analysis and decision making. His help and support was invaluable to my study and research. I would also like to thank Dr. Arena for his guidance in statistical analysis of my research data, and to his Department for the knowledge I have gained in biostatistics. I would also like to thank Dr. Edmundowicz for the research data and for his expertise and invaluable advices in the area of cardiovascular disease.

I would like to thank Dr. Judy Lave for introducing me to health economics and health insurance, which became invaluable for my future career, and her guidance and support throughout my PhD program.

I would like to thank Dmitri, for his love, inspiration, patience and support. Finally, I would like to thank my parents for their support and care throughout my graduate studies.
Abstract

Systemic lupus erythematosus (SLE) affects a significant portion of young women of childbearing age worldwide, in particular those of non-white descent. In the United States, the incidence ranges 8.1-11.4 per 100,000 among African-American, and 2.5-3.9 per 100,000 among Caucasian women. The prevalence is estimated 56-283 per 100,000 among African-American, and 17-71 per 100,000 among Caucasian women. Understanding the natural history of systemic lupus, its major complications such as cardiovascular disease and associated risk factors, identifying major biomarkers for timely and accurate diagnosis of the disease itself and its manifestations is of great public health importance, since this will help to reduce morbidity and mortality among lupus patients.

The dissertation consists of three relevant chapters. The first chapter is an overview of candidate biomarkers for diagnosis of SLE. It includes a brief review of the role of complement molecules in SLE pathogenesis, evaluation of the past and current uses of complement in monitoring SLE disease activity, and summary of recent findings that propose a novel method of measuring complement activation to specifically and sensitively diagnose SLE. The chapter concludes by discussing how this method may also support the resurgence of complement as a valuable biomarker of SLE disease activity.

The second chapter is the cost effectiveness analysis of the novel diagnostic biomarker discussed in chapter one. The analysis shows that using the novel diagnostic biomarker along with the traditional tests can be cost effective for the population of patients contemplating SLE.
Chapter three is devoted to atherosclerosis as a major complication of SLE and to coronary calcification measured by electron beam tomography (EBT) as a major biomarker of subclinical atherosclerosis. The risk factors associated with subclinical vascular disease in women with SLE are also reported. It is concluded that atherosclerosis of the coronary arteries detected by EBT is highly prevalent in patients with SLE and is related to many potentially modifiable traditional, SLE-specific and inflammatory risk factors for vascular disease.
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1 Complement Proteins as Biomarkers of SLE Disease Activity: State of Art
1.1 INTRODUCTION

Systemic lupus erythematosus (SLE) is the most clinically and serologically diverse autoimmune disease and can affect virtually all organs in the body. The clinical manifestations in SLE are thought to arise from tissue inflammation characterized by immune complex deposition, complement activation, and blood vessel abnormalities. Clinically, SLE is difficult to diagnose and manage because of its heterogeneous presentation and unpredictable course that consists of periods of remission and exacerbation (flares). Although most flares are caused by reversible inflammatory processes, over time, they can lead to irreversible organ damage that greatly affects the morbidity and mortality of patients with SLE. Earlier and more accurate detection of disease flares would not only expedite appropriate treatment, but also reduce the frequency of unnecessary interventions that are often associated with significant adverse effects.

A major barrier to the assessment of disease activity, the identification of patients at risk for flares and organ damage, and the evaluation of responses to treatment for SLE is the lack of reliable and specific biomarkers for diagnosis and monitoring of disease activity. Standard tests currently used to monitor SLE disease activity, including measurements of complement, anti-nuclear antibody (ANA), and anti-double stranded DNA (dsDNA) antibodies, are often inadequate in detecting active disease. However, recent discoveries in our laboratory of a novel method for measuring complement activation indicate that complement may yet be a promising indicator of SLE disease activity. In this article, we briefly review the role of complement in SLE pathogenesis, evaluate the past and current uses of complement in monitoring SLE disease activity, and summarize our recent findings that propose a novel method of measuring complement activation to specifically and sensitively diagnose SLE. We conclude by discussing how this method may also support the resurgence of complement as a valuable biomarker of SLE disease activity.
1.2 THE COMPLEMENT SYSTEM

The complement system, an important component of the immune system, comprises a group of more than 20 plasma and membrane-bound proteins that form three distinct pathways designed to protect against invasion of foreign pathogens (Figure 1-1).

Figure 1-1. Pathways of the complement system.
Many of the complement proteins exist as functionally inactive pro-proteins until appropriate events trigger their activation. Once activated, the proteins within each pathway undergo a cascade of sequential cleavage and release of biologically active fragments and self-assembly into multimolecular complexes. In humans and other mammals, the complement system is controlled by many regulatory proteins to prevent over activation and unwanted inflammation.

Traditionally, the complement system is traditionally thought to have four biological functions: activation of inflammation, opsonization, clearance of immune complexes, and osmotic lysis of invading microorganisms [1]. These functions are mediated by the soluble or surface-bound fragments of activated complement proteins, which in turn interact with specific membrane receptors expressed on various cell types. The soluble proteolytic fragments, C3a, C4a, and C5a, usually diffuse away from the activation site to stimulate inflammation via chemoattraction and activation of leukocytes. The larger fragments, C3b, C4b, and their derivatives (iC3b, C3d, iC4b, C4d), can opsonize microbial pathogens by remaining bound to their surface. The complement-derived ligands on the pathogen surface facilitate phagocytosis by neutrophils and macrophages by binding to complement receptor (CR) 1 (for C3b and C4b), CR3 (for iC3b and iC4b), and perhaps CR4 (for iC3b and iC4b) expressed on phagocytes. C4b and C3b can also bind to immune complexes, preventing them from aggregating into insoluble complexes and enhancing their clearance. The clearance of C3b/C4b-opsonized immune complexes is believed to be mediated by erythrocytes that express CR1 and are capable of transporting immune complexes to macrophages [2]. Our recent studies [3] and those of other investigators [4] have demonstrated that complement proteins, particularly C1, C3, and C4, can bind to apoptotic cells and blebs, and thus may play an important role in maintaining immune tolerance by facilitating the clearance of autoantigen-containing apoptotic bodies [5,6]. C3d, the end cleavage product of C3, interacts with CR2 expressed on B cells and may be involved in B cell activation and enhancement of humoral immune responses [7]. Finally, the C5b-9 membrane attack complexes (MACs) may perturb the integrity of the surface membrane of target cells, thereby stimulating various cellular functions (at sublytic doses) or causing cell lysis (at lytic doses).
1.3 COMPLEMENT ACTIVATION AND SLE

The role of complement in SLE is both complex and paradoxical. Activation of the complement system is thought to contribute to tissue inflammation and damage in SLE as a consequence of autoimmune complex deposition [8,9]. However, a hereditary deficiency of one of several components of the classical pathway (C1, C2, or C4) has been associated with the development of SLE [5,6,10]. These apparently conflicting roles can be reconciled by studies performed during the past several years demonstrating that, although the complement system plays a role in maintaining immune tolerance to prevent the development of SLE [5,6,11,12], it also participates in the inflammatory processes once SLE is established [8,9,13].

1.3.1 COMPLEMENT AND THE ASSESSMENT OF SLE DISEASE ACTIVITY

The classical complement pathway is thought to be activated during active states of SLE. As a result, one might expect the extent of parent complement proteins converted to activation products to be related to the level of disease activity. Thus, measuring serum complement levels has been considered the "gold standard" for monitoring disease activity in SLE patients. Conventionally, complement activation is assessed by functional assays quantifying hemolytic activity (CH\textsubscript{100} or CH\textsubscript{50}; measuring total complement activity) or by static measures of serum complement components such as C1q, C2, and C4 (classical pathway activation), factor B (alternative pathway activation), or C3 (representing the common terminal pathway). Since Vaughan et al. first reported an association between decreased complement and active SLE five decades ago [14], numerous studies have been conducted to evaluate the potential usefulness of these assays in monitoring SLE disease activity.

Some investigators have found CH\textsubscript{50} as well as serum C4 and C3 levels valuable as markers of SLE activity, while others have found them minimally useful (Table 1-1). Several lines of evidence support their utility. Decreased levels of CH50 and serum C3 and C4 have been associated with more severe lupus nephritis or extra-renal involvement. Moreover, increased serum C3 levels have coincided significantly with remission of nephritis and decreased levels with its relapse. In addition, decreases in serum C4 levels have preceded clinical exacerbation, and in some patients, serum C3 and C4 levels have normalized when disease flares resolved. In
contrast, serum C4 levels have remained normal in some SLE patients during disease flares, and decreased C4 levels have been documented in SLE patients with apparently inactive disease, arguing against a role for complement as indicators of SLE disease activity. Regarding the latter situation, it should be noted that the so-called “serologically active clinically quiescent (SACQ)” periods have been seen in many SLE patients [15,16]. During SACQ periods, the clinical disease is absent, but serological activity is evident. Although complement levels do not generally correlate with long-term outcome, some patients with low levels of multiple complement components appear to be at higher risk for developing renal insufficiency [17].

Collectively, these conflicting data have failed to provide convincing support for the role of low C3 and C4 or other complement abnormalities as reliable indicators or predictors of SLE disease activity.

1.3.2 COMPLEMENT ACTIVATION PRODUCTS

In view of the apparent shortcoming of measuring serum C3 and to assess SLE disease activity, many investigators have sought alternative approaches. Because measurement of C3 and C4 is primarily a static appraisal of an extremely dynamic process of activation, consumption, catabolism, and synthesis of these components, direct determination of complement activation products is thought to more precisely reflect complement activation in vivo. A number of complement activation products of the classical pathway (C1rs-C1 inhibitor complex, C2a, C4d), alternative pathway (Ba, Bb, C3b(Bb)p), and the common terminal pathway (C3a, iC3b, C3d, C5a, C5b-9 complex), have been investigated for their potential as disease markers or predictors in SLE (Table 1-2).

Several investigators have reported that plasma concentrations of complement activation products, including C1-C1 inh complex, C3a, C4a, C5a, C3d, C4d, C5b-9 complex, Ba, and Bb, increase prior to or during clinical exacerbation. However, increased C3d levels have been detected in a significant number of patients without clinical disease. A number of studies have reported little or no correlation between plasma levels of complement activation products and SLE disease activity. As was the case in the numerous studies evaluating complement component measurements, the studies of complement activation products as markers of SLE disease activity
have been inconclusive. The search for the ideal marker of disease activity in SLE warrants more intensive, coordinated efforts from both clinicians and basic science researchers.

Potential reasons why complement measures do not correlate with SLE disease activity

The discrepant reports regarding the value of measuring serum C4, C3, and various complement activation products to monitor SLE disease activity may originate from several biochemical or genetic factors. First, the range of serum C3 and C4 levels among healthy individuals varies widely and overlaps with that observed in SLE patients. Second, standard laboratory tests measure the concentration of C3 and C4 molecules in circulation irrespective of their functional status. Third, the acute phase response during inflammation may lead to an increase in C4 and C3 synthesis [18], thereby counterbalancing and masking their consumption during activation. Fourth, increased catabolism independent of inflammation [19-21] as well as reduced synthesis of C4 and C3 [22,23] have been reported to occur in patients with SLE, a situation that likely interferes with static measures of C4 and C3 levels. Fifth, genetic variations such as a partial deficiency of C4, commonly present in the general population as well as among SLE patients [24-26], may lead to lower than normal serum C4 levels in some patients because of decreased synthesis rather than increased complement consumption during disease flares. Sixth, when immune complexes deposit in tissues, complement activation may be localized and not reflected by the levels of complement products in the systemic circulation. Similarly, there are factors that could influence the measurements of complement activation products. Many of the activation products have an undefined, most likely short, half-life both in vivo and in vitro. Moreover, complement activation may occur in vitro after blood sampling. As a result, current measures of complement activation products may not accurately reflect complement activation in the patient.

Variability in clinical factors and study design may also contribute to inconclusiveness of the study results. SLE patient populations are inherently heterogeneous with respect to disease activity and organ involvement. Such heterogeneity within a given population of patients may obscure subtle yet clinically significant results in a cross-sectional study. Furthermore, it has been difficult to standardize the accurate diagnosis of SLE and definition of a disease flare. Before the recent development of several activity assessment instruments (e.g., SLE Disease Activity Index, Systemic Lupus Activity Measure, British Isles Lupus Assessment Group), widely different criteria
were used in defining SLE disease activity and flares. In many of the early studies, investigators used qualitative definitions, such as the occurrence of exacerbating manifestations and the physician’s assessment and decision to change medications. Such discrepancies in study design undoubtedly further contribute to the difficulties in interpreting and comparing results obtained from different studies.

Considering the numerous confounding factors outlined above, it is not surprising that the role of complement as a biomarker of SLE disease activity remains inconclusive. However, it is important to point out that serum complement measures can be informative when performed over time in the same patient and when the results are interpreted based on the specific genetic and clinical status of the patient. As discussed below, they may also be of value in assessing disease activity or predicting outcome in particular circumstances, such as with certain organ involvement and during pregnancy.

1.3.3 Complement and Organ-Specific Involvement in SLE

One of the most serious clinical manifestations of SLE is nephritis. Nephritic flare has been shown to be a predictor of poor long-term outcome in SLE patients. The pathogenic mechanism of lupus nephritis likely involves the deposition of immune complexes in the kidney and subsequent in situ activation of the complement system. Thus, measuring complement components and activation products in the plasma or urine may be useful for evaluating the extent of active inflammation in the kidneys. These measurements may also be useful in SLE patients with CNS involvement, another serious, although less common, manifestation of SLE. Although a few studies have suggested that elevations in complement activation products are related to CNS involvement in SLE, further study is warranted. A summary of studies investigating complement measures in organ specific manifestations of SLE is depicted in Table 1-3.

Since SLE is a prototypic multi-organ autoimmune disease, one would expect measurements of complement in tissue fluid obtained from specific sites of inflammation to be informative. However, only a few, mostly anecdotal, studies have been performed to date examining complement levels in synovial fluid [27], pleural fluid [28,29], pericardial fluid [30,31], and cerebrospinal fluid [32-34]. These were primarily small studies and yielded variable results.
Thus, the value of complement levels in body fluids as indicators of specific organ involvement remains unclear. Systematic investigation in this area is warranted.

1.3.4 Complement and Pregnancy in SLE

A majority of SLE patients are women of childbearing potential; therefore, physicians treating pregnant SLE patients frequently face the dilemma of differentiating a disease flare from a pregnancy-associated complication. Renal involvement in SLE patients may be exacerbated at various stages of pregnancy. If occurring in the third trimester, a nephritic flare may be difficult to distinguish from preeclampsia, a complication of pregnancy occurring most frequently at this stage, since the proteinuria and hypertension associated with preeclampsia may mimic an SLE flare. It is extremely important to distinguish these two conditions because their management is quite different. Laboratory tests, such as complement measurements, have been employed in this regard; however, they should be interpreted cautiously since the physiological changes associated with pregnancy vary widely. For example, it has been shown that during the gestational period in a normal pregnancy, serum levels of C3, C4, and CH$_{50}$ gradually increase from 10% to 50% above non-pregnant levels [35-37]. Such increases may counterbalance the consumption of complement during an SLE flare, making the C3 and C4 levels appear normal in a pregnant SLE patient with more severe disease. Conversely, decreased serum C3, C4, and CH$_{50}$ have been observed in a fraction of pregnant women with SLE not experiencing a disease flare [36]. The depressed complement profile in these women could be mistakenly attributed to an SLE flare when in fact preeclampsia is present.

Table 1-6 represents the results of several studies examining complement in pregnancy. In general, CH$_{50}$ appears to be a key determinant in differentiating SLE flares from preeclampsia [38]. Moreover, increased plasma concentrations of Ba and Bb appear to be highly specific (specificity 100% and 75%, respectively), sensitive (sensitivity 100% for both), and predictive of a disease flare in pregnant SLE patients [38,39]. In terms of diagnosing a flare, conventional measures of serum C3 and C4 were neither sensitive nor specific [38]. Therefore, it appears that the laboratory tests of choice in such a diagnostic and therapeutic dilemma concerning pregnant SLE patients should be a combination of measures of CH$_{50}$ and plasma concentrations of complement activation products, particularly Ba and Bb.
1.3.5 A NOVEL ERYTHROCYTE-BOUND COMPLEMENT ASSAY – A PATH TO THE FUTURE

1.3.5.1 Complement and erythrocytes

Most complement activation products have a short half-life and likely are cleared from the plasma and other tissue fluid in an efficient manner. These variables may contribute to the inconsistent reports regarding the correlation between levels of complement activation products and clinical disease activity. Complement proteins are abundant in the circulation and can readily interact with cells circulating in blood. Complement products covalently bound on cellular components likely last longer in circulation and hence may be detected more reliably. In fact, isoforms of the complement C4d fragment have been identified as two minor blood group antigens, Rodgers and Chido, on erythrocytes of apparently healthy individuals [40-42]. In general, the amount of C4 on the surface of erythrocytes of healthy individuals is low but somewhat variable. These findings indicate that that complement C4 and particularly its stable end product of activation, C4d, can bind to and persist on erythrocytes of normal individuals. The mechanisms of the deposition of C4 on erythrocytes, however, are not well understood.

Erythrocytes are the most abundant circulating blood cells and have easy access to products of systemic as well as localized inflammation. Therefore, we hypothesized that they may serve as biological beacons of the in vivo inflammatory state and of disease activity in SLE patients and those with other inflammatory diseases. It should be pointed out that few, if any, studies investigating the presence of C4 and C4-derived activation products on erythrocytes of patients with inflammatory diseases have been conducted [41,43,44]. Accordingly, the mechanism and pathophysiological significance of the binding of C4 to erythrocytes in disease are largely unknown. Because such information may potentially be important not only to accurately diagnose and monitor disease activity but also to better understand the molecular pathogenesis of SLE, we decided to investigate whether erythrocytes from patients with SLE bear specifically abnormal levels of complement activation product C4d. In view of the involvement of erythrocyte-expressed complement receptor 1 (CR1) in the clearance of
complement-bearing immune complexes, we also explored the possibility that SLE-specific defects may be reflected by abnormal CR1 levels on erythrocytes.

1.3.6 **ERYTHROCYTE-BOUND C4d AND SLE**

To test the above-stated hypothesis, we developed a flow cytometric assay in our laboratory to rigorously measure the levels of C4d and CR1 on the surface of erythrocytes. We conducted a cross-sectional study that included 100 patients who met the American College of Rheumatology criteria for the diagnosis of SLE, 133 patients with other diseases, and 84 healthy controls (Manzi et al., in press). Several remarkable findings have emerged from this study. First, significantly higher C4d levels were detected on erythrocytes of SLE patients than on those of patients with other diseases or healthy individuals (P<0.001). Second, high C4d levels on erythrocytes appeared to be specifically associated with SLE but not other inflammatory diseases (P<0.001). Third, CR1 levels were significantly lower in SLE patients than in patients with other diseases or in healthy controls (P<0.001). Fourth, the erythrocyte-C4d/CR1 (E-C4d/CR1) test was 81% sensitive and 91% specific in distinguishing SLE patients from healthy controls, and 72% sensitive and 79% specific in distinguishing patients with SLE from patients with other diseases, and an overall negative predictive value of 92%. This study demonstrated for the first time, in a systematic fashion, that considerable amounts of a complement activation product are present on the surface of erythrocytes in patients with an inflammatory disease such as SLE. In addition, the results obtained from this study are consistent with previous studies showing that decreased levels of CR1 on erythrocytes may be a general feature in SLE [45,46]. More importantly, this study demonstrated that measurement of E-C4d and E-CR1 is a simple, rapid, and inexpensive method that has greater combined sensitivity and specificity than any currently available tests for diagnosing SLE. This assay should ultimately have significant impact on the accuracy and timing of SLE diagnosis in general clinical practice, and may lead to earlier and more appropriate therapeutic interventions.

Of even greater value would be the ability of this assay to quantitatively detect ongoing disease activity in an SLE patient at a specific point of time. In this regard, we have found that E-C4d levels detected on erythrocytes of healthy controls were remarkable constant over days, weeks, and even months within a given individual (Manzi et al., in press), suggesting that even slight fluctuations in E-C4d levels may reflect changes in complement activation. Interestingly, in
contrast to the constant E-C4d levels observed in healthy individuals, the E-C4d levels in the same SLE patient examined on different study visits varied considerably (Manzi et al., manuscript in preparation). We have postulated that E-C4d levels may correlate with the extent of complement activation and SLE disease activity in a quantitative manner, rendering them not only a specific diagnostic aid but also a sensitive indicator of evolving SLE disease activity. A prospective study is currently underway to verify this hypothesis. If validated, this assay may prove to be superior to current standard tests, such as anti-dsDNA and serum C3 and C4, for diagnosis, assessment of disease activity, and prediction of disease flares in patients with SLE.

1.3.7 PERSPECTIVES - THE FUTURE

A wide variety of biological molecules have been investigated as potential biomarkers of disease activity in SLE. The search for complement-unrelated surrogate markers, such as anti-dsDNA, cytokines, soluble cytokine receptors, and soluble cell adhesion molecules, has been propelled in part by the unsatisfactory results of complement measures. In spite of intensive studies, however, the value of most of these molecules is still questionable, and most of the tests are not generally applicable in routine clinical settings. Our recent study of erythrocyte-bound complement in SLE as an accurate and timely diagnostic tool will undoubtedly rekindle enthusiasm for complement not only in the clinical management of SLE but also in complement biology.

Enthusiasm for the newly developed E-C4d/E-CR1 assay stems from its many advantages over current methodology. Obvious advantages include its simplicity, applicability, accuracy, and cost-effectiveness. Less obviously but more importantly, this assay measures complement products that associate stably with erythrocytes for a long period of time, in striking contrast to conventional assays that measure soluble complement components and their highly unstable activation products. Consequently, measurement of E-C4d is expected to mirror sensitively and faithfully, in a quantitative way, the degrees of complement activation and hence the disease activity in patients with SLE. This is an unsurpassed feature of the E-C4d assay compared to conventional complement measures. Furthermore, because complement products, once generated, probably remain bound to erythrocytes for the entire lifespan of those cells, E-C4d levels detected on a given day may reveal not only the ongoing disease activity but also the cumulative activity of the preceding days. In this context, the E-C4d assay is invaluable because it provides physicians with
power to promptly diagnose SLE, carefully assess current as well as recent disease activity, institute needed treatment, and accordingly terminate unnecessary interventions. Prospective studies underway in our laboratory will attest to this potential of the E-C4d assay.

There are additional, yet-to-be-validated uses of the E-C4d assay. For example, measurement of E-C4d may assist in identifying SLE patients with hematological manifestations including impending attack of hemolytic anemia. Because it appears to be more sensitive and specific than conventional complement assays, E-C4d measurement may also allow identification of SLE patients with “smoldering” disease activity and timely installation of preventive interventions. These features of the E-C4d assay, nevertheless, await further investigation.

In summary, we see that in the past, complement had a promising future as a valuable biomarker of SLE disease activity, but in reality has been a disappointment. The role of complement may soon be vindicated, however, as new discoveries of the utility of erythrocyte-bound complement as a novel diagnostic tool in SLE may translate to its unsurpassed value as an indicator, and possibly predictor, of SLE disease activity. Realization of this potential hinges on large-scale cross-sectional as well as long-term prospective studies that demand coordinated efforts from both investigators and physicians. With the intensive research ongoing in our and other laboratories, the comeback of complement as a valuable disease biomarker should occur in the foreseeable future.
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<td>CH50, C3, C4 levels lower in patients with active renal disease than in patients with extra-renal involvement; Decreasing C4 levels proceeded disease flares</td>
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<td>Valentijn et al. [48]</td>
<td>Retrospective study using serial serum samples obtained from 33 SLE patients; Serological test: CH50, C3, C4, immune complexes (C1q binding); Disease activity: scoring system designed by authors</td>
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<td>Ricker et al. and the Lupus Nephritis Collaborative Study Group (LNCSG) [49]</td>
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<td>Mills et al. [50]</td>
<td>Prospective study of 29 SLE patients; Serological tests: C1q, c4, C4a, C2, C3, C3a, C5, CH50, C4a:C4 ratio, C3:C3a ratio; Disease activity: clinical and serological assessment</td>
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<td>Cameron et al. [51]</td>
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<td>Swakk et al. [52]</td>
<td>Prospective study of 143 patients; Serological tests: C1q, C3, C4, C5, C9, anti-dsDNA:</td>
<td>Decreased C4, C1q, and C3, in a sequential order, detected in patients with renal exacerbation; Decreasing C4 detectable 20-25 weeks before the flare; Decreased C1q and C3 detected during but not before the flare</td>
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<td>Ting and Hsieh [53]</td>
<td>Long term study of 16 patients with childhood onset SLE; Serological tests: C3, C4, ANA, anti-dsDNA; Disease activity: Lupus activity criteria count (LACC)</td>
<td>Decreased C3 levels detected during, but rarely before, the active stage of disease; Decreased C4 levels detected prior and during active disease</td>
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<td>Abrass et al. [54]</td>
<td>Prospective study following 48 SLE patients; Serological tests: C3, anti-dsDNA, immune complexes; Disease activity: clinical record of disease manifestations and medications</td>
<td>C3 and anti-dsDNA neither associated with nor predictive of changes in disease activity</td>
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<td>Morrow et al. [55]</td>
<td>Prospective follow up of 35 SLE patients; Serological tests: CH50, C3, C4, anti-dsDNA, immune complexes; Disease activity: graded as severely active, moderately active, and inactive using the UCH/Middlesex criteria</td>
<td>None of the serological tests reliably distinguishing the three clinical groups</td>
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<td>Esdaile et al. [56,57]</td>
<td>Retrospective analysis of serum samples collected from 202 patients; Serological tests: C3, C4, anti-dsDNA, immune complexes; Disease activity: SLEDAI, a rise of &gt; or = 6 defined as a flare</td>
<td>All serological parameters tested poor predictor of SLE flares; some values of patient-based serial measures in association with specific types of flares (e.g., decreased C3 and renal involvement)</td>
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<td>Complement activation product(s) tested</td>
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<td>C1rs-C1inh, C3d</td>
<td>Sturfelt et al. [58]</td>
<td>Serial (at 6-8 wk intervals) samples from 33 SLE patients; Increased C1rs-C1inh consistently found during flares; Increased C1rs-C1inh detected before flares, especially extra-renal flares; Increased C3d associated with severe disease flares</td>
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<td>Cirs-C1inh, C3(Bb)P, C5b-9</td>
<td>Nagy et al. [59]</td>
<td>Plasma samples obtained from healthy controls and 65 SLE patients with active and inactive disease; All 3 activation products elevated in SLE patients with inactive disease compared to healthy controls; C3(Bb)P and C5b-9, but not C1rs-C1inh, distinguishing active disease from inactive disease</td>
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<td>C4a, C3a</td>
<td>Wild et al. [60]</td>
<td>Plasma samples of 24 SLE patients; Elevated C4a levels detected in 20 patients; C4a levels correlated with disease activity; Elevated C3a levels found in 2 patients only</td>
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<td>C3a, C5a</td>
<td>Belmont et al. [61]</td>
<td>Plasma samples of 76 SLE patients with severe, moderate, or inactive disease; C3a significantly elevated in patients with severe or moderate disease activity and quantitatively correlated with disease severity; C5a significantly elevated in patients with severe disease activity</td>
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<td>C3a, C5a</td>
<td>Hopkins et al. [62]</td>
<td>Serial plasma samples from 23 SLE patients (7 pregnant; 5 CNS involvement); C3a levels significantly higher in patients with a flare than in those with stable disease; Rising C3a levels predictive of disease flares; Highly elevated C3a and C5a in patients with CNS involvement; C3a levels elevated in most pregnant patients</td>
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<td>C3a, C4a, iC3b, C5b-9</td>
<td>Porcel et al. [63]</td>
<td>Plasma samples of 61 SLE patients (22 inactive disease; 39 active disease; defined by SLEDAI); C3a, C4a, and C5b-9 significantly elevated in patients with active disease, with a positive correlation with disease activity scores; C5b-9 being most sensitive and specific; iC3b not correlated with disease activity</td>
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<td>C4d, C3d</td>
<td>Senaldi et al. [64]</td>
<td>Plasma samples of 48 SLE patients (11 inactive, 23 mildly active, 14 moderately/severely active); Elevated C4d levels correlated with disease activity in a linear fashion; C3d levels elevated but not linearly correlated with disease activity</td>
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<td>Ba, Bb, C4a, C3d</td>
<td>Kerr et al. [65]</td>
<td>2-year follow-up of 51 SLE patients; Increase of Ba, C4a, and C3d in the same patients associated with severe disease activity</td>
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<td>Ba, Bb, C4d, C5b-9</td>
<td>Buyon et al. [38]</td>
<td>380 serial plasma samples from 86 SLE patients with inactive, stable/moderate, or severe disease; Ba levels significantly elevated and positively correlated with disease activity; Elevated C4 and increased Bb predictive of subsequent flares</td>
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<td>Bb, C4d, C5b-9</td>
<td>Manzi et al. [66]</td>
<td>21 SLE patients prospectively followed for 1 year; C4d and Bb sensitive indicator of moderate-to-severe disease activity; C4d and Bb sensitive at predicting increasing disease activity</td>
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<td>C5b-9</td>
<td>Falk et al. [67]</td>
<td>108 serial plasma samples from 14 SLE patients; C5b-9 levels significantly elevated in SLE patients, and positively correlated with disease activity</td>
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<td>C5b-9</td>
<td>Gawryl et al. [68]</td>
<td>120 plasma samples from 28 SLE patients; Elevated C5b-9 levels correlated with disease exacerbation; C5b-9 a more sensitive marker of disease marker than C3 and C4</td>
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<td>Lloyd et al. [47]</td>
<td>Prospective study of 27 SLE patients, with and without renal manifestations; Serological tests: C1q, C3, C4; SLE activity: clinical and serological assessment</td>
<td>SLE patients with renal involvement have reduced serum C3 and C4 more frequently than patients with extra-renal involvement only; nevertheless, the lowest levels of C3 and C4 are detected in patients with both renal and extra-renal disease. Low levels of C1q are in patients with active renal disease</td>
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<tr>
<td>Valentijn et al. [48]</td>
<td>Longitudinal study of 33 female patients with SLE; Serological tests: CH50, C3, C4 SLE activity: clinical and serological assessment</td>
<td>Mean C3 and CH50 levels are significantly lower in patients with active renal disease than when renal involvement is absent. The mean level of C4 is slightly lower during active renal disease than during inactivity</td>
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<td>Pillemer et al. [69]</td>
<td>Longitudinal study of 55 patients with lupus nephritis; Serological tests: C3; Activity of lupus nephritis: scoring system designed by authors</td>
<td>Low C3 are associated with increased renal biopsy scores for cellular crescents, necrosis and endocapillary proliferation; Higher renal chronicity index is associated with longer duration of abnormal C3; Depressed C3 predicts the extent of persistently active glomerular disease in lupus nephritis</td>
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<td>Manger et al. [70]</td>
<td>Prospective study of 338 SLE patients; Serological tests: C3, C4; SLE activity: ECLAM, SDI</td>
<td>Low C3 at disease onset is a significant risk factor for subsequent end stage renal disease</td>
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<td>Gunnarsson et al. [71]</td>
<td>Prospective study of 18 SLE patients with proliferative nephritis; Serological tests: C3, C4, C1q; Disease activity: clinical and serological assessment</td>
<td>At first biopsy, low C1q is found; At repeated biopsy, low C1q combined with albuminuria predicts persistent histopathological activity. So serum C1q levels at both first and repeated renal biopsies are predictive marker of the histopathological outcome</td>
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<td>Sturfelt et al. [58]</td>
<td>Prospective study of 33 patients with SLE; Serological tests: C1q, C3, C4, C3d; SLE activity: clinical and serological assessment</td>
<td>C3d is consistently elevated during active glomerulonephritis; Persistently increased C3d predicts subsequent renal failure; C1q levels are transiently low during flare-ups of lupus glomerulonephritis</td>
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<td>Perrin et al. [72]</td>
<td>41 SLE patients; 31 cases of membranoproliferative glomerulonephritis (MPGN); 26 other types of GN cases, 6 cases of severe alcoholic cirrhosis of the liver; Serological tests: C3, C3d</td>
<td>Elevated plasma C3d is found in 68% SLE cases, in 87% MPGN cases, in 62% patients with other hypocomplementic nephritis, but only in 15% patients with normocomplementic nephritis and in 33% patients with liver cirrhosis; Inverse correlation between C3d levels and levels of C3 in plasma</td>
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<td>Negi et al. [73]</td>
<td>24 SLE patients (8 inactive disease cases, 7 active non-renal disease cases, 9 active lupus nephritis), 15 healthy individuals; Tests: c3, C4, C3d (serum and urine); SLE activity: SLEDAI</td>
<td>Serum C3d is higher in lupus patients than in healthy controls; Serum C3d levels are comparable in active renal and extrarenal SLE; Urine C3d is elevated only in active SLE, with highest levels in active lupus nephritis; Urine C3d is in stronger correlation with SLEDAI than serum C3, C4, C3d</td>
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<td>Kelly et al. [74]</td>
<td>Prospective study of 28 SLE patients; Tests: plasma C3, C4, C4d, Bb, C5b-9, urine C3d; SLE activity: SLAM, SLEDAI, PGA</td>
<td>Patients with normal C3 patterns had minimal disease activity; Patients with low molecular weight C3 fragments in urine had either severe disease with active lupus nephritis, or non-renal SLE of moderate disease activity</td>
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<td>Manzi et al. [66]</td>
<td>Prospective study of 31 SLE patients; Tests: plasma C3, C4, C4d, Bb, C5b-9, urine C3d; SLE activity: SLAM, SLEDAI, PGA</td>
<td>Urine C3d is better than C3, plasma C4d, Bb, C5b-9 in distinguishing acute lupus nephritis patients from those without such disease activity</td>
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<td>Ho et al. [75]</td>
<td>Prospective study of 53 SLE patients; Serological tests: C3, C4;</td>
<td>Decreases in C3 are associated with concurrent decreases in</td>
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<td>hematocrit, platelet, and white blood cell counts; Previous</td>
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<td>increases in C3 are associated with a decrease in platelets;</td>
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<td>Decreases in C4 are associated with concurrent decrease in the</td>
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<td>hematocrit level and platelet count; Decreases in complement</td>
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<td>levels are not consistently associated with SLE flares</td>
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<td>Hopkins et al. [62]</td>
<td>Prospective study of 40 SLE patients; Serological tests: C3, C4, C3a, C5a;</td>
<td>Plasma C3a levels increase in SLE patients during a disease</td>
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<td>SLE activity: physician assessment</td>
<td>flare, and are particularly high in 5 patients who had acute CNS</td>
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<td>dysfunction. In 4 of these 5 patients, significantly elevated</td>
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<td>plasma C5a levels are also detected</td>
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<td>Rother et al. [76]</td>
<td>79 SLE patients; Serological tests: C3, C4, CH50, C3d; SLE activity:</td>
<td>SLE patients with CNS involvement have significantly higher</td>
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<td>M-LAI</td>
<td>levels of plasma C3d than do patients without CNS involvement</td>
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<td>Karassa et al. [77]</td>
<td>32 SLE patients with CNS events matched 1:3 to 96 control SLE patients</td>
<td>Low serum C3 and C4 levels are associated with CNS</td>
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<td>without CNS events; Serological tests: C3, C4; SLE activity: clinical and</td>
<td>involvement; The odds for SLE patients with low serum C3 or</td>
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<td>serological assessment</td>
<td>C4 levels to have CNS disease are 3.8 (95%CI 1.6-9.4) and 3.5 (95%CI 1.8-6.8),</td>
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<td>respectively, compared to those with normal</td>
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<td>C3 or C4 levels</td>
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<td>Petz et al. [33]</td>
<td>Cerebral spinal fluid (CSF) samples from 11 SLE patients (4 subjects –</td>
<td>C4 levels in CSF are reduced in patients with active CNS SLE,</td>
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<td>multiple samples) and from 25 healthy controls; serum samples from other</td>
<td>and are at significant correlation with clinical manifestations of the disease. C2</td>
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<td>20 healthy controls; Tests: serum C1-C4, CH50, and C4 in CSF</td>
<td>and CH50 are also sensitive indicators of disease activity, whereas C3 is less</td>
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<td>frequently reduced</td>
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<td>Sanders et al. [34]</td>
<td>CSF and serum samples from Sjogren’s syndrome (SS) patients and from SLE</td>
<td>C5b-9 is detected in 86% SLE-CNS cases and in 88% SS-CNS cases. In some of them,</td>
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<td>cases; Tests: C5b-9 in serum and CSF</td>
<td>C5b-9 is present in CSF, but not serum. It is suggested that the terminal pathway</td>
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<td>of complement is activated intrathecally in patients with CNS in both SLE and SS</td>
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<td>Jongen et al. [32]</td>
<td>12 SLE patients with diffuse CNS and 28 control subjects; Tests: SCF</td>
<td>In CNS SLE, serum C4 is reduced, which reflects low-grade</td>
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<td>and serum C3, C4</td>
<td>systemic disease activity, and C3, C4 CSF/serum ratios are</td>
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<td>increased, which reflects compensatory intrathecal production. This suggests that</td>
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<td>CSF/serum ratio is a valid tool to detect</td>
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<td>intrathecal C3 or C4 production</td>
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<td>counts, protein concentrations</td>
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<td>Hedberg (2 suppl)</td>
<td>10 SLE patients (9 cases with arthritis duration &gt;1 yr); Tests: complement levels, protein concentrations in synovial fluid</td>
<td>Elevated protein concentrations; Low complements, with values approximating those of fluids from patients with rheumatoid arthritis</td>
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<td>Pekin et al. [27]</td>
<td>26 SLE patients; Tests: white blood cells, protein, CH50 in synovial fluid and serum; SLE activity: clinical and serological assessment</td>
<td>2 Classes of SLE effusions: 1) transudative (in acute SLE with low serum CH50, but without joint complaints): low CH50, low white blood cell count and protein in synovial fluid; 2) exudative (in active SLE with inflammatory polyarthritis): low CH50 despite large amounts of other serum proteins in the joint cavity. This indicates that low complement levels in joint fluids come through two distinct mechanisms</td>
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<td>Pleural Manifestations/Pleural fluid</td>
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<td>Hunder et al. [28]</td>
<td>23 Subjects with malignant disease, 6 SLE cases, 6 rheumatoid arthritis (RA) cases, 3 congestive heart failure cases, 3 pulmonary embolism cases, 9 cases of pleural effusion of undefined cause; Tests: C3, C4, CH50 in pleural fluid</td>
<td>Pleural fluid in SLE and RA contain significantly less CH50 and C3, C4 than pleural fluid from patients with non-rheumatic diseases; Serum-to-pleural fluid complement concentration ratio is greater in SLE and RA than in non-rheumatic subjects. This indicates increased complement utilization in pleural fluid with a potential of pleuritis development in these patients</td>
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<td>Kinney et al. [29]</td>
<td>Non-SLE subjects undergoing open-heart surgery for coronary artery or rheumatic heart disease; Tests: C3, C4, CH50 in pericardial fluid</td>
<td>Normal ranges for pericardial-fluid C3, C4 and CH50 are 35-127 mg/dl, 6.3-23 mg/dl, 1.9-9.1 CH50 units, respectively</td>
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<td>Goldenberg et al. [30]</td>
<td>Series of samples from an SLE patient with a large pericardial effusion and from non-SLE control subjects undergoing heart surgery; Tests: CH50 in serum and pericardial fluid, ANA, anti-DNA</td>
<td>No hemolytic complement activity is detectable in the SLE patient, whereas in controls CH50 is &gt;25 units/ml. In SLE patient, pericardial fluid contains positive ANA and anti-DNA, and &gt;90% neutrophils. This indicates a localized immune reaction within the pericardium space in SLE patients</td>
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<td>Hunder et al. [31]</td>
<td>2 SLE patients with pericarditis and 2 non-SLE controls with valvular heart disease; Tests: CH50, C1q, C3, C4 in serum and pericardial fluid</td>
<td>In Patient 1, the pericardial C3 is found to be half that of serum; pericardial C1q and C4 are also low, CH50 is absent. In Patient 2, CH50 is also absent, even though serum CH50 is normal, and all the complement components are similar to those in controls. Activation of both classical and alternative pathways is suggested in pericardial fluid of SLE patients</td>
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<td>Baines et al. [35]</td>
<td>Prospective study using serial serum samples obtained from 50 pregnant non-SLE subjects and 116 normal nonpregnant controls; Serological tests: CH50, C3</td>
<td>Gradually increasing C3 activity with pregnancy progression, following a significant depression during the 1st trimester, and slight though significant increases in the activity of CH50</td>
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<td>Gallery et al. [37]</td>
<td>Prospective study of 173 initially normotensive pregnant non-SLE subjects and 32 non-pregnant controls; Serological tests: C3, C4</td>
<td>Significant elevation of C3 and C4 during gestation; Hypertension in the 3rd trimester in 26 pregnant women, but with the same degree of C3 and C4 elevation as in the 147 normotensive pregnant subjects. So C3 and C4 tests are of no value in prediction of pregnancy-associated hypertension</td>
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<td>Buyon et al. [36]</td>
<td>Prospective study of 12 pregnant SLE patients, 17 non-SLE preeclampsia subjects, 24 normal pregnant subjects, and 21 healthy nonpregnant controls; Serological tests: C3, C4; SLE activity: clinical and serological assessment</td>
<td>Elevated C3 and C4 in normal pregnancy; In non-SLE preeclampsia, C3 levels same as in normal pregnancy, and decreased C4; In SLE pregnancy, C3 and C4 lower than in normal pregnancy or preeclampsia; In SLE pregnancy, falling C3 or C4 predicted a flare; Preeclampsia in some of the SLE cases with rising C3, but not SLE flare. So C3 and C4 can distinguish between SLE flare and preeclampsia.</td>
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<td>Hopkinson et al. [78]</td>
<td>Cross-sectional study of 83 pregnant women and 80 controls (47 men and 33 non-pregnant women); Serological tests: C3, C4 and C4d</td>
<td>Significantly elevated C3, reduced C4, increased C4d and higher C4d:C4 ratio in pregnant women. So plasma C4d cannot be used as a marker of diseases activity in pregnant patients with connective tissue diseases as pregnancy itself leads to C4 activation</td>
</tr>
<tr>
<td>Abramson et al. [39]</td>
<td>A review article: serological markers used to differentiate SLE flare from diseases of pregnancy</td>
<td></td>
</tr>
<tr>
<td>Teisner et al. [79]</td>
<td>Prospective study of normal pregnant patients; Serological tests: C3, C4, C3c, C3d</td>
<td>Increased levels of C3 and C3d in the 2nd and 3rd trimesters; C4 not affected by pregnancy; C3c not detected. Suggested increased turnover of native C3 during pregnancy rather than activation of the complement cascade</td>
</tr>
<tr>
<td>Schena et al. [80]</td>
<td>Prospective study of 286 normal pregnant women and 30 women with preeclampsia; Serological tests: CCH50, C3, C5, C9, C1inh, C1s, C1q</td>
<td>Increase in levels of immune complexes associated with the exacerbation of the pre-eclamptic picture and a decrease after delivery. No significant difference between complement components in the 3rd trimester and in preeclampsia. High levels of C3d were observed in normal pregnancy and in preeclampsia</td>
</tr>
<tr>
<td>Jenkins et al. [81]</td>
<td>Cross-sectional study of 65 normal pregnant women and 39 controls (21 non-pregnant women and 18 men); Serological tests: C3d</td>
<td>No significant difference between the 2 groups; Similar C3d concentrations in all 3 trimesters. So C3d levels are unaffected by uncomplicated pregnancy and can be used to monitor complement activation in lupus irrespective of pregnancy</td>
</tr>
<tr>
<td>Thomson et al. [82]</td>
<td>Prospective study of 5 women with severe preeclampsia; 9 women with mild preeclampsia; 8 women with normal pregnancy; Serological tests: C3, C4, C1q</td>
<td>No significant difference in complement components bw the 3 groups</td>
</tr>
<tr>
<td>Study</td>
<td>Study Design</td>
<td>Results/Conclusions</td>
</tr>
<tr>
<td>---------------------</td>
<td>------------------------------------------------------------------------------</td>
<td>---------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------</td>
</tr>
<tr>
<td>Hopkins et al. [62]</td>
<td>Prospective study of 7 pregnant SLE patients, 16 pregnant healthy controls and 7 non-SLE subjects with preeclampsia; Serological tests: C3, C4, C3a, C5a; SLE activity: physician assessment</td>
<td>High normal C3 in healthy pregnant subjects and non-SLE preeclampsia; Normal C3 and elevated C3a in pregnant SLE; Inverse correlation bw C3 decreases and elevations in C3a over time in pregnant SLE patients. Low C3 and associated increase in split products in pregnant SLE subjects indicate the complement activation</td>
</tr>
<tr>
<td>Buyon et al. [38]</td>
<td>Prospective study of 14 pregnant SLE patients and 10 non-SLE women with preeclampsia; Serological tests: C3, C4, CH50, Ba, Bb, SC5b-9, C4d; SLE activity: clinical and serological assessment</td>
<td>During SLE flare - abnormal Ba, Bb, SC5b-9, CH50, but not C4d levels; In non-SLE preeclampsia with elevated Ba – normal CH50; Ratio CH50:Ba significantly lower in lupus flares than in non-SLE patients with preeclampsia. Ongoing activation of the alternative complement pathway can accompany SLE flares in pregnant patients</td>
</tr>
<tr>
<td>Lockshin et al. [83]</td>
<td>Prospective study of 28 SLE pregnancies; pregnant patients with non-SLE rheumatic disease; nonpregnant SLE; Serological tests: C3, C4, CH50, C1s-C1inh; SLE activity: clinical and serological assessment</td>
<td>Low complements in pregnant patients; Low CH50 and normal C1s-C1inh in pregnant patients; In pregnant SLE patients C1s-C1inh independent of CH50; In nonpregnant patients – linear relationship bw C1s-C1inh and CH50. Hypocomplementia occurs via different mechanisms in pregnant and nonpregnant patients with SLE</td>
</tr>
<tr>
<td>Lao et al. [84]</td>
<td>Prospective study of SLE patients in remission before pregnancy; Serological test: serum urate levels and serum C3; SLE activity: clinical and serological assessment</td>
<td>Pregnant SLE patients who developed preeclampsia had a significantly higher serum urate level while their serum C3 levels remained similar to those of patients without preeclampsia</td>
</tr>
</tbody>
</table>
2 Cost Effectiveness Analysis of the Novel Screen Test for the
Diagnosis of Systemic Lupus Erythematosus
2.1 INTRODUCTION

Systemic Lupus Erythematosus (SLE) is an autoimmune chronic disease very difficult to diagnose. At present, the diagnosis is often late: at the moment of diagnosis nearly 80% of the patients show major organ involvement due to the disease activity [85]. For improved prognosis and prolonged survival in SLE patients, it is necessary to shift diagnosis closer to the time of actual disease onset.

Timely diagnosis will also help to avoid aggressive treatment and complications from such treatment, and decrease healthcare costs [86]. It is not uncommon for rheumatologists to initiate treatment in patients who are thought to have lupus-like illness but do not meet the diagnostic criteria. To conclude that a patient has lupus without enough clinical and laboratory evidence can be detrimental both for the patient and physician, particularly if a mild form of lupus, is not distinguished from severe lupus. In addition to physiological effect, this can cause use of inadequate and potentially toxic therapies.

Therefore, a reliable screening test for diagnosis of lupus is needed. In routine practice, physicians currently rely on single or multiple serologic abnormalities to differentiate lupus, such as antinuclear antibody (ANA), antibodies to dsDNA, anti-Smith antibody, and complement proteins C3 and C4. However, there is no single adequate marker among these abnormalities that is both sensitive and specific. The most commonly used the ANA test, provides at least 95% sensitivity, but only 49% specificity [87]. Detection of high titer antibodies to dsDNA, on the contrary, is very specific (96.6%), but not sensitive (58.1%) and relatively expensive [88].

A novel method for SLE diagnosis has recently been developed and is based on measuring complement activation. It is a simple, rapid, and inexpensive method that has greater combined sensitivity and specificity than any currently available tests for diagnosing SLE. This assay (the RBC test) can provide added value to traditional diagnostic tests for antinuclear antibodies (the ANA test) and anti-dsDNA antibodies (the DNA test) by capturing additional SLE patients among dsDNA false negatives and by avoiding inappropriate SLE diagnosis among ANA false positives.
The RBC is 81% sensitive and 91% specific for SLE versus healthy controls, and 72% sensitive and 79% specific for SLE versus other diseases, and it has an overall negative predictive value of 92%. This assay should ultimately have significant impact on the accuracy and timing of SLE diagnosis in general clinical practice, and may lead to earlier and more appropriate therapeutic interventions.

The objective of the present study is to evaluate this new diagnostic marker from the health policy standpoint, combining clinical and economical data and testing whether a new diagnostic strategy using the new assay is more cost effective relative to existing diagnostic practices.

2.2 METHODS

2.2.1 DECISION PROBLEM

To identify the costs and effects of different screening strategies involving the RBC and current standard ANA and DNA tests. This analysis assumes that the RBC test will not replace the latter two, but will complement them to enhance the diagnosis of lupus.

2.2.2 TARGET POPULATION

Several screening strategies are examined in a target population of white female subjects aged 15-50 years, who have clinical symptoms of undefined connective tissue disease (UCTD).

2.2.3 TESTING STRATEGIES

The conventional test strategy for SLE consists of two tests: ANA and DNA, where ANA is followed by DNA in the case of positive result of the former test.

The introduction of the new RBC test may improve the overall testing performance in terms of detecting patients with high post-test probability of having disease.

We intend to design an optimal testing strategy that would include traditional tests ANA and DNA, and the new test RBC.
Parameters of the tests such as sensitivity, specificity and the costs of performing the tests are presented in Table 2-1.

<table>
<thead>
<tr>
<th>Tests</th>
<th>Sensitivity</th>
<th>Specificity</th>
<th>Cost</th>
</tr>
</thead>
<tbody>
<tr>
<td>ANA</td>
<td>0.95</td>
<td>0.49</td>
<td>38</td>
</tr>
<tr>
<td>DNA</td>
<td>0.581</td>
<td>0.966</td>
<td>108.25</td>
</tr>
<tr>
<td>RBC</td>
<td>0.86</td>
<td>0.71</td>
<td>56.57</td>
</tr>
</tbody>
</table>

If all three tests are performed consecutively, they give eight possible testing outcomes with different post-test disease probabilities (please refer to ). The post-test probabilities are the functions of pre-test probability of disease in population of interest and the sensitivity and specificity parameters of the tests involved. The post-test probability of the disease after a positive test is calculated as:

\[
P_{\text{posttest}} = \frac{p_{\text{pretest}} \cdot \text{sens}}{p_{\text{pretest}} \cdot \text{sens} + (1 - p_{\text{pretest}})(1 - \text{spec})}
\]

The post-test probability of the disease after negative test is:

\[
P_{\text{posttest}} = \frac{p_{\text{pretest}} \cdot (1 - \text{sens})}{p_{\text{pretest}} \cdot (1 - \text{sens}) + (1 - p_{\text{pretest}})\text{spec}},
\]

where

- \(p_{\text{posttest}}\) - post-test probability of disease
- \(p_{\text{pretest}}\) - pre-test probability of disease
- \(\text{sens}\) - test sensitivity
- \(\text{spec}\) - test specificity

### 2.2.4 Study Questions

1. To evaluate additional costs and effectiveness associated with adding the RBC test to the traditional testing strategy.
2. To identify the least cost method of use of the RBC test.

2.2.5 **Markov Model**

Markov (state transition) models are used in this analysis to compare costs and health effects incurred as a result of diagnostic strategies involving the RBC test and the traditional strategy (ANA test followed by DNA) over a 10-year timeframe with the 1-year cycle length. The evaluation is performed from the societal perspective.

![General comparison model](image)

**Figure 2-1. General comparison model**

2.2.6 **Data Elements in Markov Models**

Table 2-2 lists the parameters and their values used in the Markov models. The explanations of the values follow below.

**Incidence of SLE.** Since our target population consists of women coming to a rheumatologist’s office with symptoms of undefined connective tissue disease (UCTD), the incidence is estimated to be 0.005\(^1\).

---

\(^1\) Personal communication with S. Manzi, MD, MPH, March 2004
Prevalence of SLE. Since our target population consists of women coming to a rheumatologist’s office with symptoms of undefined connective tissue disease (UCTD), the prevalence is estimated to be 0.0051.


http://www.cdc.gov/mmwr/preview/mmwrhtml/mm5117a3.htm

Table 2-2. Data elements used in the analyses

<table>
<thead>
<tr>
<th>Variables</th>
<th>Values</th>
</tr>
</thead>
<tbody>
<tr>
<td>Incidence of SLE, per person</td>
<td>10/1,000</td>
</tr>
<tr>
<td>Prevalence of SLE</td>
<td>2/1,000</td>
</tr>
<tr>
<td>Probability of dying from SLE</td>
<td>0.0011</td>
</tr>
<tr>
<td>Probability of dying from SLE under early diagnosis</td>
<td>0.0001</td>
</tr>
<tr>
<td>Probability of dying from causes other than SLE</td>
<td>0.0072</td>
</tr>
<tr>
<td>Utility for having no SLE</td>
<td>0.9</td>
</tr>
<tr>
<td>Utility for having SLE</td>
<td>0.62</td>
</tr>
<tr>
<td>Utility for having early-detected SLE</td>
<td>0.73</td>
</tr>
<tr>
<td>Utility for toxicity of unnecessary treatment</td>
<td>0.84</td>
</tr>
<tr>
<td>Sensitivity of RBC test</td>
<td>0.720</td>
</tr>
<tr>
<td>Specificity of RBC test</td>
<td>0.790</td>
</tr>
<tr>
<td>Sensitivity of ANA test</td>
<td>0.950</td>
</tr>
<tr>
<td>Specificity of ANA test</td>
<td>0.490</td>
</tr>
<tr>
<td>Sensitivity of dsDNA test</td>
<td>0.581</td>
</tr>
<tr>
<td>Specificity of dsDNA test</td>
<td>0.966</td>
</tr>
<tr>
<td>Cost of RBC test</td>
<td>56.57</td>
</tr>
<tr>
<td>Cost of ANA test</td>
<td>38.00</td>
</tr>
<tr>
<td>Cost of dsDNA test</td>
<td>108.25</td>
</tr>
<tr>
<td>Expected annual cost of illness in SLE</td>
<td>12,364.06</td>
</tr>
<tr>
<td>Expected annual cost of early-detected SLE</td>
<td>9,697.34</td>
</tr>
<tr>
<td>Expected annual cost associated with side effects of unnecessary treatment</td>
<td>137.56</td>
</tr>
<tr>
<td>Expected annual cost associated with prophylactic treatment</td>
<td>808.26</td>
</tr>
</tbody>
</table>

Probability of dying from SLE under early diagnosis. The factor most closely related to mortality in SLE is exacerbation frequency [85]. Patients fulfilling less of the SLE criteria at the time of diagnosis are less prone to exacerbations in future course of the disease [90]. It is assumed that early diagnosis before the development of required four criteria and early onset of treatment will avoid exacerbations and ensure 10-year survival close to 100% [85]. We therefore
expect very low mortality in SLE patients diagnosed early, and estimate it to the 0.0001 per person.

**Probability of dying from causes other than SLE.** Source: age-adjusted death rates for white and black females from the National Vital Statistics Report.  

**Utility for having no SLE.** Based on the modified HUI3 (mHUI3) and assumed to be similar to “very good” self-rated health [91].

**Utility for toxicity of unnecessary treatment.** Based on the modified HUI3 (mHUI3) and assumed to be similar to “good” self-rated health [91].

**Utility for having early-detected SLE.** Based on the modified HUI3 (mHUI3) and assumed to be equal to the average between “fair” and “good” self-rated health [91].

**Utility for having SLE.** Based on the modified HUI3 (mHUI3) and assumed to be similar to “fair” self-rated health [91].

**Sensitivity and specificity of RBC test.** Source: Manzi S. et al., 2002 [92].

**Sensitivity and specificity of ANA test.** Source: Tan E. et al. 1982 [87].

**Sensitivity and specificity of dsDNA test.** Source: Servais G. et al. 2001 [88].

**Cost of RBC test.** Preliminary estimation done in the Lupus Center of Excellence, University of Pittsburgh, 2003.

**Cost of ANA and dsDNA.** Source: CPT code book; assumption: the tests are done at the Immunology department of the University of Pittsburgh Medical Center (UPMC).

**Expected annual cost of illness in SLE.** Source: Sutcliffe N. 2001. Both direct and indirect costs are included [86].

**Expected annual cost of early-detected SLE.** Source: Sutcliffe N. et al, 2001 [86]. The mean SLICC index reflecting cumulative organ damage is found to be 1.1 for a cohort with mean age of 40 and disease duration 10.5 years, and according to the regression results of the same study, 1 unit increase in SLICC is associated with $2,666.72 increase in total cost of disease. It was found that the rate of damage increase is most pronounced during the first year after dx [93,94]. So, if we diagnose and start controlling the disease earlier, we can hope to prevent organ damage (expressed as preventing SLICC index from increasing to >0.1). This will decrease the cost by $2,666.72, and the annual cost of SLE under early dx and treatment will be $9,697.344.
**Expected annual cost associated with side effects of unnecessary treatment.** Source: Bae, S. et al., 2003 [95]. We assume that for SLE patients, cost due to treatment toxicity is already incorporated into the cost of illness. However, for false positive, the cost associated with side effects of unnecessary treatment should specifically be taken into account.

### 2.2.7 Analytic Plan

#### 2.2.7.1 Analysis 1.

“Simple” comparison”: we will evaluate incremental cost and effectiveness of simply adding the RBC test to the traditional strategy for subjects whose ANA is positive, but DNA is negative. We assume that this can decrease the number of false DNA negatives who need the treatment but will not be treated according to testing results (Figure 2-2).

**Figure 2-2. “Simple” comparison: traditional strategy vs. strategy involving the RBC test**

However, it is possible that the negative effect of treating the RBC false positives outweighs the positive effect of subjecting the RBC true positives to necessary treatment with steroids. In this case, the decision to treat the RBC positives results in reduction in expected years of life, which would not be an appropriate health policy action.

In order for the treatment decision always to increase the expected years of life, it should be taken only when the probability of the disease after the series of test results exceeds a certain threshold. So the next step in the analytic plan is:
2.2.7.2 Analysis 2

To determine the therapeutic threshold at which SLE prophylactic treatment should be applied. It is assumed that if the posttest probability of having the disease exceeds a pre-specified therapeutic threshold, patients will thereafter be closely observed by a rheumatologist specialist and administered certain treatment such as low-dose steroids or NSAIDs, to avoid development of more lupus criteria and worsening of the disease.

An exploratory analysis is conducted to identify the potential application of the RBC test in various possible strategies. The diagnostic strategies involving RBC are developed as described below, based on the therapeutic threshold.

2.2.8 Therapeutic threshold calculation

The success of the screening tests in the Analysis 2 is based on the ability to detect lupus at a probability at which application of therapy becomes reasonable (therapeutic threshold). That is, if the therapy is applied, its expected positive effect in a patient who has disease outweighs the expected negative effect of therapy in a patient who does not have disease [96].

We determine the therapeutic threshold probability by running a “natural disease history Markov model” over 10 years of a patient’s life (Figure 2-3). In this model, no testing is implemented. Instead, we assume that a hypothetical patient from the target population comes to the model with some known probability of having the disease. Two possible decisions are considered in the model: implementing prophylactic treatment or not, and we compare effectiveness (quality-adjusted years of life saved) associated with these decisions. We repeat this comparison given different levels of disease probability to see how effectiveness is influenced by disease probability. The therapeutic threshold is determined as such probability of having the disease, at which the quality adjusted years of life are the same both with and without the treatment.
In the base case, if the base parameter values (Table 2-2) are used in the natural history model, the therapeutic threshold equals 0.33, which corresponds to the expected value (EV) of effectiveness of 6.71 quality-adjusted years of life saved (Figure 2-4).

We next run a sensitivity analysis of the therapeutic threshold model to test the impact on the results of the assumptions made regarding key model parameters (Appendix ). We found that in particular, therapeutic threshold significantly depends on the utility associated with toxic

---

2 The two curves representing effectiveness of “treatment” and “no treatment” strategies, intersect when disease probability is 0.33. In this point, both strategies are equivalent in terms of their effectiveness. When the disease probability is <0.33, the “no treatment” strategy is superior to “treatment”. When the disease probability >0.33, the treatment strategy becomes more effective.

3 The shaded areas represent the dominance of a particular strategy. If the disease probability <0.33, the “no treatment” strategy dominates; if the disease probability>0.33, the “treatment” strategy becomes dominating.
treatment side effects (uTox) (Figure 2-5). Therefore, to make the results of the analysis more comprehensive, the development of diagnostic strategies involving RBC and subsequent exploratory analysis of these strategies will be based not on a single base therapeutic threshold value, but on several ranges on therapeutic threshold corresponding to various intervals of uTox values. However, in the section below describing the development of RBC-involving testing strategies, a single arbitrary therapeutic threshold of 0.15 is used for simplicity (which provides more clear explanation than the base threshold of 0.33 as it is associated with more use of the RBC test).

![Figure 2-5. Sensitivity analysis of utility associated with treatment side effects (uTox) on therapeutic threshold.](image)

2.2.9 DEVELOPMENT OF RBC-INVOLVING TESTING STRATEGIES: ELIMINATING REDUNDANT TESTS AND THE CHOICE OF THE LEAST COST TESTING STRATEGY

We now identify various possible testing strategies that would involve the RBC test, and related post-test disease probabilities predicted from these strategies. We will make a treatment decision as follows:

- If post-test disease probability > therapeutic threshold, then TREAT
- If post-test disease probability < therapeutic threshold, then DON’T TREAT

Out of 3 tests (ANA, DNA, RBC), 6 possible sequences can be constructed. For each of these sequences, we first assume that all 3 tests are done irregardless of results of previous tests results. Then redundant tests are eliminated as described below if they don’t influence treatment.
decision under the given therapeutic threshold. This will give us 6 “abbreviated”
testing/treatment strategies.

illustrates the elimination of redundant tests from the sequence ANA-DNA-RBC using
an arbitrary value of the therapeutic threshold of 0.15. In case of positive ANA followed by
positive DNA, running the RBC test does not affect the treatment decision, since the post-test
disease probability is greater than the treatment threshold both when RBC is positive and
negative. Therefore, patients will be treated both in case of positive and negative RBC test, so
running the RBC test appears to be redundant. In case of positive ANA followed by negative
DNA, and in case of negative ANA followed by negative DNA, running the RBC test results in
the post-test disease probability less than the treatment threshold both in case of negative and
positive RBC results. Hence patients will not be treated regardless of the RBC test result, and so
this test can be eliminated again. However, running the RBC test after negative ANA and
positive DNA does influence treatment decision, since in case of positive RBC, the posttest
disease probability is higher than the therapeutic threshold, while in case of negative RBC, the
posttest probability is lower than the therapeutic threshold. Therefore, the final test sequence will
start with ANA followed by DNA, and then by RBC only in case of negative ANA, but positive
DNA.

![Flowchart showing the elimination of redundant tests in ANA-DNA-RBC sequence.](image)

Figure 2-6. Elimination of redundant tests in ANA-DNA-RBC sequence.

Similarly, the redundant tests will be eliminated from other 5 possible testing sequences.
As a next step, we will choose the final strategy out of 6 “abbreviated” ones. At this point it is important to mention that the outcome (quality-adjusted years of life) does not depend on the order of the tests. Those patients will be treated who have positive ANA and DNA and those who have negative ANA but positive DNA and RBC. However, the order of the tests determines the expected cost of testing. Therefore, the finally chosen strategy should be the one that has the least expected cost.

2.2.10 Choice of the Least Cost Testing Strategy

Expected cost of six testing strategies is calculated from cost of tests (Table 2-2) and the full probability of running each of the tests. According to Table 2-3, under an arbitrary therapeutic threshold of 0.15, the DNA-ANA-RBC strategy would be preferred as the least cost one.

Table 2-3. Expected cost of testing under various sequence of the tests (therapeutic threshold 0.15)

<table>
<thead>
<tr>
<th>Sequence</th>
<th>Cost of testing, $</th>
</tr>
</thead>
<tbody>
<tr>
<td>ANA-RBC-DNA</td>
<td>132.84</td>
</tr>
<tr>
<td>RBC-ANA-DNA</td>
<td>153.99</td>
</tr>
<tr>
<td>DNA-ANA-RBC</td>
<td>111.56</td>
</tr>
<tr>
<td>DNA-RBC-ANA</td>
<td>113.00</td>
</tr>
<tr>
<td>ANA-DNA-RBC</td>
<td>147.23</td>
</tr>
<tr>
<td>RBC-DNA-ANA</td>
<td>166.10</td>
</tr>
</tbody>
</table>

2.2.11 Therapeutic Thresholds Intervals and Related RBC-Involving Strategies

As was mentioned earlier, the therapeutic threshold is highly sensitive to the utility associated with negative treatment effects (uTox) (Figure 2-5). Different uTox values will be associated with different therapeutic thresholds. As the therapeutic threshold changes in sensitivity analysis, the treatment strategy changes discretely. For instance, as can be seen on , in the sequence ANA-DNA-RBC, running RBC test becomes redundant after ANA-DNA+ if the treatment threshold to be between 0.239 and 0.373. In general for this sequence, if the therapeutic threshold is over 0.373 but below 0.852, treatment is applied only when all three tests are positive; if the therapeutic threshold is above 0.239, treatment is also applied when ANA and DNA are positive while RBC is negative; if the therapeutic threshold is over 0.127, treatment is also applied when ANA is negative, but DNA and RBC are positive; finally, if the therapeutic
threshold is over 0.032, treatment is applied when ANA is positive, DNA is negative and RBC is positive.

We select four intervals of therapeutic thresholds. The borders of these intervals are selected so that when the therapeutic threshold crosses these borders, the treatment decision changes ( ). For instance, the when the therapeutic threshold is between 0.127 and 0.239, then treatment decisions are assigned as shown on in . However, if the therapeutic threshold is just above 0.239, we are going to change our treatment decision not treating the patients with ANA negative, DNA positive, and RBC positive.

For these four intervals of therapeutic threshold, we repeat the procedure of elimination of non-informative tests in six possible test sequences. From the six reduced testing strategies to be applied in each of these intervals, we then choose least cost strategies corresponding to the four intervals of therapeutic thresholds as described in previous sections. This will give us four least cost abbreviated strategies for each of the threshold intervals (Figure 2-8).

The structure of the reduced testing strategies and their costs are represented in Table 2-4. The expected costs of these strategies are plotted against 4 selected therapeutic threshold intervals in Figure 2-7. The reduced testing strategy resulting from the sequence ANA-RBC-DNA is least cost for all therapeutic threshold intervals but 0.127 – 0.239. In the threshold interval 0.127 – 0.239, the reduced test strategy resulting from the DNA-ANA-RBC sequence is the least-cost. The least cost testing strategies for each of the threshold intervals are presented in Figure 2-8.

### Table 2-4. Test sequences, treatment strategies, and expected cost of reduced test strategies

<table>
<thead>
<tr>
<th>Sequence</th>
<th>&lt;0.127 Threshold</th>
<th>0.127-0.239 Threshold</th>
<th>0.239-0.373 Threshold</th>
<th>0.373-0.852 Threshold</th>
</tr>
</thead>
<tbody>
<tr>
<td>ANA-RBC-DNA</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>ANA+RBC+DNA+</td>
<td>148.22</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>ANA+RBC-DNA+</td>
<td>132.84</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>ANA-RBC+DNA+</td>
<td>95.59</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>RBC-ANA-DNA</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>RBC+ANA+DNA+</td>
<td>148.22</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>RBC+ANA-DNA+</td>
<td>153.99</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>RBC-ANA+DNA+</td>
<td>95.59</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>DNA-ANA-RBC</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>DNA+ANA+RBC+</td>
<td>174.83</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>DNA+ANA-RBC+</td>
<td>111.56</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>DNA+ANA-RBC+</td>
<td>110.58</td>
<td></td>
<td></td>
<td></td>
</tr>
</tbody>
</table>
Table 2-4 (contd.)

<table>
<thead>
<tr>
<th>Sequence</th>
<th>&lt;0.127 Threshold Cost</th>
<th>0.127-0.239 Threshold Cost</th>
<th>0.239-0.373 Threshold Cost</th>
<th>0.373-0.852 Threshold Cost</th>
</tr>
</thead>
<tbody>
<tr>
<td>DNA-RBC-ANA</td>
<td>DNA+RBC+ANA+ 174.00</td>
<td>DNA+RBC+ANA+ DNA+RBC+ANA+ 113.00</td>
<td>DNA+RBC+ANA+ DNA+RBC+ANA+ 110.58</td>
<td>DNA+RBC+ANA+ 112.77</td>
</tr>
<tr>
<td></td>
<td>DNA+RBC+ANA+ DNA+RBC-ANA+ 174.00</td>
<td>DNA+RBC+ANA+ DNA+RBC+ANA+ 113.00</td>
<td>DNA+RBC+ANA+ DNA+RBC+ANA+ 110.58</td>
<td>DNA+RBC+ANA+ 112.77</td>
</tr>
<tr>
<td></td>
<td>DNA+RBC+ANA+ DNA+RBC+ANA+ 174.00</td>
<td>DNA+RBC+ANA+ DNA+RBC+ANA+ 113.00</td>
<td>DNA+RBC+ANA+ DNA+RBC+ANA+ 110.58</td>
<td>DNA+RBC+ANA+ 112.77</td>
</tr>
<tr>
<td>ANA-DNA-RBC</td>
<td>ANA+DNA+RBC+ANA+DNA+RBC+ANA+ 174.83</td>
<td>ANA+DNA+RBC+ANA+DNA+RBC+ANA+ 147.23</td>
<td>ANA+DNA+RBC+ANA+DNA+RBC+ANA+ 95.59</td>
<td>ANA+DNA+RBC+ANA+DNA+RBC+ANA+ 98.08</td>
</tr>
<tr>
<td></td>
<td>ANA+DNA+RBC+ANA+DNA+RBC+ANA+ 174.83</td>
<td>ANA+DNA+RBC+ANA+DNA+RBC+ANA+ 147.23</td>
<td>ANA+DNA+RBC+ANA+DNA+RBC+ANA+ 95.59</td>
<td>ANA+DNA+RBC+ANA+DNA+RBC+ANA+ 98.08</td>
</tr>
<tr>
<td></td>
<td>ANA+DNA+RBC+ANA+DNA+RBC+ANA+ 174.83</td>
<td>ANA+DNA+RBC+ANA+DNA+RBC+ANA+ 147.23</td>
<td>ANA+DNA+RBC+ANA+DNA+RBC+ANA+ 95.59</td>
<td>ANA+DNA+RBC+ANA+DNA+RBC+ANA+ 98.08</td>
</tr>
<tr>
<td>RBC-DNA-ANA</td>
<td>RBC+DNA+ANA+RBC+DNA+ANA+DNA+RBC+ANA+ 174.00</td>
<td>RBC+DNA+ANA+RBC+DNA+ANA+DNA+RBC+ANA+ 166.10</td>
<td>RBC+DNA+ANA+RBC+DNA+ANA+DNA+RBC+ANA+ 110.58</td>
<td>RBC+DNA+ANA+DNA+RBC+ANA+ 83.12</td>
</tr>
<tr>
<td></td>
<td>RBC+DNA+ANA+RBC+DNA+ANA+DNA+RBC+ANA+ 174.00</td>
<td>RBC+DNA+ANA+RBC+DNA+ANA+DNA+RBC+ANA+ 166.10</td>
<td>RBC+DNA+ANA+RBC+DNA+ANA+DNA+RBC+ANA+ 110.58</td>
<td>RBC+DNA+ANA+DNA+RBC+ANA+ 83.12</td>
</tr>
<tr>
<td></td>
<td>RBC+DNA+ANA+RBC+DNA+ANA+DNA+RBC+ANA+ 174.00</td>
<td>RBC+DNA+ANA+RBC+DNA+ANA+DNA+RBC+ANA+ 166.10</td>
<td>RBC+DNA+ANA+RBC+DNA+ANA+DNA+RBC+ANA+ 110.58</td>
<td>RBC+DNA+ANA+DNA+RBC+ANA+ 83.12</td>
</tr>
</tbody>
</table>

Figure 2-7. Expected cost after eliminating redundant tests for six test sequences and four intervals of therapeutic threshold.
**Therapeutic threshold:**

- **<0.127**
- **0.127 - 0.239**
- **0.239 – 0.372**
- **0.372 – 0.852**

<table>
<thead>
<tr>
<th></th>
<th>RBC</th>
<th>ANA</th>
<th>DNA</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>&lt;0.127</strong></td>
<td>N</td>
<td>N</td>
<td>N</td>
</tr>
<tr>
<td><strong>0.127 - 0.239</strong></td>
<td>N</td>
<td>N</td>
<td>T</td>
</tr>
<tr>
<td><strong>0.239 – 0.372</strong></td>
<td>N</td>
<td>T</td>
<td>N</td>
</tr>
<tr>
<td><strong>0.372 – 0.852</strong></td>
<td>T</td>
<td>T</td>
<td>N</td>
</tr>
</tbody>
</table>

**Expected cost of testing per person:**

- **$148.2**
- **$111.6**
- **$95.6**
- **$82.8**

Figure 2-8. Least-cost test strategies for four therapy threshold intervals for ANA-RBC-DNA test sequence.

### 2.2.12 Least Cost Traditional Strategy

Our goal is to compare the RBC test performed together with traditional tests with the traditional tests alone. Therefore, we also select the least cost testing strategy for the combinations of the two traditional tests ANA and DNA for different values of therapeutic threshold. However, the treatment strategy is more robust for the combinations of two tests. According the posttest probabilities in Figure 2-9, for any therapeutic threshold between 0.084 and 0.626 the treatment is applied only when both ANA and DNA are positive. The least-cost strategy to detect patients with both ANA and DNA positive would require running DNA test after ANA (Table 2-5).
Table 2-5. Test sequences, treatment strategies, and expected cost of reduced test strategies

<table>
<thead>
<tr>
<th>Sequence</th>
<th>Strategy: treat if…</th>
<th>Cost</th>
</tr>
</thead>
<tbody>
<tr>
<td>ANA-DNA</td>
<td>ANA+DNA+</td>
<td>$95.6</td>
</tr>
<tr>
<td>DNA-ANA</td>
<td>DNA+ANA+</td>
<td>$110.6</td>
</tr>
</tbody>
</table>

2.2.13 **COST-EFFECTIVENESS ANALYSIS OF USING RBC TEST FOR SLE SCREENING**

2.2.13.1 **Analysis 1**

The first analysis evaluates additional cost and effectiveness associated with simple adding of the RBC test to the traditional strategy (Figure 2-2).

2.2.13.2 **Analysis 2**

The ultimate goal of this analysis was to evaluate the incremental cost-effectiveness of using RBC test for SLE screening in conjunction with the traditional ANA and DNA tests. As follows from the previous sections, the introduction of RBC test to the traditional testing procedures in the least-cost way may result in both an increase and a decrease in the total expected cost of the testing depending on the therapeutic threshold (Figure 2-8).
In this section we run the cost-effectiveness analysis comparing the traditional SLE testing procedure based on ANA test followed by DNA test with the least cost testing strategy including RBC test. It is expected that using RBC test will result in an increase in quality adjusted years of life as a result of early disease detection and, on the other hand, avoidance of false positive diagnosis and unnecessary treatment. The additional test may also result in extra costs of SLE treatment as more patients may be tested positive. Finally, the cost of additional RBC test will contribute to the total cost increase. The proposed cost-effectiveness analysis will calculate the cost of the incremental quality adjusted year of life achieved with introduction of RBC test.

Since the choice of optimal test strategy including RBC test depends on the therapeutic threshold and estimated negative effect of prophylactic treatment on false positives, we will run four cost-effectiveness analyses for the therapeutic threshold intervals corresponding to the least cost testing strategies shown in Figure 2-8.

Each of the four cost-effectiveness analyses will use the utility of negative treatment effect consistent with the correspondent therapeutic threshold shown in Figure 2-5.

Table 2-6 shows the uTox intervals corresponding to the therapeutic threshold intervals for each of the four strategies, and the values of uTox within these intervals used for the cost effectiveness analyses.

<table>
<thead>
<tr>
<th>Table 2-6. Therapeutic threshold intervals, uTox intervals and uTox values used for the four treatment strategies</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>Strategy</strong></td>
</tr>
<tr>
<td>---</td>
</tr>
<tr>
<td>1</td>
</tr>
<tr>
<td>2</td>
</tr>
<tr>
<td>3</td>
</tr>
<tr>
<td>4</td>
</tr>
</tbody>
</table>

Cost-effectiveness analyses involve the same variables presented in Table 2-2.
2.3 RESULTS

2.3.1 ANALYSIS 1

As Table 2-7 shows, the traditional strategy dominates the RBC-involving one, since adding the RBC test is associated with greater costs (expected incremental cost is $552.3), and with lower effectiveness (expected 0.048 quality-adjusted years of life lost).

Table 2-7. Results of the cost-effectiveness analysis of the strategy involving the RBC test with respect to traditional

<table>
<thead>
<tr>
<th>Strategy</th>
<th>Cost</th>
<th>QALYs</th>
<th>ICER</th>
</tr>
</thead>
<tbody>
<tr>
<td>ANA, DNA (traditional)</td>
<td>6369.5</td>
<td>7.379</td>
<td></td>
</tr>
<tr>
<td>ANA, DNA, RBC</td>
<td>6921.8</td>
<td>7.331</td>
<td></td>
</tr>
<tr>
<td>Δ</td>
<td>552.3</td>
<td>-0.048</td>
<td>-11,602.94</td>
</tr>
</tbody>
</table>

2.3.2 ANALYSIS 2

Results of the cost-effectiveness analyses of optimal strategies with respect to traditional are presented in Table 2-8. If therapeutic threshold is estimated as <0.127, then the optimal strategy involving RBC would be Strategy 1, where ANA is always followed by RBC test, and DNA is done in case of positive ANA, but negative RBC, and in case of negative ANA, but positive DNA (Figure 2-8). The incremental cost-effectiveness of this strategy with respect to the traditional strategy (ANA test followed by DNA in case of positive result on the former) is estimated as $48,165 per quality-adjusted year of life saved.

---

4 Incremental cost-effectiveness ratio
Table 2-8. Results of the cost-effectiveness analyses of optimal strategies with respect to traditional

<table>
<thead>
<tr>
<th>Therapeutic threshold interval</th>
<th>Strategy 1</th>
<th>Cost</th>
<th>Eff</th>
<th>Incr C/E</th>
</tr>
</thead>
<tbody>
<tr>
<td>&lt;0.127</td>
<td>ANA, RBC, DNA</td>
<td>$6,778.90</td>
<td>7.394</td>
<td></td>
</tr>
<tr>
<td></td>
<td>ANA, DNA</td>
<td>$6,369.50</td>
<td>7.386</td>
<td>0.0085</td>
</tr>
<tr>
<td></td>
<td>∆</td>
<td>$409.40</td>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td><strong>Strategy 2</strong></td>
<td>$6,386.50</td>
<td>7.3831</td>
<td></td>
</tr>
<tr>
<td>0.127-0.239</td>
<td>DNA, ANA, RBC</td>
<td>$6,369.50</td>
<td>7.3828</td>
<td>0.0003</td>
</tr>
<tr>
<td></td>
<td>∆</td>
<td>$17</td>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td><strong>Strategy 4</strong></td>
<td>$6,423.90</td>
<td>7.3765</td>
<td></td>
</tr>
<tr>
<td>0.373-0.852</td>
<td>ANA, RBC, DNA</td>
<td>$6,369.50</td>
<td>7.3734</td>
<td>0.0031</td>
</tr>
<tr>
<td></td>
<td>ANA, DNA</td>
<td>$54.40</td>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td>∆</td>
<td>$409.40</td>
<td></td>
<td>$48,164.71</td>
</tr>
</tbody>
</table>

If therapeutic threshold is estimated as <0.127-0.239, then Strategy 2 would be optimal. In this strategy, DNA test is followed by ANA test in case of positive result of the former, and then RBC test follows in case of negative ANA (Figure 2-8). The incremental cost-effectiveness of this strategy compared to traditional is estimated $55,667 per quality-adjusted year of life saved.

In case of even higher therapeutic threshold equivalent to 0.239-0.373, the optimal testing strategy coincides with traditional, and RBC test does not play any role (Figure 2-8).

If therapeutic threshold reaches the values of 0.373-0.852, Strategy 4 becomes optimal. This strategy starts with ANA testing, followed by RBC in case of positive ANA result. If RBC test is positive, DNA test is done. The incremental cost-effectiveness of this strategy with respect to traditional is $17,548.
2.4 CONCLUSION

- Under traditional strategies the same decision to treat patients with both positive DNA and ANA is made for the therapeutic threshold disease probability within a wide range of 0.084 to 0.626 (see Figure 2-9). Introduction of RBC test to the traditional ANA and DNA tests complicates the treatment decision making. Now different treatment decisions must be made for therapeutic thresholds below 0.127, from 0.127 and 0.239, from 0.239 to 0.373, and from 0.373 to 0.852 (Table 2-4).

- The sensitivity analysis shows that the therapeutic threshold can significantly vary with the input parameters of the model, especially the negative effect of prophylactic treatment on false positives represented by the variable uTox (Figure 2-5).

- If the therapeutic threshold is established for prophylactic treatment, introduction of RBC to the traditional strategies is the most cost-effective when the therapeutic threshold is very high. The incremental cost-effectiveness of RBC test to the traditional tests is $17,548 per adjusted years of life for the case of high therapeutic threshold in the range of 0.373 to 0.852. The benefit from RBC test here comes from the reduction of false positives to which the treatment is applied.

- At the lower levels of therapeutic threshold either the incremental cost-effectiveness of the RBC test is significantly higher ($48,165 and $56,667 for the threshold below 0.127 and between 0.127 and 0.239) or RBC test is redundant since it does not influence the treatment decision (for the threshold within 0.239 and 0.373).
2.5 SUMMARY

In conclusion, our findings confirm that the choice of diagnostic strategy depends on the threshold disease probability. If it is low or high, the use of RBC test is warranted. Otherwise, the traditional strategy is the most reasonable one.

If threshold disease probability is high, the strategy ANA-RBC-DNA is particularly powerful, since it yields a relatively low ICER ($17,548.39). However, since no agreement currently exists on what is a reasonable threshold for cost effectiveness of a new diagnostic strategy or a therapy, and since most of authors agree that it is currently far over $50,000, the strategies recommended under lower disease probability are also reasonable to use as they yield ICER $48,164.71 and $56,666.67.
3 Prevalence of Atherosclerosis and Associated Risk Factors in
Women with Systemic Lupus Erythematosus
3.1 INTRODUCTION

Systemic lupus erythematosus (SLE) is a multisystemic autoimmune disease with a wide spectrum of clinical presentations and mortality causes. Over the last decades, with improved management of the disease, patients die due to active lupus or an infection less frequently, but cardiovascular disease (CVD) has emerged as a significant threat to their health.

The burden of CVD in SLE population is comprehensively described. An increased risk was particularly noted in young SLE patients [97-99]. Female lupus patients aged 35-44 are over 50 times more likely to have myocardial infarction than are women of similar age without lupus [97]. Pathogenesis of vascular disease in lupus population is thought to be of atherosclerotic origin, and studying early subclinical manifestations of atherosclerosis in SLE patients is important for timely and successful interventions. Subclinical vascular disease in carotid arteries is evaluated using B-mode ultrasound, which measures the degree of focal plaque and intima-media thickness (IMT) [100,101]. In the study of Roman et al., 37% SLE patients compared to 15% controls had evidence of carotid plaque [98]. Calcification of coronary arteries and aorta measured by electron beam tomography (EBT) is another marker of subclinical vascular disease. It was shown that in SLE, the prevalence of coronary calcification and the mean coronary calcium scores were both significantly increased in lupus patients [99]. Aortic stiffness is a measure of functional rather than structural abnormalities of aorta. It is evaluated using pulse-wave velocity (PWV), which measures the rate at which arterial pulse waves move along the vessel. Although all these subclinical markers were shown to be significant predictors of future cardiovascular events and mortality [102-104], few studies compared prevalence of subclinical vascular disease in different vascular beds (e.g., carotid and coronary) in women with SLE.

While premature atherosclerosis in lupus patients is an accepted fact, the relationship between lupus and CVD is less clear. There are several potential mechanisms by which atherosclerosis may be accelerated in SLE. It is recognized that in addition to traditional cardiovascular risk factors, inflammatory and lupus-related factors are involved. However, the data on some of these factors are either contradictory or scarce. For instance, while duration of steroid exposure has been identified as a significant risk factor for future events [100], some studies found no relationship between steroid use and CVD [99,105]. Increased titers of antiphospholipid (aPL) antibodies are associated with an increased risk of vascular abnormalities
in some studies [106,107], while others have shown lower prevalence of aPL in patients with carotid plaque [98] or with coronary calcification [99]. The data on inflammatory markers are limited and controversial. Expression of endothelial cells sICAM-1 and E-selectin was elevated during exacerbation of SLE in one study [108], but not in the other [109]. Plasminogen activator inhibitor (PAI) was found to be increased in lupus patients with the history of thrombotic events [110]. However, the data on the relationship between inflammatory markers and atherosclerosis in SLE patients is mostly limited to the finding that increased CRP and low white cell count and albumin are associated with subclinical vascular disease [111]. In addition, overall, no agreement exists on which clinical manifestations or biomarkers are the best predictors of CVD in lupus patients.

The present study was designed to extend our knowledge of subclinical vascular disease in patients with SLE by examining calcification of coronary arteries and aorta using EBT, and evaluating traditional, lupus-related and inflammatory risk factors associated with this phenomenon. The frequency and extent of focal carotid plaque and IMT were be evaluated using US, and the burden of atherosclerosis in the two vascular beds was be compared.

### 3.2 PATIENTS AND METHODS

#### 3.2.1 STUDY COHORT

##### 3.2.1.1 SLE Patients

The women recruited for this study are currently enrolled in the Pittsburgh Lupus Registry described previously [111]. All eligible women who were 18 years of age or older were invited to participate in the original cardiovascular study, regardless of their history of cardiovascular events. The first 300 women to respond were enrolled. Of these 300 patients, those 200 who are free of previous cardiovascular event (confirmed MI or stroke, or a physician diagnosis of angina or transient ischemic attach (TIA)), are being recruited for evaluation of calcification of the
coronary arteries and aorta using EBT. Criteria for defining a myocardial infarction, stroke, angina and transient ischemic attack were taken from the Cardiovascular Health Study [112]. The University of Pittsburgh’s Institutional Review Board approved this study, and all women provided written informed consent prior to participation. Each participant provided an authorization for release of medical information so that pertinent hospital and outpatient records could be reviewed to confirm aforementioned events. The analyses presented in this report include those CVD event-free 137 women who have all the data completed by far.

3.2.1.2 Controls

The control group recruited for this study consisted of healthy women free of previous cardiovascular event and matched to cases by age (5-year interval), race and geographical location. For the present analysis, we used the data on 106 control subjects.

3.2.2 VARIABLE MEASUREMENT

Participation in the study consisted of an interview, physical examination, laboratory tests, carotid ultrasound scan and EBT, all of which took place on the same day. Several groups of risk factors were evaluated.

3.2.2.1 Traditional Cardiovascular Risk Factors.

The questionnaires were used to obtain the information on age, race, education level, household income, smoking habits (current use and total pack-years), family history of cardiovascular disease (MI, stroke, or sudden death in a first-degree relative before the age of 60 years), menopausal status (follicle-stimulating hormone levels were obtained when menopausal status was uncertain), use of estrogen replacement therapy and diabetes (use of oral hypoglycemic agents or insulin). Body mass index (calculated from height and weight) and waist-to-hip ratio were obtained using a standard protocol. Current blood pressure status was determined using an average of 2 consecutive sitting blood pressure readings. Levels of total cholesterol, low-density lipoprotein (LDL) cholesterol, high-density lipoprotein (HDL) cholesterol, triglyceride, glucose,

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5HEARTS Study: Heart Effects of Atherosclerosis and Risks of Thrombosis in SLE. Principal Investigator: S. Manzi, MD, MPH; Research Support: NIH RO1 AR46588-01

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insulin and homocysteine levels were measured in fasting blood samples using standardized laboratory tests. Hypertension was defined as an average systolic blood pressure $\geq 140$ mmHg or an average diastolic blood pressure $\geq 90$ mmHg or the use of antihypertensive agents. Diabetes was defined according to the American Diabetes Association criteria. Hyperlipidemia as defined either by diagnosis, or by the use of antidiabetic drugs. A blood clot was confirmed by medical records upon the report of a clot made by a patient.

3.2.2.2 SLE-Related Disease Factors.

SLE-disease activity was measured using the Systemic Lupus Activity Measure (SLAM) [113] and the Systemic Lupus Erythematosus Disease Activity Index (SLEDAI). Cumulative organ damage was measured by the Systemic Lupus International Collaborating Clinics (SLICC) damage index [114] and a modified version of SLICC that excluded several of the cardiovascular variables, such as angina, MI, and coronary artery bypass surgery. In order to complete these indices, we have obtained a routine cell sedimentation rate (ESR), antibodies to native DNA (double-stranded DNA (dsDNA) determined by immunofluorescence with *Crithidia lucillae* kinetoplast assay), and C3 and C4 complement levels. Information on corticosteroid therapy (current use, maximum dose, cumulative dose, and duration of use), hydroxychloroquine and immunosuppressant use was collected. Renal disease was defined using the SLICC renal variable, which requires the presence of nephrotic-range proteinuria ($\geq 3.5$ grams/24 hours) or renal insufficiency (glomerular filtration rate < 50%). Other laboratory studies included a lupus anticoagulant test [partial thromboplastin time (APTT, APTT with mix) or dilute Russell’s viper venom time (dRVVT)], as well as tests for anticardiolipin antibodies (IgG $> 15$ GPL units, IgM $> 10$ MPL units; Incstar, Stillwater, MN).

3.2.2.3 Immune and Inflammatory Markers.

Serum albumin (dye binding assay), C-reactive protein (ultrasensitive colometric ELISA), fibrinogen (modified clot-rate assay), soluble intercellular adhesion molecule-1 (sICAM-1), E-selectin, soluble CD40L (ELISA), and plasminogen activator inhibitor-1 (PAI-1) levels were measured.

Each laboratory test was done in the same lab for all the study participants.
3.2.3 **Vascular Disease Measurements**

**EBT** was used to provide 2 disease measures: coronary calcification and aortic calcification. The EBT scanning protocol was done in the Preventive Cardiology Center in the Division of Cardiology at the University of Pittsburgh Medical Center directed by Dr. D.Edmundowicz. The scanning was done using an Imatron C-150 Ultrafast CT Scanner (Imatron, South San Francisco, CA) and standard protocol. The calcium scoring method was the same for coronary and aortic images. The total calcium score was calculated with a densitometric program available on the Imatron C-150 scanner using the Agatston method. We tested within-reader variability and between-reader reliability on an ongoing basis.

**Carotid ultrasound** was performed at the University of Pittsburgh Epidemiology Ultrasound Research Laboratory under the direction of Dr. K. Sutton-Tyrrell as previously described [100]. Briefly, a Toshiba SSA-270A scanner (Tustin, CA) equipped with a 5-MHz linear array imaging probe was used to image the right and left common artery, carotid bulb, and the first 1.5 cm of the internal and external carotid arteries. Plaque was defined as a distinct focal area protruding into the vessel lumen, with at least 50% greater thickness than that found in surrounding areas. For each segment scanned, the degree of plaque was graded as follows: 0 = no observable plaque; 1 = one small plaque (less than 30% of the vessel diameter); grade 2 = one medium plaque (between 30% and 50% of the vessel diameter) or multiple small plaques; grade 3 = one large plaque (greater than 50% of the vessel diameter) or multiple plaques with at least one medium plaque. The grades were summed across the right and the left carotid arteries to create the combined plaque index (possible range 0-30), the overall measure of the extent of focal plaque.

Intima-media thickness (IMT) was measured across 1-cm segments of both the right and left sides of the near and far walls of the distal common carotid artery and the far wall of the carotid bulb and the internal carotid artery. Values from each location were averaged to produce an overall measure of IMT. The reproducibility of carotid scanning has previously been documented [100].
3.2.4 Statistical Analysis

The distributions of aortic and coronary calcification scores, the main outcomes of interest, were heavily skewed. The calcium scores were dichotomized by the cutpoint of 0 score (absence/presence of calcium), or divided by natural cutpoints for the coronary arteries (0, 10 and 100 calcium score) and aorta (0 and 300 calcium score). The descriptive analyses were summarized as means, medians and ranges for continuous variables and as percentages for categorical variables. All risk factors were screened univariately, and those variables significantly associated with the presence of coronary and aortic calcium at the $\alpha=0.20$ level of significance, were subsequently assessed in stepwise multivariate analyses. Statistical tests used for the univariate analyses included t-test for normally distributed continuous data, Wilcoxon rank sum test when the assumptions of the t-test were not met, and chi-square tests or Fisher’s exact test for categorical data. Continuous risk factors were either used in their original forms or split into categories based on means, quartiles, or natural cut-off points. SAS-pc (SAS institute, Cary, NC) was used to perform all statistical procedures.

Stepwise binary logistic regression was utilized to build models evaluating independent risk factors related to the presence of coronary or aortic calcium, in the total study cohort and in the subsets of pre- and postmenopausal patients. Ordinal logistic regression (cumulative logit) was used to identify risk factors associated with severity of calcification. Model assumptions (Hosmer and Lemeshow goodness-of-fit and score test for proportional odds) were used to assess model fit [115]. All the multivariate models were controlled by age, a known determinant of EBT-detected vascular disease [116,117].

Other outcomes included the presence (yes/no) and severity (plaque index 0, 1, 2, or $\geq 3$) of carotid plaque and IMT. On the subset of patients (N=70) whose carotid ultrasound and EBT were done within one year, we evaluated the association between the two measures of atherosclerosis.

3.2.5 Statistical Power

Based on our power computations, we designed a study that recruited 100 cases. Power calculations for the multiple logistic regression models incorporated the multiple correlation coefficient $\rho^2$ relating the specific covariate of interest to the remaining covariates, $P_0$ - the
probability of events at mean value of all covariates (using 0 value for binary covariates), and the
OR of disease corresponding to a one standard deviation change from the mean value of the
specific covariate⁶, given the mean values of the remaining covariates [118]. The calculations are
performed using \( P_0 \) for the coronary calcification is equal to 0.31 [119], 2-tailed test,
alpha=0.05.

With 100 cases, and assuming \( P_0 \) of 0.31, we had 80% power to detect an OR for a main
effect (e.g., cardiovascular risk factor of ESR) of 1.97 at alpha=0.05, assuming the multiple
correlation coefficient relating the main effect of interest and other covariates in the model is 0.2.
Even if the multiple correlation coefficient was as high as 0.4, we still had 80% power to detect
an OR of 2.19 for one standard deviation change in ESR. PASS software was used to perform
power estimation (NCSS Statistical Software, Kaysville, UT).

3.3 RESULTS

3.3.1 PATIENT POPULATION

The 137 women with SLE were predominantly Caucasian (86%), and had a mean age of
49.5±10.0 years, and disease duration of 16.3±7.2 years. Compared with all women in the
Pittsburgh Lupus Registry (n=983), the study participants were slightly younger (49.9±10.18
years versus 51.8±14.7 years⁷ [111]), had somewhat longer disease duration (16.7±7.60 years
versus 14.8±7.1 years [111]) and higher proportion of Caucasian (86% versus 84.4% [111]). 56%
of the study participants were postmenopausal. The presence of coronary calcium was detected
in 49.6% of patients and aortic calcium was found in 80.3%.

3.3.2 COMPARISON OF CORONARY AND AORTIC CALCIFICATION AMONG CASES AND
CONTROLS

Coronary and aortic calcification data were available for 106 control subjects. They were not
significantly different from cases in terms of age (controls: 50.94±8.02 years, cases: 49.5±10.0

⁶ Therefore, OR for a 1 unit change should be transformed as \( \text{OR} \times \text{std} \)
years, p=0.25). However, there were more Caucasians among controls than among cases, (93.4% vs. 86.4%, p=0.078) and fewer postmenopausal subjects (45.3% vs. 57.1%, p=0.065).

The presence of coronary calcium was detected in 50.9% of control subjects, which was not significantly different from cases (49.6%, p=0.88). However, among controls, there were significantly fewer subjects with coronary calcium score ≥ 10 than among cases (15.1% vs. 27.9%, p=0.017). Aortic calcium was significantly less prevalent among controls than among cases (67.9% vs. 80.3%, p=0.02).

### 3.3.3 Risk Factors Associated with Coronary and Aortic Calcification in SLE Patients

#### 3.3.3.1 Univariate Analysis

Univariate analysis compares traditional cardiovascular, SLE-specific and inflammatory variables between SLE subjects with and those without calcium in coronary artery and aorta (calcium score cutpoint of 0). The study participants with either coronary or aortic calcium tended to be older compared to those without calcification (p<0.01). The group with coronary calcification was characterized by lower percentage of Caucasians (80.9% vs. 91.3%, p<0.1) and higher percentage of postmenopausal patients (69.1% vs. 43.5%, p<0.01) compared to the group without coronary calcium. Patients with either type of calcification had higher systolic, diastolic and pulse pressure, higher insulin levels, cumulative organ damage assessed by SLICC, and higher levels of inflammatory markers, such as CRP, PAI-1, sICAM-1 (both for subjects with coronary and aortic calcification), ESR, fibrinogen, E-selectin (for subjects with coronary calcification) (Table 3-1).

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7 The numbers for the Pittsburgh Lupus Registry are derived from literature; therefore, no p value is calculated
Table 3-1. Comparison of traditional cardiovascular, SLE-specific and inflammatory risk factors in the 137 female SLE patients with versus without coronary calcification (Ca), and with versus without aortic calcification*

<table>
<thead>
<tr>
<th>Variable</th>
<th>No coronary Ca (N=69)</th>
<th>Coronary Ca (N=68)</th>
<th>No aortic Ca (N=27)</th>
<th>Aortic Ca (N=110)</th>
<th>Overall (N=137)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Age, years</td>
<td>45.5±8.3</td>
<td>53.6±10.0†</td>
<td>42.8±8.1</td>
<td>51.2±9.7†</td>
<td>49.5±10.0</td>
</tr>
<tr>
<td>Race, % white</td>
<td>91.3</td>
<td>80.9§</td>
<td>81.5</td>
<td>87.3</td>
<td>86.1</td>
</tr>
<tr>
<td>SLE duration, years</td>
<td>16.2±7.6</td>
<td>16.4±6.8</td>
<td>14.4±6.8</td>
<td>16.7±7.3§</td>
<td>16.3±7.2</td>
</tr>
<tr>
<td>Postmenopausal, %</td>
<td>43.5</td>
<td>69.1†</td>
<td>44.4</td>
<td>59.1</td>
<td>56.2</td>
</tr>
<tr>
<td>Body mass index, kg/m²</td>
<td>26.7±15.0</td>
<td>31.4±6.1†</td>
<td>27.9±23.0</td>
<td>29.3±6.6†</td>
<td>29.0±11.7</td>
</tr>
<tr>
<td>Waist ratio</td>
<td>0.8±0.1</td>
<td>0.9±0.1†</td>
<td>0.8±0.1</td>
<td>0.9±0.1</td>
<td>0.8±0.1</td>
</tr>
<tr>
<td>Systolic BP, mm Hg</td>
<td>110.9±13.4</td>
<td>129.7±19.1†</td>
<td>106.1±11.7</td>
<td>123.7±18.7†</td>
<td>120.2±18.9</td>
</tr>
<tr>
<td>Diastolic BP, mm Hg</td>
<td>74.0±9.0</td>
<td>80.3±10.8†</td>
<td>71.4±10.2</td>
<td>78.5±10.0†</td>
<td>77.1±10.4</td>
</tr>
<tr>
<td>Pulse pressure, mm Hg</td>
<td>36.9±8.8</td>
<td>49.4±13.8†</td>
<td>34.7±6.0</td>
<td>45.2±13.5†</td>
<td>43.1±13.1</td>
</tr>
<tr>
<td>Hypertensive, %</td>
<td>36.2</td>
<td>70.6†</td>
<td>29.6</td>
<td>59.1†</td>
<td>53.3</td>
</tr>
<tr>
<td>Total cholesterol, mg/dl</td>
<td>187.6±41.2</td>
<td>191.2±38.5</td>
<td>176.2±35.1</td>
<td>192.6±40.3§</td>
<td>189.4±39.8</td>
</tr>
<tr>
<td>Median glucose, mg/dl</td>
<td>87.0</td>
<td>89.0§</td>
<td>86.0</td>
<td>89.0</td>
<td>89.0</td>
</tr>
<tr>
<td>Median triglycerides, mg/dl</td>
<td>108.0</td>
<td>126.5‡</td>
<td>109.0</td>
<td>118.0</td>
<td>116.0</td>
</tr>
<tr>
<td>Insulin, µU/ml</td>
<td>12.0±6.8</td>
<td>18.6±11.2†</td>
<td>11.7±9.3</td>
<td>16.1±9.7†</td>
<td>15.2±9.8</td>
</tr>
<tr>
<td>SLE cumulative organ damage</td>
<td>1.0±1.2</td>
<td>1.7±1.9‡</td>
<td>0.9±1.3</td>
<td>1.4±1.6§</td>
<td>1.3±1.6</td>
</tr>
<tr>
<td>C3, mg/dl</td>
<td>93.0±23.0</td>
<td>107.2±31.7†</td>
<td>94.0±21.3</td>
<td>101.5±29.9</td>
<td>100.0±28.5</td>
</tr>
<tr>
<td>C4, mg/dl</td>
<td>18.0±6.4</td>
<td>21.6±8.0†</td>
<td>18.8±6.3</td>
<td>20.1±7.6</td>
<td>19.8±7.4</td>
</tr>
<tr>
<td>WBC count, 10³/mm³</td>
<td>4.9±1.8</td>
<td>5.9±2.0†</td>
<td>5.2±2.2</td>
<td>5.5±1.9</td>
<td>5.4±1.9</td>
</tr>
<tr>
<td>Median CRP, mg/ml</td>
<td>1.5</td>
<td>4.1†</td>
<td>1.9</td>
<td>2.6§</td>
<td>2.6</td>
</tr>
<tr>
<td>Median ESR, mm/h</td>
<td>9.0</td>
<td>13.0§</td>
<td>12.0</td>
<td>10.0</td>
<td>10.0</td>
</tr>
<tr>
<td>Median PAI-1, ng/ml</td>
<td>11.9</td>
<td>23.4†</td>
<td>11.8</td>
<td>16.6§</td>
<td>14.6</td>
</tr>
<tr>
<td>Median fibrinogen, mg/dl</td>
<td>306.0</td>
<td>356.0†</td>
<td>302.0</td>
<td>343.5</td>
<td>340.0</td>
</tr>
<tr>
<td>E-selectin, ng/ml</td>
<td>42.0±18.8</td>
<td>51.9±21.9†</td>
<td>46.8±24.2</td>
<td>50.0±20.5</td>
<td>49.4±21.2</td>
</tr>
<tr>
<td>Median sICAM-1, ng/ml</td>
<td>255.0</td>
<td>285.7§</td>
<td>243.2</td>
<td>269.4§</td>
<td>264.9</td>
</tr>
</tbody>
</table>

*Except where otherwise indicated, values are the mean ± SD. BP = blood pressure.
† P ≤ 0.01 versus those with no calcification.
‡ P ≤ 0.05 versus those with no calcification.
§ P ≤ 0.10 versus those with no calcification.
# Women were considered to have hypertension if they were taking antihypertensive medication or their sitting systolic blood pressure was >140 mm Hg or their diastolic blood pressure was >90 mm Hg.

### 3.3.3.2 Multivariate Analysis

In the binary logistic regression, risk factors independently associated with the presence of coronary calcification included older age (OR=1.11, p<0.01), race (being Caucasian appeared to have protective effect, OR=0.14, p=0.02), elevated systolic blood pressure (OR=1.09, p<0.01), WBC count (OR=1.85, p<0.01), ESR (OR=1.04, p=0.05) and PAI-1 (OR=3.09, p<0.01). The
independent determinants of severity of coronary calcification were older age (OR=1.07, p<0.01), pack-years of smoking (OR=1.03, p=0.09), and, similar to the regression on the presence of coronary calcium, elevated systolic blood pressure (OR=1.04, p<0.01), WBC count (OR=1.27, p=0.02), ESR (OR=1.03, p=0.03) and PAI-1 (OR=1.93, p<0.01) (Table 3-2).

Independent determinants of the presence of aortic calcium in the multivariate analysis were older age (OR=1.07, p=0.03), elevated systolic blood pressure (OR=1.07, p<0.01), and PAI-1 (OR=1.47, p=0.1). Factors independently associated with severity of aortic calcification included older age (OR=1.12, p<0.01), pack-years of smoking (OR=1.05, p=0.03), higher SLE-related cumulative organ damage as measured by SLICC damage index (OR=1.31, p=0.03), decreased platelets (OR=0.99, p=0.04) and sICAM-1 (OR=1.01, p=0.01) (Table 3-3).

Table 3-2. Logistic regression analysis of variables associated with the presence and severity of coronary calcification in women with SLE (N=137)*.

<table>
<thead>
<tr>
<th>Explanatory variable</th>
<th>Presence of coronary calcium</th>
<th>Severity of coronary calcification</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Odds ratio</td>
<td>90% CI</td>
</tr>
<tr>
<td>Age, years</td>
<td>1.11</td>
<td>1.05-1.16</td>
</tr>
<tr>
<td>Race**</td>
<td>0.14</td>
<td>0.04-0.60</td>
</tr>
<tr>
<td>Pack-years of smoking</td>
<td>-</td>
<td>-</td>
</tr>
<tr>
<td>Systolic blood pressure, mm Hg</td>
<td>1.09</td>
<td>1.05-1.13</td>
</tr>
<tr>
<td>WBC, 10^3/mm^3 ng/ml</td>
<td>1.85</td>
<td>1.39-2.47</td>
</tr>
<tr>
<td>ESR, mm/h</td>
<td>1.04</td>
<td>1.01-1.07</td>
</tr>
<tr>
<td>PAI-1, ng/ml</td>
<td>3.09</td>
<td>1.94-4.91</td>
</tr>
</tbody>
</table>

*Presence of coronary calcium: calcification score>0; severity of coronary calcification: 0 – calcification score=0 (N=69); 1 – calcification score 1-10 (N=27); 2 – calcification score 10-100 (N=22); 3 – calcification score >100 (N=19).

**0 – non-Caucasian, 1 – Caucasian
Table 3-3. Logistic regression analysis of variables associated with the presence and severity of aortic calcification in women with SLE (N=137)*.

<table>
<thead>
<tr>
<th>Explanatory variable</th>
<th>Presence of aortic calcium</th>
<th>Severity of aortic calcification</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Odds ratio</td>
<td>90% CI</td>
</tr>
<tr>
<td>Age, years</td>
<td>1.07</td>
<td>1.02-1.12</td>
</tr>
<tr>
<td>Pack-years of smoking</td>
<td>-</td>
<td>-</td>
</tr>
<tr>
<td>Systolic blood pressure, mm Hg</td>
<td>1.07</td>
<td>1.03-1.10</td>
</tr>
<tr>
<td>SLE cumulative organ damage</td>
<td>-</td>
<td>-</td>
</tr>
<tr>
<td>Platelets, 10^3/mm^3</td>
<td>-</td>
<td>-</td>
</tr>
<tr>
<td>sICAM, ng/ml</td>
<td>-</td>
<td>-</td>
</tr>
<tr>
<td>PAI-1, ng/ml</td>
<td>1.47</td>
<td>1.00-2.17</td>
</tr>
</tbody>
</table>

*Presence of aortic calcium: calcification score>0; severity of aortic calcification: 0 – calcification score=0 (N=27); 1 – calcification score 1-300 (N=74); 2 – calcification score >300 (N=36).

3.3.4 Risk Factors for Coronary and Aortic Calcification by Menopausal Status

Comparing risk factors associated with calcification in pre- and postmenopausal SLE patients is a promising area. However, considering the sample size, this report is limited to the univariate rather than multivariate analysis. In the group of premenopausal patients (N=60), those with calcification tended to be have higher body mass index and waist-to-hip ratio compared to women of the same group without calcification. Premenopausal women with coronary calcium were likely to be hypertensive, to have increased systolic and diastolic blood pressure, pulse pressure, elevated triglycerides and insulin levels. Their complement C3 and WBC count tended to be significantly increased, as well as all the inflammatory markers measured. In the group of postmenopausal women, those with coronary calcification were older, tended to be heavier smokers and to have higher body mass index than patients of the same group without calcification. Postmenopausal patients with coronary calcium also were likely to be hypertensive and had increased systolic blood pressure, pulse pressure and insulin. They tended to have higher SLE-related cumulative organ damage as measured by SLICC, elevated complement C3 and C4, and WBC count. Among all inflammatory markers measured, only CRP, PAI-1 and E-selectin were significantly associated with coronary calcification in postmenopausal women (Table 3-4).
Table 3-4. Characteristics of pre- and postmenopausal SLE patients, according to the presence or absence of calcification in the coronary artery*

<table>
<thead>
<tr>
<th>Variable</th>
<th>Premenopausal (N=60)</th>
<th>Postmenopausal (N=77)</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>No Calcification (N=39)</td>
<td>Calcification (N=21)</td>
</tr>
<tr>
<td>Age, years</td>
<td>41.7±6.0</td>
<td>44.1±6.8</td>
</tr>
<tr>
<td>Pack-years of smoking</td>
<td>1.7±5.2</td>
<td>1.8±6.8</td>
</tr>
<tr>
<td>Body mass index, kg/m²</td>
<td>27.7±19.5</td>
<td>33.5±5.8†</td>
</tr>
<tr>
<td>Waist hip ratio</td>
<td>0.8±0.1</td>
<td>0.9±0.1§</td>
</tr>
<tr>
<td>Systolic BP, mm Hg</td>
<td>107.7±11.3</td>
<td>124.1±16.5†</td>
</tr>
<tr>
<td>Diastolic BP, mm Hg</td>
<td>71.6±8.3</td>
<td>79.5±10.3†</td>
</tr>
<tr>
<td>Pulse pressure, mm Hg</td>
<td>36.0±7.3</td>
<td>44.6±10.2†</td>
</tr>
<tr>
<td>Hypertensive, %#</td>
<td>25.6</td>
<td>52.4‡</td>
</tr>
<tr>
<td>Median triglycerides, mg/dl</td>
<td>101.0</td>
<td>119.0‡</td>
</tr>
<tr>
<td>Insulin, µU/ml</td>
<td>11.9±8.1</td>
<td>20.8±11.1†</td>
</tr>
<tr>
<td>SLE cumulative organ damage</td>
<td>0.7±1.0</td>
<td>1.0±1.2</td>
</tr>
<tr>
<td>C3, mg/dl</td>
<td>88.4±23.6</td>
<td>101.6±35.6‡</td>
</tr>
<tr>
<td>C4, mg/dl</td>
<td>17.6±6.1</td>
<td>19.3±7.5</td>
</tr>
<tr>
<td>WBC count, 10³/mm³</td>
<td>5.0±2.0</td>
<td>5.9±2.0‡</td>
</tr>
<tr>
<td>Leukopenic, %</td>
<td>33.3</td>
<td>14.3</td>
</tr>
<tr>
<td>Median ESR, mm/h</td>
<td>6.0</td>
<td>15.5§</td>
</tr>
<tr>
<td>Median CRP, mg/ml</td>
<td>1.0</td>
<td>5.6†</td>
</tr>
<tr>
<td>Median PAI-1, ng/ml</td>
<td>12.6</td>
<td>24.5†</td>
</tr>
<tr>
<td>Median fibrinogen, mg/dl</td>
<td>302.0</td>
<td>357.0†</td>
</tr>
<tr>
<td>E-selectin, ng/ml</td>
<td>43.4±20.6</td>
<td>53.5±18.2‡</td>
</tr>
<tr>
<td>Median sICAM-1, ng/ml</td>
<td>241.1</td>
<td>277.7‡</td>
</tr>
</tbody>
</table>

*Except where otherwise indicated, values are the mean ± SD. BP = blood pressure.
† P ≤ 0.01 versus those with no calcification.
‡ P ≤ 0.05 versus those with no calcification.
§ P ≤ 0.10 versus those with no calcification.
# Women were considered to have hypertension if they were taking antihypertensive medication or their sitting systolic blood pressure was >140 mm Hg or their diastolic blood pressure was >90 mm Hg.

Table 3-5 represents characteristics of pre- and postmenopausal patients according to the presence or absence of calcium in aorta. Among premenopausal patients, variables significantly associated with the presence of aortic calcium were older age, higher body mass index, elevated systolic and diastolic blood pressure, pulse pressure, and insulin. No significant relationship was found between aortic calcium and lupus-specific or inflammatory markers in this group of patients. Risk factors significantly associated with aortic calcification among postmenopausal patients were older age, heavier smoking, higher body mass index, systolic blood pressure, pulse
pressure, median glucose, insulin, hypertension, and elevated WBC count. Among inflammatory markers, only CRP, PAI-1 and sICAM-1 were increased in postmenopausal women with aortic calcium compared to those without calcification (Table 3-5).

Table 3-5. Characteristics of pre- and postmenopausal SLE patients, according to the presence or absence of calcification in aorta*

<table>
<thead>
<tr>
<th>Variable</th>
<th>Premenopausal (N=60)</th>
<th>Postmenopausal (N=77)</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>No Calcification (N=15)</td>
<td>Calcification (N=45)</td>
</tr>
<tr>
<td>Age, years</td>
<td>39.0±6.5</td>
<td>43.7±5.8‡</td>
</tr>
<tr>
<td>Pack-years of smoking</td>
<td>0.8±1.9</td>
<td>2.1±6.5</td>
</tr>
<tr>
<td>Body mass index, kg/m²</td>
<td>31.2±30.8</td>
<td>29.3±7.3‡</td>
</tr>
<tr>
<td>Systolic BP, mm Hg</td>
<td>103.1±9.5</td>
<td>116.8±15.6†</td>
</tr>
<tr>
<td>Diastolic BP, mm Hg</td>
<td>68.3±8.4</td>
<td>76.4±9.4†</td>
</tr>
<tr>
<td>Pulse pressure, mm Hg</td>
<td>34.8±4.2</td>
<td>40.4±10.1‡</td>
</tr>
<tr>
<td>Median glucose, mg/dl</td>
<td>89.0</td>
<td>86.0</td>
</tr>
<tr>
<td>Insulin, µU/ml</td>
<td>12.5±11.9</td>
<td>15.7±9.4‡</td>
</tr>
<tr>
<td>Hypertensive, %#</td>
<td>20.0</td>
<td>40.0</td>
</tr>
<tr>
<td>WBC count, 10³/mm³</td>
<td>5.7±2.6</td>
<td>5.2±1.9</td>
</tr>
<tr>
<td>Leukopenic, %</td>
<td>26.7</td>
<td>26.7</td>
</tr>
<tr>
<td>Median CRP, mg/ml</td>
<td>1.9</td>
<td>1.6</td>
</tr>
<tr>
<td>Median PAI-1, ng/ml</td>
<td>11.0</td>
<td>14.4</td>
</tr>
<tr>
<td>Median sICAM-1, ng/ml</td>
<td>225.6</td>
<td>257.7</td>
</tr>
</tbody>
</table>

*Except where otherwise indicated, values are the mean ± SD. BP = blood pressure.
† P ≤ 0.01 versus those with no calcification.
‡ P ≤ 0.05 versus those with no calcification.
§ P ≤ 0.10 versus those with no calcification.
# Women were considered to have hypertension if they were taking antihypertensive medication or their sitting systolic blood pressure was >140 mm Hg or their diastolic blood pressure was >90 mm Hg.

3.3.5 **ASSOCIATION BETWEEN CORONARY AND AORTIC CALCIFICATION (EBT) AND CAROTID PLAQUE (DOPPLER ULTRASOUND) IN SLE PATIENTS**

The relationship between calcification of the coronary arteries and aorta measured by EBT and carotid atherosclerosis assessed by the ultrasound was evaluated on the subset of 70 study participants who had carotid ultrasound scan and EBT done within one year. According to the carotid ultrasound scans, 18 of these 70 women (25.7%) had at least 1 focal plaque (plaque index >0). The overall mean IMT for far and near walls of the common carotid artery (CCA), carotid bulb and internal carotid artery (ICA) was 0.64±0.09 for these 70 patients, and mean IMT for bulb and ICA only 0.63±0.11.
As shown on Figure 3-1, there was significant association between presence of at least 1 focal carotid plaque (plaque index >0) and presence of calcium in the coronary artery (p=0.09). The presence of carotid plaque was also significantly related to the aortic calcification score above the median (>30.5) (p=0.02); however, no association was found between carotid plaque and aortic calcium score >0.

Figure 3-1. Association between the presence of coronary and aortic calcification (EBT) and carotid plaque (Doppler US)

IMT scores (carotid US) were averaged for far and near walls of the common carotid artery, carotid bulb and ICA (estimate 1), and separately for the bulb and ICA (estimate 2). Both IMT estimates were found to be significantly associated with the presence of calcium both in coronary artery and aorta as measured by EBT (Figure 3-2).

*Far and near CCA, bulb and ICA

Figure 3-2. Association between carotid IMT (Doppler US) and presence of coronary and aortic calcium (EBT)
3.4 DISCUSSION

The study represents new data on the presence, severity and determinants of coronary and aortic atherosclerosis in women with SLE using EBT; this is the first study that compares the prevalence of EBT-detected atherosclerotic burden among SLE patients and age- and race-matched controls. We found coronary calcium in 49.6% of patients. Asanuma et al. recently reported the 31% rate of coronary calcium detected by EBT in their 65 lupus patients [99], which is lower than in our study. This difference can be related to older mean age of our study cohort (49.5±10.0 years versus 40.3±11.6) and longer disease duration (16.3±7.2 years vs. 9.9±8.7 years). Using a myocardial perfusion technique, Bruce et al. found abnormalities of coronary artery in 35% of SLE patients [105], which is also lower than our EBT-detected estimate of coronary atherosclerosis. It should be noted that perfusion techniques rely on flow-limiting stenosis and may underestimate the prevalence of coronary atherosclerosis.

We found no significant difference among cases and controls in terms of the presence of the coronary calcium. However, among controls, there were fewer subjects with clinically significant coronary calcium score $\geq 10$ than among cases (15.1% vs. 27.9%, p=0.017).

Aortic calcium was also significantly less prevalent among controls than among cases (67.9% vs. 80.3%, p=0.02). Our estimates are consistent with previous findings that SLE subjects are at higher risk for coronary and aortic atherosclerosis than non-lupus population [120,121].

In our study, the prevalence of calcium in the aorta was significantly higher than in the coronary artery (80.3% vs. 49.6%). Although no data on EBT-detected calcium in aorta was previously reported for SLE population, higher prevalence of aortic calcification compared to coronary is consistent with previous observations in non-SLE middle-aged women [120] and with autopsy studies which reported a smaller percentage of the coronary artery surface involved with atherosclerotic lesions than either the thoracic or abdominal aorta surface [122]. This can be explained by earlier onset of aortic calcification compared to coronary. Younger age of patients with aortic calcification (51.9±9.8 years of age) compared to patients with coronary calcification
(54.2±10.2 years of age) in our study is consistent with this tendency. It was noted that aorta is not protected by estrogen as much as the coronary artery, and in this sense, represents a longer exposure to the adverse risk factors [123,124]. It can therefore be suggested that in young women with SLE, the measurement of aortic rather than coronary calcium may provide earlier evaluation of underlying atherosclerosis.

Risk factors associated with development of atherosclerosis in SLE cohort are traditional cardiovascular, lupus-related and inflammatory. In our study, traditional cardiovascular risk factors independently associated with the presence and severity of atherosclerosis in the coronary artery and aorta were older age, non-Caucasian race, smoking history and elevated systolic blood pressure. Most of these results are consistent with findings of other authors. Age is a known risk factor for atherosclerosis of coronary and carotid arteries and aorta in SLE [100,111,125]. Smoking was found to be significantly associated with coronary calcification in SLE patients [125] and with focal carotid plaque among non-lupus women [101]. Elevated systolic blood pressure was identified as a risk factor associated with atherosclerosis in previous SLE studies [100,111] and in the study conducted on premenopausal non-SLE women [101]. However, observing higher prevalence of atherosclerosis among non-Caucasians than in whites is less common. In the study of Newman et al. on non-SLE population, older black subjects (80+ year old) had lower coronary artery calcium (CAC) score than did white, although these differences were less pronounced in younger cohorts (75-79 year old) [126].

Among SLE-specific risk factors, elevated WBC count was noted to be associated with the presence and severity of coronary calcification. Cumulative organ damage (SLICC index), as well as decreased platelet count, were found to be significantly associated with severity of aortic, but not coronary calcification. This confirms that, as was noted earlier, aorta is protected by estrogen less than the coronary artery, and thus is more exposed to adverse risk factors. Overall, our findings are consistent with results of other studies. Patients with more cumulative organ damage were found to be more likely to have a stiffer aorta [127]. Damage-index score was found to be an independent determinant of carotid plaque detected by ultrasonography and B-mode ultrasound [98,100]. Decreased platelet count is a typical indicator of systemic lupus. Elevated WBC count suggests the ongoing inflammatory process and thought to be associated with atherogenesis. Although the data on association of WBC count and subclinical vascular
disease in SLE patients is scarce, elevated WBC count was shown to be a significant predictor of cerebral infarction incidence in the general population [128].

The multivariate analyses of our study showed no significant relationship between calcification and lupus activity indices, such as SLAM and SLEDAI, or typical laboratory assays, such as complement C3 and C4 and dsDNA. This can be related to the fact the systemic lupus is a chronic disease characterized by flares and remissions, and measures of disease activity and laboratory tests fluctuate respectively. As a result, their cross-sectional measures cannot reliably reflect a chronic process of atherosclerosis in SLE. In contrast, SLICC index reflects irreversible, cumulative effect of SLE, and its independent association with atherosclerosis is particularly informative and thus important.

**Inflammatory markers.** We found an independent association of increased ESR and PAI-1 with presence and severity of coronary calcification, and association of PAI-1 and sICAM-1 with the presence and severity of aortic calcification, respectively. This is consistent with findings reported by other researchers. ESR was found significantly increased in SLE patients with a history of CVD compared to SLE controls without such a history and to non-SLE controls [129]. Elevated PAI-1 levels worsen the prognosis of myocardial infarction and are associated with an increased risk of cardiovascular disease in general population [130]. Increased PAI-1 was also found to be significantly associated with a history of thrombosis [110]. The data on sICAM-1 in SLE are limited. Upregulated expression of sICAM was reported to be associated with increased disease activity in SLE [108]. The role of sICAM-1 in the atherogenesis in the general population is recognized. It was reported that sICAM-1 was expressed in human atherosclerotic plaques [131]. However, our study was the first to demonstrate the relationship between sICAM-1 and subclinical vascular disease in SLE.

Other inflammatory markers evaluated in our study, such as CRP, fibrinogen, albumin, E-selectin and CD40L, were not found to be independently associated with the presence or severity of EBT-detected atherosclerosis. The existing data on these variables are either limited or contradictory. For instance, on the one hand, studies conducted on SLE patients showed that CRP was independently associated with intima-media thickness of the carotid artery [111], with congestive heart failure (CHF) [132], vascular events (cardio-, cerebrovascular and peripheral vascular) [133], and with a history of CVD [129]. On the other hand, it was also reported that in patients with SLE, elevated CRP is associated with increased risk of death but not with
atherosclerosis [134]. Also, no association was found between elevated CRP and coronary calcification [99] and presence of carotid plaque [98]. The lack of relationship of inflammatory markers with atherosclerosis observed in lupus, particularly cross-sectionally, can be partially explained by elevated baseline level of these parameters in SLE patients in comparison to non-lupus subjects. This latter phenomenon was described by Svenungsson et al. [129], and confirmed by the descriptive analysis of our data showing abnormally high CRP levels. Prospective studies are needed to establish the true relationship between the inflammatory markers and development of atherosclerosis in SLE population.

Since the development of vascular disease is recognized to be associated with menopausal loss of estrogen protective effect, we described characteristics of pre- and postmenopausal patients according to the presence or absence of calcification in the coronary artery and aorta. Some trends in risk factors were noticed in pre- and postmenopausal groups of women. Both groups of patients were almost equally subjected to traditional risk factors for coronary calcification; in premenopausal women, calcification was associated with fewer SLE-related variables but with more inflammatory markers compared to postmenopausal group. In fact, in the premenopausal group, all inflammatory markers measured were significantly associated with coronary calcification, while in the postmenopausal group, only half of the markers was significantly associated with the outcome. These findings may suggest that in premenopausal women, an otherwise low-risk demographic group, inflammatory mechanisms particularly contribute to atherogenesis. A larger sample size is required to study the independent relationship between calcification and inflammatory markers in multivariate analysis.

We found that the development of atherosclerosis in the two vessel beds, coronary and carotid, is strongly correlated. This finding is consistent with systemic nature of atherosclerosis and its ability to affect several vascular beds. Previously conducted autopsy series have shown an association between carotid and coronary atherosclerosis [135,136]. Significant association between focal plaque, increased IMT, and prevalent and incident coronary events has also been reported in non-lupus population-based cohorts [137-140].

The findings are weakened by a few study limitations. Although SLE is more prevalent in black population, the cohort of this study mostly consisted of white women. This reduces generalizability of the study results. The true calcification frequency in the lupus population
could have been underestimated, and other risk factors associated with coronary and aortic calcification could have been not detected due to this problem.

In addition, the cross-sectional study design makes it impossible to establish temporal relationships or causality. Further prospective studies may help not only to resolve this problem, but also to address the issue of fluctuating SLE activity, which can be associated with changes in many atherogenic-related factors.

3.5 SUMMARY

In conclusion, our findings confirm that the prevalence of atherosclerosis is significantly increased among patients with lupus. As in general population, atherogenesis starts earlier in the aorta than in the coronary artery. There is a significant correlation in the development of atherosclerosis in the carotid and coronary vessel beds. Traditional cardiovascular, lupus-related and inflammatory risk factors constitute predictors of coronary and aortic calcification in SLE cohort. In premenopausal SLE patients, development of atherosclerosis is significantly determined by chronic inflammation. This suggests additional potential targets to reduce the risk of cardiovascular disease in these patients.
APPENDIX SENSITIVITY ANALYSIS OF THE THERAPEUTIC THRESHOLD

The sensitivity of the therapeutic treatment with respect to the SLE incidence, probability of dying from SLE, probability of dying from SLE under early diagnosis, and probability of dying from causes other than SLE are presented in Figure A-3 to Figure A-6.

The therapeutic threshold probability also depends on the ratio between utilities of patients with SLE (uSLE), patients with early diagnosed SLE (uEarlySLE), and patients with no SLE who nevertheless undergone prophylactic SLE treatment and suffer from side effects (uTox). Average utility of patients with SLE is based on the literature [91] and is fixed at 0.62. The utility of SLE with early diagnosis and utility of side effects are assumed to be higher. We run sensitivity analyses on these variables (Figure A-7 and Figure A-8).
The therapeutic threshold proves to be the most sensitive to the utility of side effects denoted as uTox (see Figure A-8).

Figure A-7. Sensitivity of therapeutic threshold with respect to the utility of early diagnosed SLE

Figure A-8. Sensitivity of Therapeutic threshold with respect to the utility of side effect
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