Serologically Active Clinically Quiescent Systemic Lupus Erythematosus: Clinical and Immunological Correlates

by

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Abstract

Aim: Some remitted systemic lupus erythematosus (SLE) patients are serologically active (by elevated anti-double-stranded DNA antibodies and/or hypocomplementemia) despite longstanding clinical quiescence ("SACQ"), thus presenting a management dilemma. We described the SACQ period and its patients, and sought biomarkers heralding disease flare in them.

Methods: Patients with a prolonged SACQ period were followed prospectively, with clinical and laboratory data collected at each visit. Serologically and clinically active (SACA) and serologically and clinically quiescent (SQCQ) patients served as positive and negative controls, respectively. Descriptive statistics and logistic regression analyses were utilized.

Results: SACQ patients were phenotypically unique prior to remission onset, and did not accrue subclinical organ damage over the quiescent period. Fluctuation in immunoglobulin isotype did not predict flare. The SACQ interferon and cytokine/chemokine profiles mirror SQCQ patients.

Conclusions: SACQ patients' active serology should not guide treatment decisions, and these patients are best managed conservatively. Alternate biomarkers must be sought.

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Chapter 1: Literature Review

Remission in systemic lupus erythematosus (SLE)

In early reports, systemic lupus erythematosus (SLE) was classically described as an unrelenting disease that would often culminate in death(1). However, the disease has been increasingly recognized as a chronic, albeit potentially fatal, relapsing-remitting disease. Given the propensity for severe manifestations and organ damage over time, remission is a very desirable outcome, and, as such, efforts have been made to describe it and understand its correlates. In fact, studies have revealed that the propensity for flare or remission in the initial years of disease are predictive of long term outcome, with those remitting earlier having a more favourable disease course(2,3). Substantial variability exists, however, in the nature and duration of remission, likely attributable to differences in patient cohorts and inconsistent remission definitions.

Dubois provides one of the first descriptions of remission in a cohort of 163 lupus patients in 1956(4). He reported that 38% of the patients experienced at least one "spontaneous remission" prior to treatment with antimalarials or corticosteroids, including one patient with a 26-year remission, and up to 16% with multiple remissions. He admitted, however, that most of these patients "did not have the full picture of systemic lupus erythematosus," but rather had a rheumatoid arthritis-like presentation (presumably prominent joint symptomatology in the absence of major organ manifestations). There was no definition of remission proffered in this historic paper, but it seemed to be based upon the physician's global clinical impression. In

1964 he and Tuffanelli then corroborated this considerable remission rate, reporting that 35% of 520 SLE patients experienced “spontaneous remission,” lasting up to 26 years in one case(5). The definition of remission was similarly implicit in this study.

Heller and Schur described serologic and clinical remission in a cohort of 305 patients followed between 1967 and 1981, defined on the basis of change in ANA or LE cell test from positive to negative in patients who were asymptomatic and without obvious active organ involvement(6). By contrast with Dubois and Tuffanelli, they found only 13 (4%) had developed a combined clinical and serologic remission, ranging in duration from 6 months to 13 years. Their definition of remission included patients on no therapy, those on hydroxychloroquine, as well as those requiring low doses of corticosteroids “because they became Addisonian if not receiving therapy.” Thus, their frequency of remission was much lower than in previous studies, in spite of having included patients both on and off of immunosuppressive medications. This disparity is likely attributable to the stringent serologic criteria for remission in this study, especially as ANA typically persists once present, and is not known to fluctuate with disease activity(7).

More consistent numerically with Heller and Schur’s findings (but disparate in definition) was Tozman and colleagues’ determination that the rate of “prolonged complete remission” in SLE, defined as the absence of clinical manifestations of disease and off all immunosuppressive therapy, was 4/160 (2.5%)(8). They utilized both clinical and laboratory variables in their assessment including, for the first time

in the setting of remission, the absence of anti-DNA antibodies and C3 hypocomplementemia, both of which are known to run a concordant course with disease activity in some lupus patients(9-11). These patients had remitted from previously severe disease, with median remission duration 75 months. Thus, considerable disparity in duration, definition and frequency of remission existed in the earlier literature.

In 1996 Drenkard and colleagues published an extensive review encompassing over 2000 person/years of data, and defined remission as “at least one year during which lack of clinical disease activity permitted withdrawal of all treatment for lupus proper”(3). They found 23% of their cohort met these criteria, with increasing proportions of remissions in subsets with longer disease duration. Thus they found that disease activity waned over time. Unlike Dubois’s observation of frequent spontaneous remissions, 87% of Drenkard’s cohort required at least some quantity of prednisone, with or without immunosuppressive agents, to control their disease prior to remission onset. Survival was highly correlated with remission, with those achieving at least one remission having a 12.5-fold smaller chance of dying from SLE than those who did not, regardless of the severity of past SLE manifestations.

Quantifying remission

Since Dubois’s qualitative description of remission, presumably based on his global assessment of SLE activity, attempts have been made to standardize and quantify SLE activity through the use of validated disease activity measures(12). The most

commonly used among these include the SLE Disease Activity Index (SLEDAI) or its modification, the SLEDAI-2000 (SLEDAI-2K), Safety of Estrogen in Lupus Erythematosus National Assessment-SLEDAI (SELENA-SLEDAI), British Isles Lupus Assessment Group (BILAG), the Systemic Lupus Activity Measure (SLAM) and European Consensus Lupus Activity Measurement (ECLAM). Each of these scales provides a quantitative tool for physicians to systematically describe and compare the extent of lupus disease manifestations in a patient longitudinally(12). Notably, in 1992 the original SLEDAI was validated, and provided clinicians with a simple and quantitative method of describing lupus activity(13). However, despite the availability of these quantitative tools and standardized definitions of flare in SLE, consensus surrounding the definition of remission has not been met, with the impact of serologic activity on remission status as well as the use of concomitant medications remaining the subjects of debate(14). Still, despite these tools, given the multisystemic nature of SLE, and the importance of distinguishing accurately between active disease and irreversible disease- and treatment-associated damage, the facility with which remissions are defined in other medical specialties, such as Oncology, remains elusive(15).

Remission descriptors and correlates

Researchers began to delve deeper into the nature of remission in SLE, its correlates and its predictors. Barr and colleagues described patterns of disease in a cohort of 204 consecutive SLE patients, as defined by the SLEDAI score, modified to include only clinical (and excluding serologic) components of disease activity,

compared to the physician's global assessment (PGA)(16). They described three patterns of SLE: relapsing-remitting, chronic active and long quiescent, with chronic active being the pattern accounting for the largest proportion of patient years among those studied, and long quiescent being the least frequent. Long quiescent was defined semi-quantitatively, by the physician's global assessment score, and quantitatively, via SLEDAI, as a score of 0 for at least 1 year, irrespective of serologic features or treatment. It was found that 28% of patients (16% of total person years) experienced long quiescence, the rarest of the three patterns, by PGA, and 44% (25% of total person years) by SLEDAI, even though a more permissive definition of quiescence was tolerated, without heed given to concurrent treatment. Thus, even within the same study, considerable disparity existed between measures of disease activity. Furthermore, because a modified SLEDAI score was used, the long quiescent cohort encompassed those with and without concurrent serologic activity in patients who, because treatment was permitted, may have simply been in a state of disease *suppression* as opposed to true remission.

Similarly, in a cohort comprised of ten European rheumatology centres and 187 patients, it was found that remission, even after ten years of disease, when activity is thought to wane(2,3), was a rare outcome(17). The SLEDAI and ECLAM were employed in this study, but not explicitly utilized to define remission. Rather, remission was defined as "the absence of disease-related signs, without the need for any treatment." By these criteria, none of the patients studied were in remission, as all continued to require some form of treatment, including prednisolone in 72%.

The number of patients without any clinical disease activity while taking medications was not specified, thus an important subset of quiescent patients in whom treatment was gradually being withdrawn was not described.

As a corollary, Mok and colleagues studied the rate of disease flare in post-menopausal women, who typically have longer disease duration, as SLE is most commonly diagnosed during the reproductive years(18). They found that post-menopausal, compared to premenopausal, flares were significantly less common, occurring in 44% versus 94%. Their flare definition included new serologic positivity (in the absence of clinical disease activity), and disease quiescence was not explicitly defined. Furthermore, while SLEDAI was used to define disease activity, the definition of flare was based upon the dose of corticosteroid and/or immunosuppressive agent used, not upon a prospectively determined fluctuation in SLEDAI score. It is thus difficult to compare the outcomes among these studies, in spite of the use of a consistent disease activity outcome measure.

As early SLE disease course is predictive of outcome(2,3), Formiga and colleagues studied remissions among those with high disease activity early in their disease course(19). They defined remission as disease activity permitting the withdrawal of all SLE-related treatment over at least one year, and asymptomatic serologic fluctuations were permissible. Twenty-four percent of their exclusively Caucasian cohort (of 100 patients) achieved such a remission, at mean 64 months after diagnosis, and the remissions persisted, on average, over more than 4.5 years.

While there were differences in baseline SLEDAI value between those who achieved remission and those who did not (with those with higher initial SLEDAI scores less likely to remit), these did not attain statistical significance.

Thus they observed remissions in patients with all disease manifestations, including major organ involvement. They found a significant correlation between SLEDAI values and time to remission onset: remission occurred later among those with more severe baseline disease. It is worthwhile noting that the cohort was comprised exclusively of Caucasian patients, who have been shown to have consistently more mild disease – and thus, one would anticipate, more frequent remission - than Blacks, Hispanics and Asians(20). A comparable study exploring these outcomes in these ethnicities would be of interest to determine whether remission rates were as high.

A European study(21) followed an inception cohort for five years, and found 27.5% of a cohort of 200 patients achieved remission, as defined by Global Physician Assessment, within the first year of disease. They found no differences, compared to those with persistently active disease, in age of onset, number of American College of Rheumatology (ACR) SLE classification criteria fulfilled, or maximum corticosteroid dose required. They did, however, find that cumulative corticosteroid dose, maximum SLEDAI score achieved, and organ damage accrual, all over the first year, were significantly lower in those achieving early remission. While approximately half of the patients achieving early quiescence maintained their

remission through a period of more than a mean four years of follow up, only 25% of those 145 patients with persistent disease activity within the first year eventually achieved remission. Those achieving earlier remission evolved to milder disease than those without, with less active disease and fewer relapses. The frequency of remission is thus comparable to that described in Formiga's study(19), but the comparability of the cohorts is less clear, as a precise remission definition was not proffered, nor was there mention made of ethnicity (presumably, predominantly Caucasian).

Wais and colleagues studied laboratory correlates of remission in 57 Caucasian outpatients with SLE(22). Remission was defined as a BILAG score ≤ 5, and variables included proinflammatory cytokines, such as IL-6, IL-10 and TNF-α; adhesion molecules, such as sICAM and E-selectin; and conventionally concordant serologic markers, such as complement levels and anti-double stranded DNA antibodies (anti-dsDNA). They found that, overall, the only difference in lab indicators between the 39 inactive patients and the 18 active patients was lower CRP in the former group ($p<0.001$), concluding that clinically inactive patients continue to experience some degree of immunoinflammatory activity. The authors acknowledged, however, that in contrast to others' studies in patients with SLE, the level of cytokines was not significantly increased from background levels in many cases. This finding, of course, may have been indicative of a skew in the cohort towards relatively quiescent disease, or reflective of differences in definitions of disease activity.

This finding also begets the question of whether organ damage accrues subclinically in patients with SLE in clinical remission. While that question was beyond the scope of Wais's paper, this issue was later addressed and refuted(23), thus supporting the impression that the "active" cohort was atypically quiescent, and not that the inactive cohort had increased immunoinflammatory activity. Furthermore, given the enormous complexity of SLE pathophysiology, one must recognize that Wais's findings do not exclude the possibility of significant differences occurring between groups in other unmeasured immunoinflammatory molecules.

In 2005, Urowitz and colleagues took significant strides toward addressing the inconsistencies that had plagued the SLE remission literature by quantifying and describing disease quiescence using incrementally less restrictive criteria(24). Thus, they defined prolonged remission as at least a five-year period without disease activity (SLEDAI-2K = 0), while not taking corticosteroids, immunosuppressives or antimalarials. They found that remission, thus defined, was a rare event, occurring in only 12 of 703 (1.7%) patients in their cohort. As would be expected, when progressively less stringent criteria were applied to the remission definition, encompassing one to five years' disease quiescence, permitting the presence of hypocomplementemia and/or anti-dsDNA positivity, and permitting the use of antimalarials, corticosteroids and immunosuppressive medications, remission prevalence increased as stringency decreased. When defined as clinical quiescence (by SLEDAI-2K) for one year, permitting active serology, and requiring

the use of medications (the least restrictive definition), remission prevalence was 24.5%. Thus, as elegantly demonstrated by this paper, the question to be asked is not, “What is the prevalence of remission?” -- a vague and subjective construct -- but rather, “What type of remission is being quantified?” -- as “remission” is in the eye of the investigator.

Thus the generalizability of the literature about SLE remission is limited by differences in definition, with duration, disease activity measure used, the permissibility of treatment, and serologic activity all being variables that may significantly affect the result. Furthermore, given the heterogeneity of lupus presentation, and the impact of ethnicity upon disease manifestations, severity and prognosis, differences inherent to a cohort, itself, may prove central in the duration and type of remission achieved. Regardless of how defined, remission remains a desirable outcome in SLE, but is relatively rarely achieved. A more complete understanding of SLE pathophysiology may lead to improved management.

The significance of organ damage accrual in SLE over time

Remission, most stringently defined, is a desirable disease state as the patient is free from both the onslaught of inflammation-associated disease activity and the untoward effects of treatment. In addition to the immediate manifestations of disease and treatment, each can impact the patient more permanently, a construct referred to as “damage.” This outcome is particularly important as mortality has decreased over the years in the setting of aggressive treatment with corticosteroids

and immunosuppressive medications, but the propensity for irreversible organ damage, attributable to both the disease itself and to its treatment(25), has kept pace with the increasing survival rate. The Systemic Lupus International Collaborating Clinics/American College of Rheumatology Damage Index (SDI) is a reliable and well-validated index which measures damage in individual organ systems(26,27).

Particularly germane to remission studies, Swaak and colleagues described damage accrual using the SDI in patients with disease duration of more than 10 years(28), as disease activity is known to wane over time, as highlighted above. They found the three most common signs of damage were hypertension (in 40%), osteoporosis and cardiovascular disease (each in 15%), all of which may be largely attributable to corticosteroid use. In 2003, Gladman and colleagues tracked the SDI longitudinally in an inception cohort of SLE patients, followed prospectively for at least 15 years(29). They found that damage accrued linearly over time. In keeping with the observations of Swaak and colleagues, they found that within the first year of SLE, 42% of damage was disease-attributable, and 58% was possibly or definitely corticosteroid-attributable. Later in the disease course, only 20% of damage was disease-attributable, and 80% was possibly or definitely attributable to corticosteroid use. That much of late damage accrual can be attributed to treatment effects – as opposed to disease - serves to reinforce the importance of corticosteroid withdrawal, whenever possible, and the desirability of unmedicated remission.

The significance of serologic abnormalities in monitoring SLE disease activity

The ultimate goal of evaluation in the patient with SLE is to monitor for disease activity, and manage and treat any signs thereof, in hopes of preventing worsening illness and, ultimately, irreversible organ damage. To diagnose remission, the clinician must confirm an absence of clinical manifestations of disease, yet the complex and often non-specific protean manifestations of SLE can be difficult to interpret. In some patients, serologic activity can provide corroborative evidence of disease activity, as some biomarkers can fluctuate with disease activity.

Anti-dsDNA

Anti-dsDNA was first described in 1957(30,31), and subsequently implicated in SLE pathogenesis and organ damage when Koffler and colleagues eluted these immune complexes from the kidneys of patients with lupus nephritis(32). The presence of these immune complexes is a highly specific diagnostic marker for SLE, that occurs in up to 83% of patients(33). Furthermore, studies have correlated fluctuations in anti-dsDNA titre with clinical disease activity (see below) in all but a unique subset of patients, termed serologically active clinically quiescent (SACQ), in whom anti-dsDNA and/or complement levels run a discordant course. Given their utility in diagnosis and monitoring, commercial kits for measuring the presence of anti-dsDNA are widely available. These assays, however, are not created equally, and have varying levels of sensitivity and specificity.

The three most commonly employed tests are the *Crithidia*, ELISA, and Farr assays. The *Crithidia luciliae* immunofluorescent test (CLIFT) capitalizes upon this hemoflagellate's kinetoplast (that contains circular dsDNA), which is incubated with patient serum, and an anti-immunoglobulin is applied. The enzyme-linked immunosorbent assay (ELISA) test is simple, relatively inexpensive and easily reproducible, utilizing DNA-coated polystyrene plates as substrate for colorimetric quantitation of serum anti-dsDNA antibodies. Finally, the Farr assay separates bound and free DNA through ammonium sulphate immunoprecipitation, with bound, radioactive DNA precipitating with immunoglobulins, and free DNA remaining in the supernatant(34). Of these, the Farr assay and CLIFT are the most specific, with the Farr assay best correlating with detection of anti-dsDNA of highest avidity(35,36). Antibodies of high avidity, in turn, are correlated with the presence of active renal disease(37).

It has been suggested that not all anti-dsDNA is created equally, with anti-dsDNA of high avidity, of IgG isotype and complement-fixing IgG sub-class best correlating with disease activity and renal involvement(38-45). As a corollary, IgM isotype and non-complement-fixing IgG sub-class best correlate with disease quiescence.

Complement

Measurement of decreasing complement components C3, C4 and CH50 have long been appreciated to correlate with active lupus, and with the presence of associated glomerulonephritis(46,47). This is consistent with what is known of their

pathophysiologic role, with circulating complement activation products stimulating an inflammatory cascade, resulting in tissue damage. The measurement of complement components is most useful when performed serially and thus comparatively, as in some SLE patients the disease process may not be concordant with complement values, and in others C4 is chronically low in the context of the C4 null allele, which is not infrequently associated with SLE(46,48,49).

Anti-nucleosome antibodies

There is the general consensus that anti-nucleosome antibodies are both sensitive and highly specific for SLE(50-53). In a systematic literature review and meta-analysis, Bizzaro and colleagues determined that the presence of anti-nucleosome antibodies conferred a 41-fold increase in the risk for SLE (versus 28-fold with anti-dsDNA)(50). Suleiman et al found that anti-nucleosome antibodies were 98% specific for the diagnosis of SLE(53). They also found anti-nucleosome antibodies 98% sensitive and 86% specific for detecting active SLE, versus 61% and 84% for anti-dsDNA sensitivity and specificity, respectively(53). Several studies have investigated the role of anti-nucleosome antibodies in renal disease. Most found anti-nucleosome antibodies elevated in the setting of lupus nephritis(51,54,55), with some finding them of improved sensitivity compared to anti-dsDNA antibodies(51,56).

Serologically active clinically quiescent (SACQ) systemic lupus erythematosus (SLE)

Recognition of SLE patients with clinical/serologic discordance can first be found in the literature in 1979, when Gladman and colleagues described 14 patients with persistently positive lupus erythematosus (LE) preparations and antinuclear antibodies, hypocomplementemia, and high levels of DNA binding(57). These patients had displayed typical lupus manifestations in the past, including major organ manifestations, such as renal or central nervous system involvement, but had since evolved to a clinically quiescent state. In spite of their clinical quiescence, these patients had impaired lymphocyte response to concavalin A, suggestive of a defect in cell-mediated immunity, as was typically seen in patients with active lupus.

These findings were, themselves, discordant from other studies of the time, which revealed serologic abnormalities were often harbingers of or associated with active disease(58-60). None of Gladman's patients, termed "serologically active clinically quiescent," or "SACQ," were taking corticosteroids or immunosuppressive medications, and had not done so for mean 4.25 years (range 2-11 years). Given seven years of follow-up revealing no flares among these individuals, Gladman and colleagues suggested that, in these individuals, close clinical follow-up without preemptive treatment with corticosteroids or immunosuppressive medications may be appropriate.

These suggestions contrasted starkly with those made by Swaak and colleagues in the very same year(61) in their paper describing rise and subsequent precipitous fall in anti-dsDNA as predictive of flare in SLE patients. In their discussion, Swaak and

colleagues advocated for the role for adoption of therapy on the basis of anti-dsDNA fluctuations. Perhaps even more divergent was a paper published prior to Gladman's SACQ observations, describing treatment of SLE patients with up to 100 mg of prednisone daily, guided by immunologic abnormalities, such as depressed serum complement levels, until these had normalized(62).

Ter Borg and colleagues studied fluctuations in anti-dsDNA and complement levels prior to SLE flare, and found 27/33 disease exacerbations were accompanied by elevations in anti-dsDNA antibodies(63). While the test was neither perfectly sensitive nor specific for disease flare, they concluded that serial assessment of anti-dsDNA levels, especially by Farr assay, was a reasonable approach to the monitoring of SLE disease activity. They found anti-dsDNA to be more sensitive for predicting exacerbations than C3 or C4 levels.

Thus there were two factions emerging among lupus practitioners – those who felt treatment might be appropriate on the basis of fluctuations in anti-dsDNA and/or complement levels, and those who argued for close clinical monitoring in lieu of preemptive treatment.

In 1994 Walz LeBlanc and colleagues conducted a prospective cohort study to identify SACQ patients and study their clinical predictors of flare(64). SACQ patients were defined as those in whom "serologic abnormalities – either low C3, C4 or CH50, or elevated anti-dsDNA antibodies – were present on three consecutive clinic

visits in the absence of clinical disease activity as measured by the SLEDAI." There were no requirements for medication withdrawal. One hundred-and-six SACQ periods were analyzed in 74 patients.

The SACQ period was terminated by flare within a year of the 3rd SACQ assessment in 46 instances: in 31 patients, the SACQ period was observed to occur between two flares, with both clinical, and serologic activity; in the remaining 15, the SACQ period followed a serologically and clinically quiescent period and preceded a clinical flare(64). The remaining 60 SACQ episodes were not associated with disease flare within the year following the 3rd SACQ assessment. Of these, 35 were preceded by clinical and serologic activity; 25 were preceded by clinical and serologic quiescence.

Comparisons were then made between those SACQ patients who flared and those who did not(64). The only features distinguishing these two groups were increased use of steroids, and at higher doses, during the SACQ period, a shorter median duration of the SACQ period, and a slightly greater incidence of vasculitis in those who flared. There were no differences in demography, or in fluctuations in serology between groups. Perhaps this suggests that in those who flared SACQ merely represented a transitional state between flares as opposed to true discordance. Thus Walz LeBlanc and colleagues' work emphasized and delineated the difference between disease quiescence, with true clinical-serologic discordance, versus disease suppression between flares.

In the same year, long-term follow-up was pursued in Gladman's patients, and ultimately obtained in 11 of the original 14(64). Seven patients remained clinically well, off all corticosteroids or immunosuppressive medications. Four patients had minor clinical flares of SLE requiring intervention that occurred only after a mean of 5.5 years from SACQ onset. This lent further support to the authors' original contention that in some patients, serologic abnormalities and clinical disease run discordant courses, thus the former need not dictate medical intervention. These findings were situated at a particularly poignant time, given that other authors were then further experimenting with preemptive dosing of prednisone against SLE flare in the face of rising titres of anti-dsDNA antibodies.

For instance, in 1995, with a cohort of 156 SLE patients, Bootsma and colleagues randomly assigned those with an observed increase in anti-dsDNA level to either conventional treatment, or an addition of 30 mg prednisone to their preexisting regimen(65). They found the relapse rate was higher in the conventionally-treated group, with comparable cumulative corticosteroid dosing between groups. Thus the SACQ cohort appeared to represent a unique subset in whom serology and clinical features run a discordant course, unlike the patients in the Bootsma study.

A similar study was carried out years later by Tseng and colleagues, in patients who were serologically active and clinically stable(66). They conducted a prospective, randomized, double-blind, placebo-controlled trial of prophylactic steroid dosing in

154 patients evaluated monthly for up to 18 months. In this study, patients who were clinically inactive or clinically stable/active, defined as a SELENA-SLEDAI score of ≤4 or 5-12, respectively, and who were not receiving more than 15 mg prednisone per day were eligible. Those with serologic evidence of flare, namely 25% elevation in anti-dsDNA and 50% elevation in C3a, were randomized to receive either a three-week course of prednisone, with starting dose 30 mg per day, or placebo. They found that significantly more flares occurred in the placebo group than in the treatment group (six versus none among 41 patients who experienced serologic flare, p=0.007). Severe caution must be exercised, however, in extrapolating these findings to SACQ patients, as Tseng's patients could have had active disease requiring up to 15 mg (a moderate dose) of prednisone daily and still have met inclusion criteria. Since this cohort included patients who continued to have evidence of active disease despite treatment with corticosteroid, as well as patients whose clinical manifestations may have been merely suppressed by their baseline corticosteroid dosing, they were fundamentally different than the SACQ patients defined by Gladman and colleagues, and this study's findings cannot be generalized to them.

Since SACQ patients' disease activity runs a discordant course from conventional serology, and since some SACQ patients ultimately do flare, efforts were made to identify more reliable biomarkers in this unique subset of patients. Ng and colleagues studied the role for anti-nucleosome antibodies, compared to anti-dsDNA, in the monitoring of patients with "SACQ" SLE(67). In this study, SACQ was

defined as a BILAG score less than 6 for at least six months in the context of anti-dsDNA positivity by ELISA method. Most patients were taking steroids and/or immunosuppressive medications. Nine percent (27/290) of the cohort was thus defined.

The investigators simultaneously measured anti-nucleosome antibodies, and found that time to first flare after a SACQ period was significantly correlated with their presence (p=0.0012), with higher titres thereof (p=0.0006), and with the presence of anti-dsDNA antibodies greater than five times above the upper limit of normal (p=0.02). They concluded that anti-nucleosome antibodies might be better predictors of flare in SACQ patients than anti-dsDNA. While numerous past studies have determined that anti-nucleosome antibodies are reliable indicators of flare in SLE patients, in general, their reliability in a stringently-defined SACQ group remains to be determined, as Ng's SACQ patients were comprised of those whose disease may have been merely stable, or suppressed by treatment. For instance, a patient with worsening arthritis and stable, localized discoid skin lesions (BILAG score 5), on 15 mg of prednisone would have met Ng's criteria for "SACQ." However this patient fundamentally differs from Gladman's SACQ patient, with persistent pathogenic active serology, on the one hand, but free of any disease activity, off all corticosteroids and immunosuppressive medications, on the other.

These stringently-defined SACQ patients thus present a conundrum: How can the clinician reconcile the presence of potentially pathogenic serologic activity with the

clinical picture of complete quiescence? How is this patient best managed? The more fundamental question to be asked is, ”What are these patients’ outcomes?” as these dictate management. Two potential SACQ outcomes exist – continued quiescence or flare. If the former, and SACQ can persist, or evolve to serologic and clinical quiescence, then it would be prudent for the clinician to spare the patient from exposure to corticosteroids and immunosuppressive medications and their associated risks and side effects. Alternately, the SACQ patient who evolves to flare will ultimately require such treatment. Thus a method to distinguish which SACQ patients will remain quiescent, versus those who will ultimately flare would be of clinical utility.

With this goal in mind, I strove to describe the SACQ period and its patients(68). SACQ was defined as at least two years of persistent serologic activity in the absence of clinical activity (SLEDAI-2K score of 2 or 4 from anti-dsDNA positivity by Farr assay and/or hypocomplementemia), with visits ≤18 months apart, during which antimalarial medications were permitted, but corticosteroids and immunosuppressives were not. This restrictive definition was applied in order to exclude both those patients who had active SLE merely suppressed by ongoing immunosuppression, and those who were in evolution from or to imminent flare. Thus this definition was meant to include only those with *bona fide* clinical/serologic discordance.

Fifty-six of 924 (6.1%) of the cohort were SACQ, accounting for 70 SACQ periods among them. Median SACQ duration was 158 weeks (mean 182 weeks), and, on average, occurred more than a decade after SLE diagnosis. The SACQ period was characterized by both elevations in anti-dsDNA and hypocomplementemia in the majority (62.5%) of patients. SACQ patients had milder disease (SLEDAI-2K at presentation = 7.3 versus 10.1, p=0.01) and required less corticosteroid and immunosuppressive medications at first clinic visit than did non-SACQ patients. There was a trend toward fewer deaths in the SACQ group. SACQ patients had less musculoskeletal, dermatologic and central nervous system involvement over their disease course.

Of interest, in light of Farr assay positivity in many of the SACQ patients and its correlation with active renal disease, there was no difference in the prevalence of renal involvement in patients prior to SACQ onset compared to non-SACQ patients. Furthermore, that this major organ was comparably involved in SACQ and non-SACQ patients suggests that SACQ does not merely represent the eventual outcome of a milder disease course. Anti-dsDNA antibodies were in the moderate and high range in 20.9% and 9.3% of the SACQ cohort, respectively, thus the elevations therein were not simply reflective of marginal increases from normal range.

The first SACQ period was terminated by disease flare in 33 (59%) of patients at median 155 weeks. Flare most frequently manifested as arthritis, mucous

membrane involvement or sterile pyuria. Of the remaining SACQ patients, six (10.7%) became serologically and clinically quiescent at median 236 weeks, and 17 (30.4%) remained SACQ at their most recent clinic visit. Flares could not be predicted from fluctuations in anti-dsDNA antibodies and/or complement levels in the preceding visits. Thus SACQ patients could not be identified *a priori*. Outcomes within the thusly-defined SACQ group could not be predicted on the basis of serologic fluctuations, as was the case in the studies conducted by Swaak, Ter Borg, Tseng and Bootsma.

In 2012, Conti and colleagues evaluated the frequency of SACQ within their cohort, using the definition we had proposed in 2010(69). Specifically, SACQ was defined as at least a two-year period without clinical activity and with persistent serologic activity, by SLEDAI-2K, during which patients could be taking antimalarials, but could not be taking corticosteroids or immunosuppressive medications. They found only 1/45 (2.2%) patient met their SACQ definition, which represents a value slightly lower than the 6.1% observed in the larger Toronto cohort. The most probable cause for these discrepant findings lies in Conti and colleagues' duration of prospective follow-up, which was only two years, compared to up to 38 years of follow-up in our longitudinal study. Given that the proportion of time a SLE patient would spend in a SACQ state is relatively low compared to their overall length of disease, maximizing the duration of longitudinal follow-up will yield the most accurate estimate of SACQ prevalence for any patient.

Clinically active serologically quiescent (CASQ) SLE

A second discordant cohort has been studied in the clinically active, serologically quiescent (CASQ) lupus patients. Gladman and colleagues described these patients, defined as those with three or more consecutive visits with clinical activity in the absence of serologic activity (hypocomplementemia and elevations in anti-dsDNA) by SLEDAI-2K score(70). CASQ patients comprised 62/514 (12.1%) of their cohort, with the CASQ period lasting median eight months, and was associated with a mean SLEDAI-2K score of 8.9±5.3, indicative of mild to moderate disease activity. Approximately one-third of CASQ patients ultimately evolved to serologically and clinically active disease (and thus two-thirds did not). Major organ involvement, defined as renal, vasculitic or central nervous system manifestations, occurred in 43 (69%) CASQ patients; of these, 31 (50% of all the CASQ patients) had renal manifestations during the CASQ period.

Thus, in spite of pathophysiologic plausibility of immune complex deposition leading to damage in SLE, with anti-dsDNA antibodies having been eluted from the kidneys of patients(32), this study suggests that (measurable) anti-dsDNA is not the *sine qua non* of renal lupus, which had once been dogma. Rather, it lends further support to the notion that serologic and clinical concordance does not occur in all lupus patients, and thus other mechanisms, including perhaps as yet unmeasured antibodies and inflammatory molecules, are the likely drivers of SLE pathophysiology in some patients.

Thus individuals with clinical-serologic discordance represent a clinically significant minority of SLE patients. The mechanism for this discordance, in spite of the purported pathogenicity of anti-dsDNA antibodies and/or complement components remains to be determined. One potential mechanism for discordance may be pathogenicity of alternate immune complexes that remain unmeasured in the conventional clinical setting, such as anti-nucleosome antibodies, described above. A second mechanism, which may be of import in light of its prominence in SLE pathogenicity, is through the so-called interferon signature, which has been shown to play an important pro-inflammatory role in SLE pathogenesis(71,72). These two mechanisms may prove critical in our understanding and clinical follow up of these discordant patients.

Interferon alpha in SLE

Plasmacytoid dendritic cells (pDCs) are the primary source of interferon-alpha (IFN-α), which, in health, is produced in the setting of viral defense. This occurs through the recognition and subsequent internalization of nucleic acids, such as single-stranded RNA (ssRNA) or hypomethylated viral or bacterial DNA (73,74). The nucleic acid is then trafficked, via endosomal compartment, to meet with Toll-Like Receptors (TLRs) – typically TLRs 7 and 9, which then activate Interferon Regulatory Factors (IRFs) 5 and 7, whose activation, in turn, results in the production of IFN-α. Excess TLRs 7 and 9 signalling, however, leads to a state of SLE-like autoimmunity, and the IRF 5 polymorphisms that predispose to SLE are gain-of function mutations, and induce transcription of IFNα mRNA. Indeed, there

are strong genetic links with IRF 5 variants and the SLE phenotype, in the setting of autoantibodies, which appear to act as a chronic stimulus for IFN-α production in this context(75).

It is thought that self nucleic acids, atypically exposed to the extracellular milieu as a result of impaired apoptosis thought fundamental to the disease, are the drivers of the copious IFN production that defines SLE. This was supported by an early study in which Bave and colleagues induced IFN-α expression in normal subjects' peripheral blood mononuclear cells (PBMCs) by exposure to both apoptotic cells and IgG from SLE patients *in vitro*(76). The apoptotic cells were sources of nucleic acid, and the IgG proteins were presumed to be inherent to autoantibodies in the SLE patients. Neither component alone was sufficient for IFN-α induction.

IFN-α then enhances TLRs 7 and 9 signalling, resulting in a positive feedback loop which, in turn, drives pathogenic inflammation(71,77). Thus, in SLE increased levels of IFN-α and IFN-responsive genes are observed, likely in the context of pDC activation. IFN-α production has pleotropic effects, including maturation of dendritic cells; CD8+ T-cell activation, with presentation of self-antigens and resultant loss of self-tolerance; and differentiation of B cells into long-lived plasma cells that produce the autoantibodies that are the hallmarks of SLE(71,77). IFN-α, its associated gene transcripts, and IFN-associated cytokines and chemokines thus figure prominently and pathogenically in SLE.

Of note, even the earliest studies recognized correlations between levels of IFN-α and disease activity. Prior to recognition of nucleic acid apoptotic debris as the potent IFN-α-inducing factor, Bengtsson and colleagues found a positive correlation between SLEDAI, anti-dsDNA and IL-10, and a negative correlation between complement and leukocyte levels, and serum levels of IFN-α(78).

Thus there was early evidence for the correlation between levels of IFN-α and disease activity. This was further substantiated in 2005, when Dall'Era and colleagues corroborated a positive correlation between levels of type I IFN (i.e., IFN-α) and SLEDAI score, anti-dsDNA, as well as cutaneous disease manifestations. There was negative correlation with levels of C3, and a trend toward association with renal disease(79). What remains unanswered in these studies, however, is the role of IFN-α in patients with clinical-serologic discordance, who clearly generate the pathogenic substrate to drive IFN-α production and the resultant SLE active phenotype, but in the setting of durable clinical quiescence.

With correlates observed between serum IFN-α and SLE phenotype, investigators then studied serologic and clinical factors associated with interferon-inducible genes. When SLE patients with high and low IFN-inducible gene expression were compared, those in the former group were notable for increased general and renal disease activity and increased damage, as well as hematologic involvement and hypocomplementemia(80). There was an association in the IFN-high group with anti-Ro antibody positivity, which was thought to be IFN-inducible. It was found that

pulse glucocorticoid administration markedly decreased IFN-inducible gene expression, which was not true of oral corticosteroid or other immunosuppressive medications. There was a trend toward decreased IFN-inducible gene expression with the use of antimalarials, which is of interest as antimalarials purportedly act through alteration of endosomal pH, thus interfering with a critical step in the process leading to increased IFN-α production(81).

Feng and colleagues similarly studied IFN-inducible genes *LY6E, OAS1, OASL, MX1* and *ISG15*, which were found to explain 98% of the total variation among 14 IFN-associated genes selected(82). They found that these genes were associated with disease activity scores, and showed increased expression in those with lupus nephritis; *LY6E* distinguished active from inactive nephritis, thus implying these genes may serve as useful biomarkers of disease activity.

However, in 2009 Landolt-Marticorena and colleagues conducted a longitudinal analysis of IFN-inducible gene expression, or the so-called “IFN signature”(83). They studied *LY6E, OAS1, IFIT1, ISG15* and *MX1* expression over time in SLE patients compared to healthy controls. They found that IFN-associated gene expression was significantly higher in SLE patients than in healthy controls, and that three individual genes, as well as a composite score of the five, were significantly elevated in patients with active disease, and correlated with SLEDAI-2K score. The composite score was also associated with elevated anti-dsDNA and severe active renal disease. Despite these findings, the longitudinal portion of their analysis

revealed that those with marked fluctuation in SLEDAI-2K score over time had minimal fluctuation in composite IFN score. They thus concluded that changes in IFN-associated gene expression had no predictive utility, since there was no significant correlation between fluctuations in the IFN signature and SLEDAI-2K score. Similar to serum IFN levels, then, it appears that the IFN signature correlated with aspects of clinical disease, however there are no studies investigating its correlation with or utility in patients with clinical-serologic discordance.

Similarly, Morimoto and colleagues performed hierarchical clustering to define patients who differed in type 1 IFN bioactivity and gene expression, and identified a group with low IFN activity, in the absence of anti-IFN autoantibodies, which they proved were capable of neutralizing type 1 IFN activity in vitro(84). Levels of anti-dsDNA, anti-RNP, anti-Sm, and cytokines MCP-1 and IP-10 differed in the IFNhigh and IFNlow subgroups. This suggests fundamental variation in pathophysiology, as both groups exhibit the SLE phenotype, with no significant difference in SLEDAI score, presumably through varied mechanisms. Niewold and colleagues observed increased IFN-α activity in patients with a risk/risk or risk/neutral IRF 5 genotypes compared to those with protective/protective or protective/neutral haplotypes(85). They subsequently found a novel risk haplotype of IRF 5 that was associated with anti-dsDNA positivity and predicted high IFN-α activity. They concluded that “these autoantibodies are directly pathogenic in SLE” and lead to increased IFN-α in concert with specific combinations of IRF 5 functional genetic elements(86).

The findings of Landolt-Marticorena, Morimoto and Niewold, suggestive of a characteristic IFN signature which may be stable over time, despite fluctuations in disease activity, highlights the possibility of SLE, as a clinical syndrome, being driven by more than one mechanism, with the IFN pathway being more prominent in some patients than others. Studying the IFN signature in patients in remission – especially those with discordant serology, and especially in light of Niewold's observation of anti-dsDNA pathogenicity in its context – may provide useful insights into disease pathophysiology.

There have been two studies to date that may implicate the interferon pathway specifically in patients with SACQ remission. Pau and colleagues studied a bicongenic mouse which phenotypically resembles SACQ patients(87). This mouse avidly generates autoantibodies, but not IFN-α, in spite of abundant pDCs. While bone cell stimulation with TLRs leads to IFN-α production, similar splenic cell stimulation does not, suggesting inhibition of IFN-α production. This has been theorized to result in a process known as TLR tolerance, wherein continued exposure to a (nucleic acid) stimulus results in blunting of the IFN response.

The second study, conducted by Kwok and colleagues, revealed that SLE peripheral blood mononuclear cells (PBMCs) had diminished IFN-α production on TLR 9 stimulation compared to healthy controls(88). However, the serum of SLE patients in whom the IFN response was most blunted generated the most robust IFN-α response when incubated with the PBMCs of healthy controls. Furthermore, when

PBMCs from healthy controls were incubated with SLE serum, washed, and then re-exposed immediately, the IFN-α response was blunted, but recovered incrementally if a latency of 24 or 48 hours was permitted before re-exposure. They theorized that persistent TLR 9 stimulation with DNA-containing immune complexes induced pDC tolerance, and resultant decreased IFN-α production. Might persistent exposure to immune complexes – as in the bicongenic mice and in these human healthy controls - be the driving force behind clinical quiescence in SACQ patients through blunting of the interferon response?

Chapter 2: Hypothesis and Research Aims

In disease, outliers often can provide insights not readily apparent in those whose illness runs a conventional course. SLE is a classically relapsing/remitting disease, in which prolonged remissions are rare. The pathophysiology, which results in the disease process being "turned off" in these instances, is not well understood. Even more unusual is a subset of remissions in which clinical and serologic activity runs a discordant course:

Anti-double stranded DNA antibodies (anti-dsDNA) are highly specific diagnostic markers for SLE, and often exhibit close concordance with disease activity. In many SLE patients, elevations therein herald disease flare, so it is beneficial to follow anti-dsDNA levels in them closely, and treat those known to be concordant with steroids or immunosuppressives, on the basis of active serology at the first clinical suggestion of disease activity, in order to avert more severe flare(65). However, a subset of patients displays persistent serologic activity, as evidenced by elevated anti-dsDNA and/or hypocomplementemia, despite clinical quiescence. These serologically active clinically quiescent (SACQ) patients, who represent at least 6% (if stringently defined) of our SLE cohort, pose a management dilemma for clinicians(68).

Our group has found that approximately 60% of SACQ patients ultimately flare, but do so only after a mean of three years of disease clinical quiescence(68). Given the significant morbidity associated with steroid and immunosuppressive use, minimizing patients' exposure, especially in the context of disease quiescence, is

imperative, provided that damage does not accrue subclinically during a remission period off corticosteroids and immunosuppressives. Furthermore, a method to predict which of the SACQ SLE patients will go on to flare and require medication, and when, would be a boon to the treating clinician.

Thus the purpose of my investigations was to first describe remission in adult SLE patients, with a focus upon patients with a prolonged remitted course, and those with discordant serology. Then, after having confirmed that patients with active serology can experience a prolonged clinical remission, I strove to determine if disease-related organ damage accrued subclinically in SACQ patients during SACQ periods, because if it did, then “clinical remission” would be illusory. Finally, once it was confirmed that damage did not accrue subclinically during SACQ remission, I sought clinically relevant biomarkers that would predict flare in SACQ patients, because to date, I have found that fluctuation in conventional biomarkers (anti-dsDNA or complement levels) during a SACQ period are not predictive of flare(68).

It has been suggested that not all anti-dsDNA is created equally, with anti-dsDNA of high avidity, of IgG isotype and complement-fixing IgG sub-class best correlating with disease activity and renal involvement(38-45). As a corollary, IgM isotype and non-complement-fixing IgG sub-class best correlate with disease quiescence. There is the general consensus that anti-nucleosome antibodies are both sensitive and highly specific for SLE, and may correlate better with SLE activity than conventional serum biomarkers(50-53). Interferon-α (IFN-α) is thought to play a central role in

lupus pathogenesis via abnormal stimulation, differentiation and maturation of dendritic cells, and has been shown to be altered in SLE patients compared to healthy controls(78,83,89).

A bicongenic mouse, phenotypically resembling SACQ patients, has been developed. It avidly produces autoantibodies, but not IFN-α, in spite of abundant plasmacytoid dendritic cells (pDCs). While bone cell stimulation with Toll-like receptors (TLRs) leads to IFN-α production, similar splenic cell stimulation does not, suggesting inhibition of IFN-α production(87). Others have observed that persistent TLR 9 stimulation with DNA-containing immune complexes induces pDC tolerance, and resultant decreased IFN-α production. This was reversible when pDCs were removed from the immune stimulus, then re-exposed(88).

On these bases, I hypothesized that

a) prolonged SACQ periods occur

b) SACQ SLE patients will not accrue damage during a SACQ period

c) their anti-dsDNA and anti-nucleosome immunoglobulin profiles will differ from non-SACQ SLE patients and

d) their serologic/clinical discordance stems from altered expression of interferon-inducible genes (compared to those of concordant patients) and resultant altered cytokine/chemokine production.

The specific aims of this thesis have been

a. To confirm that prolonged remission can occur among SACQ patients

b. To determine if damage accrues subclinically during a SACQ period
c. To determine if immunoglobulin profiles differ in SACQ patients compared to other SLE patients
d. To quantify levels of five interferon-α-responsive genes, which have been shown to have increased expression in non-SACQ SLE patients compared to healthy controls (*LY6E*, *OAS1*, *IFIT1*, *ISG15* and *MX1*), in SACQ SLE patients during a SACQ period
e. To compare the levels of expression of these genes in SACQ SLE patients during a SACQ period to these levels in
    i. Serologically and clinically active (SACA) SLE patients
    ii. Serologically and clinically quiescent (SQCQ) SLE patients
f. To compare levels of relevant downstream cytokines and chemokines in SACQ patients during a SACQ period to
    i. Serologically and clinically active (SACA) SLE patients
    ii. Serologically and clinically quiescent (SQCQ) SLE patients

Chapter 3: Prolonged remission in patients with systemic lupus erythematosus

This work has been accepted for publication to the Journal of Rheumatology.

Abstract

Objectives: Systemic lupus erythematosus (SLE) is typically a relapsing/remitting disease, however, some patients experience prolonged remission. These patients may provide further insights into SLE pathophysiology. In this study we characterize their clinical course.

***Methods*:** Prolonged remission was defined as SLEDAI-2K=0 (serologically and clinicially quiescent, SQCQ), or =2 or 4 (based on serology, serologically active clinically quiescent (SACQ)) for ≥5 consecutive years, with visits ≤18 months apart. The patients could be taking antimalarials, but not corticosteroids or immunosuppressives. Flare was defined as clinical activity on SLEDAI-2K, or by corticosteroid/immunosuppressive initiation. Each patient's pre-remission course was classified as monophasic, relapsing/remitting, or chronic active. These patients were compared to matched SLE controls and patients achieving remission on medications.

Results: 38/1613 (2.4%) patients achieved prolonged remission off medications. The mean duration was 11.5±6.4 years. Twenty-seven (71.0%) patients had relapsing/remitting disease, 11 (28.9%) had monophasic illness, none had chronic active disease prior to remission. They differed from matched controls in ethnicity, disease activity at first visit and cumulative organ damage. 34/1613 (2.1%) patients achieved prolonged remission on steroids and/or immunosuppressives, with mean duration 8.5±2.9 years. Twelve (35.3%) flared. They were younger at diagnosis, with more disease activity prior to remission than patients off medications.

Conclusions: Prolonged remission is an infrequent outcome among patients and is preceded by an atypically monophasic clinical course in a significant minority. Those on

medications represent a heterogeneous group: those who will tolerate eventual taper, and those whose disease activity was merely suppressed by ongoing immunosuppression. Prolonged remission may reflect unique pathophysiologic mechanisms, and warrants further investigation.

Introduction

In early reports, systemic lupus erythematosus (SLE) was classically described as an unrelenting disease that would often culminate in death (1). However, the disease has been increasingly recognized as a chronic, albeit potentially fatal, relapsing-remitting disease. Given the increased risk of organ damage with disease activity over time, remission is a very desirable outcome. Studies have revealed that the propensity for flare or remission in the initial years of disease are predictive of long term outcome, with those remitting earlier having a more favourable disease course (2,3). Substantial variability exists, however, in the nature and duration of remission, likely attributable to differences in patient cohorts and inconsistent remission definitions (Table 3-1) (3-6,8,16,17,19,21,24,57,64,68,69).

One important discrepancy between studies is that of the significance of isolated, potentially pathogenic serologic activity, that is, elevation in anti-dsDNA antibodies and/or hypocomplementemia, in the setting of clinical quiescence. A description of these patients, termed “serologically active clinically quiescent,” can first be found in the literature in 1979, when Gladman and colleagues described 14 patients who were clinically quiescent but had persistently positive lupus erythematosus (LE) preparations and antinuclear antibodies, hypocomplementemia, and high levels of DNA binding (57). These patients had displayed typical lupus features in the past, including major organ manifestations, such as renal or central nervous system involvement

Serologically active clinically quiescent patients present a clinical conundrum of reconciling the presence of potentially pathogenic serologic activity with the clinical picture of complete quiescence. Are these patients similar to those who are both

serologically and clinically quiescent and thus could be spared exposure to corticosteroids and immunosuppressive medications and their associated side effects? A method to distinguish which serologically active clinically quiescent patients will remain quiescent, versus those who will ultimately flare would be clinically beneficial. Another important group of potentially remitted patients are those who have evolved to clinical quiescence, with or without serologic quiescence, while being treated with corticosteroids and/or immunosuppressive medications. While such patients enjoy a disease-free state, they do so under the coverage of medications, which bear significant associated risks. In these patients it is only with medication taper and withdrawal that the clinician can determine if the patient has truly remitted, or, alternately, if their disease is merely suppressed by a quantity of corticosteroid or immunosuppressive medication. If the former, then drug discontinuation is the goal to minimize treatment-associated damage; however if the latter, medications must be maintained to minimize disease-associated morbidity. Thus these patients comprise a mixed group of two disease states necessitating very different approaches to management.

In order to gain insights into the nature and extent of prolonged remission among patients with SLE, we have defined remission as at least five years of clinical quiescence in patients with and without active serologic markers, and those on and off corticosteroids and immunosuppressive medications.

Methods

Setting: The University of Toronto Lupus Clinic at the Centre for Prognosis Studies in the Rheumatic Diseases, Toronto Western Hospital was established in 1970 to study

clinical-laboratory correlations in SLE. All patients entered fulfill 4 or more of the 1971 or 1982 ACR classification criteria, or 3 criteria and a typical biopsy lesion of SLE. The Lupus Clinic is a tertiary care facility affiliated with the University of Toronto. It also serves as a primary and secondary care facility in downtown Toronto. The Clinic's patients range from those with acutely active disease of variable manifestations to patients with inactive disease on maintenance therapy to patients in complete remission, off all therapy (90). All patients sign informed consents to allow their clinical, serologic and genetic material to be studied and reported.

Patient Selection: SLE patients are followed with clinical and laboratory information collected using a standardized protocol at clinic visits, typically at 2 to 6-month intervals, which occur regardless of disease activity. Patients registered in the Lupus Clinic database between July 1970 and October 2011 were identified. Serologically and clinically quiescent and serologically active clinically quiescent lupus patients were selected from this population.

Definitions:

Serologically and clinically quiescent was defined as at least a five-year period without clinical and serologic activity (SLE disease activity index 2000, SLEDAI-2K score = 0) where clinic visits were no more than 18 months apart. *Serologically active clinically quiescent* was defined as at least a five-year period without clinical activity and with persistent serologic activity (SLEDAI-2K score = 2 or 4, from positive anti-dsDNA antibody and/or hypocomplementemia only, at each clinic visit) where clinic visits were no more than 18 months apart. A *mixed* remission period was defined as one during which a patient's serology fluctuated between serologically and clinically quiescent and

serologically active clinically quiescent status. Patients were then divided into those off all corticosteroids and immunosuppressives for the duration of quiescence (No Medication group), and those who remained on one or both of these classes of medications (Medication group). Patients in all groups could be taking antimalarials. *Disease flare* was defined as any increase in SLEDAI-2K score not accounted for by either hypocomplementemia or anti-dsDNA, or the initiation of (No Medication group) or increase in (Medication group) corticosteroid and/or immunosuppressive therapy.

Disease course was defined as either monophasic, relapsing-remitting, or chronic active.

A *monophasic disease* course was defined as a single flare (clinical SLEDAI-2K activity at ≥1 consecutive visit(s)) followed by clinical quiescence, as defined above.

A *relapsing-remitting* course was defined as at least two discrete episodes, separated by periods of clinical quiescence.

A *chronic active* course was defined as persistent clinical activity, without any intervening period of quiescence.

Organ manifestations were defined by SLEDAI-2K descriptors; diagnosis of "cardiac – atherosclerotic," "thrombotic" and "pulmonary" manifestations were made clinically and through the use of imaging modalities.

Serologic studies: Anti-dsDNA antibodies were quantified by the Farr assay (normal ≤7 U/mL) (91). Serum complement components C3 and C4 were evaluated by

nephelometry (normal range C3 0.9-1.8 g/L; C4 0.1-0.4 g/L, Siemens Healthcare Diagnostics Inc. Newark DE, 19714 USA) (92).

Analysis: Patients in the No Medication group were matched 1:3 to SLE controls on the bases of sex, age at first clinic visit, decade of entry into the clinic, length of clinic follow up and disease duration at first remission visit. A second, unmatched control group was selected from the remainder of the SLE cohort (with sole criterion for inclusion being >5 years of follow up in clinic) to ensure that matching criteria of the first control group were not, in fact, driving the rare outcome. Adjusted mean SLEDAI (AMS), a validated measure accounting for variable duration between clinic visits in reporting SLE disease activity over time (93), was calculated for each patient from clinic entry until remission; in matched controls, AMS was calculated from clinic entry to a visit of matched duration. Descriptive statistics were used. Comparisons were made using t-tests and McNemar's test. Logistic regression analysis was pursued, guided by the findings of the univariate analysis. Charts were reviewed to elucidate the rationale for continued corticosteroid and/or immunosuppressive use among those patients in the Medication group.

Results

No Medication group:

1613 patients with visits were identified in the SLE clinic database. Thirty-eight of 1613 (2.4%) patients achieved prolonged, medication-free remission. One patient experienced two discrete prolonged medication-free serologically active clinically quiescent remission periods, (with approximately eight years between the end of the

first and start of the second remission period). For this patient only the first prolonged remission was included in the analysis. Thirty-two (84%) patients were female.

Mean duration of SLE clinic follow up was 21.8 ± 10.3 years, and the mean time to remission from clinic entry was 9.1 ± 8.8 years. The mean prolonged remission duration was 11.5 ± 6.4 years. Seventeen remission periods were serologically and clinically quiescent, 10 were serologically active clinically quiescent and 11 were mixed serologically and clinically quiescent/serologically active clinically quiescent. When subdivided by type, mean remission duration was 9.8 ± 5.7, 9.2 ± 3.3 and 16.5 ± 6.4 years for those who were serologically and clinically quiescent, serologically active clinically quiescent and mixed remissions, respectively. All but one of the 28 patients who continue to be followed contemporarily were in remission at their last clinic visit.

Antimalarials were used by 16 (42%) patients at remission onset, with a further 5 (13%) using them at some point during their remission.

Twenty-seven (71%) patients had had relapsing/remitting disease, 11 (29%) had monophasic illness, and none had chronic active disease prior to remission. The clinical manifestations in those patients with a monophasic course are outlined in Table 3-2. Mean AMS from clinic entry until remission onset was 3.02 ± 1.93.

Case-control analysis:

There were more Caucasian cases than controls (82% vs. 72%, p=0.02). Cases had significantly lower SLEDAI-2K at first clinic visit (8.03 ± 9.47 vs. 10.6 ± 9.04, p=0.02), and their AMS until remission onset (versus clinic visit of matched duration from clinic entry) was similarly significantly lower (3.02 ± 1.93 vs. 5.95 ± 3.56, p<0.0001). Among those patients with organ damage, significantly less had accrued in cases (1.08 ± 1.32

vs. 1.60 ± 2.06, p=0.03). There were significantly fewer skin, central nervous system and pulmonary manifestations over the patients' disease courses among cases. There was no difference in antimalarial use between groups, but overall prednisone use and cumulative dose was significantly lower among cases at the start of their prolonged remission period, as was the use of immunosuppressive agents (Table 3-3).

Logistic regression models were built comparing cases to matched and unmatched controls. In a model where all potential risk factors were included, no association between presence of remission and sex, age at diagnosis, disease duration at first visit, race, disease activity (by SLEDAI-2K) at first visit, or renal, pulmonary or dermatologic involvement were statistically significant. A stepwise regression suggested remitted patients were less likely to have dermatologic involvement (OR 0.27 (0.10, 0.71), p = 0.008). Including all risk factors in a model using the unmatched controls was similarly unrevealing for any association with sex, age at diagnosis, disease duration or disease activity at first visit, and race with remission status. The stepwise regression associated older age at diagnosis with remission status (OR 1.03 (1.00, 1.05), p = 0.02).

To further characterize the remitted patients, we then embarked upon analyses comparing demographic and clinical characteristics of the cohort to the Medication group (see also, below), and to both matched and unmatched controls, at several time points, to provide a measure of disease evolution over time (Supplementary Tables 3-1-5). Given the goal of the paper was descriptive, and that multiple comparison were made in these analyses, we focused upon only those results which were highly

significant (which we defined as $p<0.01$). With this lens we found the No Medication patients had lower disease activity, by SLEDAI-2K, at various time points in their disease course, and that steroid use was less prevalent than in both matched and unmatched controls. They had less renal disease than matched and unmatched controls at 5 years from clinic entry ($p = 0.003$ for both), but this difference did not persist at other time points studied.

Medication group:

Thirty-four patients who achieved prolonged remission while taking corticosteroids and/or immunosuppressives were identified among the 1613 eligible patients (2.1%). The mean duration of prolonged clinical quiescence in this group was 8.5 ± 2.9 (range 5.1-16.3) years. This prolonged clinically quiescent period was terminated by flare in 12 patients (35%). In the remaining 22 (65%) patients whose prolonged clinically quiescent period did not end in flare, medications were eventually successfully discontinued in five (15%). Medications were being tapered in six (18%) patients, and were being maintained in two (6%) with organ transplants necessitating ongoing immunosuppression. Six (18%) patients were maintained on a stable regimen, with no standardized drug withdrawal algorithm specified. Three patients (9%) were lost to follow up. (Figure 3-1).

Comparison of No Medication versus Medication groups:

When the groups were compared, Medication group patients were younger at diagnosis (27.9 ± 11.7 versus 36.1 ± 15.2; $p=0.01$) and required more immunosuppressives and

corticosteroid (100% versus 58%; p<0.0001) at higher cumulative doses (42.9 ± 39.7 versus 20.7 ± 17.2 grams (among those requiring corticosteroids; n=22); p=0.006) from clinic entry to the onset of prolonged clinical quiescence. Their disease was more active prior to remission onset (AMS 4.24 ± 2.67 versus 3.02 ± 1.93; p = 0.03). There were no between–group differences in ethnicity, SLEDAI-2K at presentation, antimalarial use, time to prolonged clinical quiescence, organ manifestations to remission onset, or SLICC damage index (Tables 3-4 and 3-5). The two groups similarly did not differ in terms of hematologic involvement or autoantibody profiles (Supplementary Tables 3- 2-5).

Discussion

Remission is an elusive and often ill-defined goal in SLE. The generalizability of the SLE remission literature is limited by differences in definition, with duration, disease activity measure used, the inclusion of treatment, and serologic activity all being variables that may significantly affect the result. Furthermore, given the heterogeneity of lupus presentation, and the impact of ethnicity upon disease manifestations, severity and prognosis (20), differences inherent to a cohort itself may prove central to the type and duration of remission achieved. Regardless of how it is defined, remission remains a desirable outcome in SLE, but is rarely achieved. Table 1 summarizes past studies exploring remission in SLE, highlighting the similarities and differences between these efforts.

Dubois provided one of the first descriptions of remission in a cohort of 163 lupus patients in his 1956 paper (4). He reported that an astounding 38% of the patients

experienced at least one “spontaneous remission” prior to treatment with antimalarials or corticosteroids, including one patient with a 26-year remission, and up to 16% with multiple remissions. He admitted, however, that most of these patients “did not have the full picture of systemic lupus erythematosus,” but rather had a rheumatoid arthritis-like presentation. There was no definition of remission offered in this historic paper, but it seemed to be based upon the physician’s global clinical impression. In 1964 he and Tuffanelli then corroborated the considerable remission rate, reporting that 35% of 520 SLE patients experienced “spontaneous remission,” lasting up to 26 years in one case (5). The definition of remission was similarly vague in this study.

By contrast, and highly consistent with this study’s findings, Tozman and colleagues determined that the rate of “prolonged complete remission” in SLE, defined as the absence of clinical manifestations of disease and off all immunosuppressive therapy, was 4/160 (2.5%) (8). They utilized both clinical and laboratory variables in their assessment including, for the first time in the setting of remission, the absence of anti-DNA antibodies and C3 hypocomplementemia, both of which are known to run a concordant course with disease activity in some lupus patients (9-11). These patients had remitted from previously severe disease, with median remission duration 75 months. Thus, considerable disparity in duration, definition and frequency of remission existed in the earlier literature.

In 2005, Urowitz and colleagues addressed the inconsistencies that had plagued the SLE remission literature by quantifying and describing disease quiescence using incrementally less restrictive criteria (24). Thus, they defined prolonged remission as at least a five-year period without disease activity (SLEDAI-2K = 0), while not taking

corticosteroids, immunosuppressives or antimalarials. They found that remission, thus defined, was a rare event, occurring in only 12 of 703 (1.7%) patients in their cohort. As would be expected, when progressively less stringent criteria were applied to the remission definition, encompassing one to five years' disease quiescence, permitting the presence of hypocomplementemia and/or anti-dsDNA positivity, and permitting the use of antimalarials, corticosteroids and immunosuppressive medications, remission prevalence increased as stringency decreased. When defined as clinical quiescence (by SLEDAI-2K) for one year, permitting active serology, and permitting the use of medications (the least restrictive definition), remission prevalence was 24.5%. Thus, as demonstrated by this paper, the important issue to be decided is the type of remission to be quantified.

In this study, our goal was to describe those patients who had achieved prolonged remission, which we defined as at least a five-year period without clinical activity. While any remission definition is somewhat arbitrary, we felt this cut-off, borrowed from the oncology literature, where 5-year survival rates abound and cancers quiescent for five years are presumed cured, was clinically significant. Furthermore, five years provides a considerable window for damage accrual secondary to disease or medication use and therefore reprieve of this duration would likely yield an appreciable difference compared to a patient with active disease, requiring treatment with corticosteroids (27).

While prolonged complete remissions were rare, durable remission of a decade or more can be anticipated, even among those whose anti-dsDNA and/or complement levels fluctuated from normal range. In fact, the mixed remission group had the longest average remission duration of nearly 17 years. Thus in patients whose serology proves

discordant, fluctuations from normal range during prolonged remission may simply be observed without the introduction of corticosteroids or immunosuppressive medications, as remission can persist in spite of these changes. This finding is consistent with past studies, which reveal that, among SACQ patients, fluctuations in anti-dsDNA and/or complement levels were not predictive of disease flare (68).

It should also be emphasized that these patients fundamentally differ from those described by Tseng and colleagues, who were serologically active and clinically stable (66). In their study, those with serologic evidence of flare, namely 25% elevation in anti-dsDNA and 50% elevation in C3a, were randomized to receive either a three-week course of prednisone, with starting dose 30 mg per day, or placebo. They found that significantly more clinical flares occurred in the placebo group than in the treatment group (six versus none among 41 patients who experienced serologic flare, p=0.007). Severe caution must be exercised, however, in extrapolating these findings to our SACQ patients, as Tseng's patients could have had active disease requiring up to 15 mg of prednisone daily and still have met inclusion criteria. Since this cohort included patients who continued to have evidence of active disease despite treatment with corticosteroid, as well as patients whose clinical manifestations may have been merely suppressed by their baseline corticosteroid dosing, they were fundamentally different than the serologically active clinically quiescent patients as we had defined them.

Monophasic course is a rare outcome among the rheumatic diseases; review of the literature yields rare description thereof in few disease entities (systemic juvenile idiopathic arthritis, myositis, adult-onset Still's disease and polyarteritis nodosa)(94-97). Thus a unique finding of this study was the significant subset of patients, representing

nearly one-third of the No Medication group, whose illness was atypically monophasic. None of these patients' SLE diagnosis was thought to be attributable to drug use, thus they did not appear to have a reversible etiology. To our knowledge, there are no other studies that report this unusual pattern of disease activity in this classically relapsing-remitting disease. These patients may provide unique pathophysiologic insights into SLE, if not autoimmunity, more generally, and thus warrant further investigation at genotypic and phenotypic levels.

We noted disease duration of nearly a decade at remission onset, in keeping with past observations that likelihood of remission increases with disease duration(3). Our case-control analysis also demonstrates that the remitted patients had milder disease, with less need for corticosteroids and/or immunosuppressives, and less resultant damage accrual early on. This is consistent with the notion that early disease activity is the harbinger of what is to come: Formiga and colleagues studied remissions among those with high disease activity early in their disease course (19). They defined remission as disease activity permitting the withdrawal of all SLE-related treatment over at least one year, and asymptomatic serologic fluctuations were permissible. Twenty-four percent of their exclusively Caucasian cohort (of 100 patients) achieved such a remission, at mean 64 months after diagnosis, and the remissions persisted, on average, over more than 4.5 years. While there were differences in baseline SLEDAI value between those who achieved remission and those who did not (with those with higher initial SLEDAI scores less likely to remit), these did not attain statistical significance. Thus they observed remissions in patients with all disease manifestations, including major organ involvement, and found a significant correlation between SLEDAI values and time to

remission onset: remission occurred later among those with more severe baseline disease. We acknowledge that, like in Formiga's cohort, there were no Blacks among our cases. This, of course, may limit the generalizability of our findings, on the one hand, but may speak to an important and defining phenotypic clue to prolonged remission, on the other, which may be borne out in future, multicentred, collaborative studies.

We found that cases did not differ from controls with respect to prevalence of renal manifestations at the start of their remission period. This is consistent with past investigations of serologically active clinically quiescent patients, revealing no difference in nephrologic involvement compared to a large group of SLE controls (n=868) (68), and commensurate with widely-cited renal lupus prevalences (98). (We did find that they differed from matched and unmatched controls at one time point (5 years from clinic entry, Supplementary Table 3-3). We observed a lower prevalence of CNS manifestations in cases than controls at the start of the remission period, however, this difference was not reflected at other time points investigated. While these findings may be suggestive of differing major organ involvement in those SLE patients achieving prolonged remission, they should be borne out in a larger sample of remitted patients, ideally over multiple centres, internationally, especially given the notoriously variable prevalences reported in these organ systems (98-100).

Our analysis of patients who had remitted while taking corticosteroids and/or immunosuppressives is suggestive of two subsets within this cohort: those patients in true remission, for whom medications being successfully tapered will be withdrawn, on the one hand; and those patients in whom disease was merely suppressed by

treatment, on the other. In fact, a significant minority of these patients were evolving to the No Medication group, but had not yet fulfilled the five-year duration criterion for drug-free remission. Comparison of these remitted/suppressed subsets at genetic and/or biochemical levels may yield important differences which may be applied in the future to disease prognostication and treatment.

The pathophysiology of SLE remission, in general, and especially in the face of persistent, purportedly pathogenic serologic activity is not understood. A pilot study comparing autoantibody levels in patients with serologically active clinically quiescent disease who ultimately flared compared to those who did not failed to elucidate a difference between groups(101). A fascinating experiment performed by Pau and colleagues involved a lupus-prone mouse phenotypically resembling serologically active clinically quiescent patients, and explored the centrality of interferon-alpha expression in SLE. They found that, despite marked plasmacytoid dendritic cell expansion, there was decreased interferon alpha production peripherally, even in the face of Toll-like receptor stimulation(87). Inspired by these unique findings, we plan to explore the interferon response in this rare and perhaps instructive cohort.

Conclusions

Prolonged clinical remission off corticosteroids and/or immunosuppressive medication is an infrequent outcome among SLE patients, occurring in only 2.4%. It lasts more than a decade, and is preceded by an atypically monophasic clinical course in a significant

minority. These occurrences may be reflective of unique pathophysiologic mechanisms, and warrant further investigation.

2.1% of our cohort achieves prolonged clinical quiescence on medication. This group, however, appears heterogeneous: those who flared, representing a group whose disease activity is merely suppressed by ongoing medication use, and those who tolerated/were tolerating medication withdrawal, reflective of true prolonged clinical quiescence (as in the No Medication group).

Remission in SLE may be reflective of unique pathophysiologic mechanisms, and thus warrants further investigation.

Table 3-1: Past remission studies

Authors	Year	Journal	Remission Definition	Serologic activity permissible	Treatment permissible	Remission achieved % (total n)	Remission duration
Dubois	1956	Ann Int Med	N/D	N/D	N/D	38% (156)	Up to 26 years
Dubois & Tuffanelli	1964	JAMA	N/D	N/D	N/D	35% (520)	Up to 26 years
Gladman et al	1979	J Rheumatol	Asymptomatic	Yes (all patients)	None	7.8% (180)	4.25 years (mean), 2 – 11 years (range)
Tozman et al	1982	Ann Rheum Dis	Absence of clinical manifestations of disease	No	None	2.5% (160)	75 months (median)
Heller & Schur	1985	J Rheumatol	Asymptomatic without active organ involvement	No	AM, "low dose" CS	4% (305)	0.5 – 13 years (range)
Walz Leblanc et al	1994	J Rheumatol	Clinical SLEDAI = 0 over ≥3 consecutive clinic visits	Yes (all patients)	Any	N/D	N/D
Drenkard et al	1996	Medicine	≥1 year during which lack of clinical disease activity permitted withdrawal of all SLE treatment	Yes	None	23% (667)	4.6 ± 3.6 years (mean ± SD), 1 – 17.3 years (range)
Barr et al	1999	Arthritis Rheum	Clinical SLEDAI or PGA = 0 for ≥1 year (one PGA to <1.0 permissible)	Yes	N/D	16% of patient-years of follow up (204)	2.3 ± 1.1 years (mean ± SD), 1.0 – 5.7 years (range)
Formiga et al	1999	Rheumatology	≥1 year during which lack of disease activity permitted SLE treatment withdrawal	Yes	None	24% (100)	55 months (mean)
Swaak et al	1999	Rheumatology	Absence of disease-related signs with no need for treatment	N/D	None	0% (187)	N/A
Urowitz et al	2005	J Rheumatol	Clinical SLEDAI = 0 for ≥ 5 years	Yes	None	1.7% (703)	7.1 ± 5.3 years (mean ± SD), 5 – 17 years (range), 6 years (median)
Nossent et al	2010	Lupus	"By PGA" not otherwise defined, within 1st year of SLE diagnosis	N/D	N/D	27.5% (200)	N/D; 49% achieving remission maintained over 5 year follow up
Steiman et al	2010	J Rheumatol	Clinical SLEDAI-2K = 0 for ≥ 2 years	Yes (all patients)	AM	6.1% (924)	182 weeks (mean), 158 weeks (median)
Conti et al	2012	PLoS ONE	Clinical SLEDAI-2K = 0 for ≥ 2 years	Yes (all patients)	AM	2.2% (45)	N/D

AM = antimalarials; CS = corticosteroids; N/A = not applicable; N/D = not described; PGA = Physician Global Assessment; SLEDAI = SLE Disease Activity Index

Table 3-2: SLEDAI-2K clinical characteristics of flare in patients with monophasic course:

Patient	Clinical characteristics in flare	Remission type	Remission duration to most recent visit (yrs)	Race	Age at diagnosis (yrs)	Ever CS	Ever AM	Ever IS
1	Arthritis, fever, headache, pericarditis, pleurisy	SQCQ	25.1	Caucasian	41.5	Yes	No	No
2	Organic brain syndrome, fever, mucosal ulcers, pleurisy	mixed	22.3	Caucasian	60.6	Yes	No	No
3	Fever, rash	SACQ	7.1	Caucasian	83.1	No	No	No
4	Leukopenia, mucosal ulcers, pericarditis, rash, renal	mixed	27.7	Caucasian	50.4	No	No	No
5	Alopecia, rash, renal	SQCQ	8.7	Caucasian	52.4	Yes	No	Yes
6	Alopecia, fever, headache, organic brain syndrome, rash, renal	mixed	25.1	Asian	22.1	Yes	No	No
7	Arthritis, rash	SQCQ	11.0	Caucasian	52.1	No	Yes	No
8	Arthritis, leukopenia	mixed	12.1	Other	31.9	No	Yes	No
9	Alopecia, arthritis, pleurisy, rash, renal, vasculitis	SACQ	8.0	Other	16.8	Yes	Yes	No
10	Arthritis, fever, rash, thrombocytopenia	Mixed	11.5	Caucasian	12.1	No	Yes	No
11	Leukopenia, renal	SQCQ	5.8	Other	36.2	Yes	Yes	No

SQCQ = serologically and clinically quiescent; SACQ = serologically active clinically quiescent; CS = corticosteroids; AM = antimalarials; IS = immunosuppressives

Table 3-3: No Medication group matched case-control analysis:

	Cases (n=38)	Controls (n=114)	Matched p value
Demographics:			
Sex (F)	32 (84.2%)	96 (84.2%)	N/A
Age at diagnosis (years)	36.1 ± 15.2	36.6 ± 14.9	N/A
Length of follow-up at remission onset (years)	9.13 ± 8.79	8.89 ± 8.50	N/A
Race Caucasian Black Asian Other	 32 (82.4%) 0 (0%) 2 (5.3%) 4 (10.5%)	 82 (71.9%) 16 (14.0%) 10 (8.8%) 6 (5.3%)	 0.02 (Caucasian vs. all others)
SLEDAI-2K at 1st clinic visit	8.03 ± 9.47	10.6 ± 9.04	0.02
AMS (from clinic entry to remission onset)	3.02 ± 1.93	5.95 ± 3.56	< 0.0001
SLICC Damage Index Score > 0 Mean score	 20/37 (54.1%) 1.08 ± 1.32	 67/109 (61.5%) 1.60 ± 2.06	 0.37 0.03
Organ involvement (ever), by SLEDAI-2K, from clinic entry to remission onset (or matched visit):			
Musculoskeletal	16 (42.1%)	50 (43.9%)	0.73
Skin	28 (73.7%)	104 (91.2%)	0.0004
Vasculitis	10 (26.3%)	42 (36.8%)	0.08
Renal	26 (68.4%)	88 (77.2%)	0.12
Central nervous system	14 (26.8%)	65 (57.0%)	0.002
Cardiac – SLE-related	12 (31.6%)	34 (29.8%)	0.74
Cardiac – atherosclerotic*	4 (10.5%)	19 (16.7%)	0.16
Thrombotic*	3 / 25 (12.0%)	10 / 84 (11.9%)	0.59
Pulmonary*	5 (13.2%)	34 (29.8%)	0.0009
Medication use from clinic entry:			
Corticosteroids	22 (57.9%)	91 (79.8%)	<0.0001
Antimalarials	23 (60.5%)	73 (64.0%)	0.55
Immunosuppressives	9 (23.7%)	54 (47.4%)	0.0003
Cumulative corticosteroid dose (grams)	(n=22)** 20.7 ± 17.2 (n=38)*** 12.0 ± 16.6	(n=90)** 42.7 ± 37.8 (n=113)*** 34.0 ± 37.9	 <0.0001 <0.0001

* Diagnosed clinically and/or radiographically; not a component of SLEDAI-2K
**Cumulative corticosteroid dose in patients on corticosteroids at some point
***Cumulative corticosteroid dose all patients. (Assume = 0 in patients never on corticosteroids)

Table 3-4: Clinical characteristics of Medication (MED) compared to No Medication (NO MED) groups at remission start:

	MED (n=34)	NO MED (n=38)	Unmatched p value
Sex (F)	33 (97.1%)	32 (84.2%)	0.11
Age at diagnosis (years)	27.9 ± 11.7	36.1 ± 15.2	**0.01**
Length of follow-up at remission onset (years)	9.13 ± 8.74	9.13 ± 8.79	1.00
Race Caucasian Black Asian Other	 25 (73.5%) 4 (11.8%) 4 (11.8%) 1 (2.9%)	 32 (84.2%) 0 (0%) 2 (5.3%) 4 (10.5%)	 0.27 (Caucasian vs. all others)
SLEDAI-2K at 1st clinic visit	8.15 ± 7.72	8.03 ± 9.47	0.95
AMS (from clinic entry to remission onset)	4.24 ± 2.67	3.02 ± 1.93	**0.03**
SLICC Damage Index* Score > 0 Mean score	 18/31 (58.1%) 1.68 ± 1.87	 20/37 (54.1%) 1.08 ± 1.32	 0.14
Organ system involvement (ever), by SLEDAI-2K, from clinic entry to remission onset:			
Musculoskeletal	11 (32.4%)	16 (42.1%)	0.39
Skin	28 (82.4%)	28 (73.7%)	0.38
Vasculitis	6 (17.7%)	10 (26.3%)	0.38
Renal	19 (55.9%)	26 (68.4%)	0.27
Central nervous system	18 (52.9%)	14 (36.8%)	0.17
Cardiac – SLE-related	9 (26.5%)	12 (31.6%)	0.63
Cardiac – atherosclerotic*	4 (11.8%)	4 (10.5%)	1.00
Thrombotic*	4/28 (14.3%)	3/25 (12.0%)	1.00
Pulmonary*	10 (29.4%)	5 (13.2%)	0.09

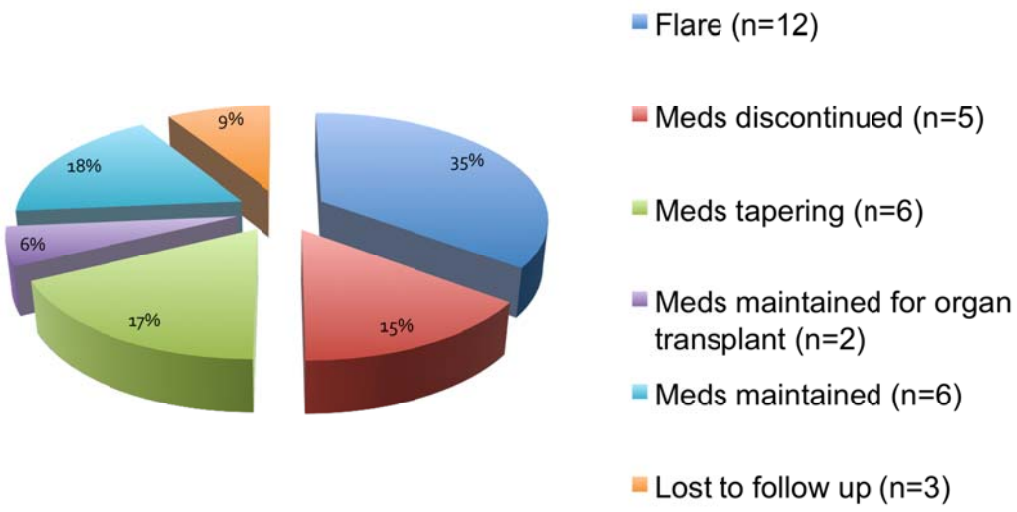
* Diagnosed clinically and/or radiographically; not a component of SLEDAI-2K

Table 3-5: Medication use from clinic entry in Medication (MED) compared to No Medication (NO MED) group:

	MED (n=34)	NO MED (n=38)	Unmatched p value
Corticosteroids	34 (100%)	22 (57.9%)	**<0.0001**
Antimalarials	21 (61.8%)	23 (60.5%)	0.91
Immunosuppressives	18 (52.9%)	9 (23.7%)	**0.01**
Cumulative corticosteroid dose (grams)	(n=34)** 42.9 ± 39.7 (n=34)*** 42.9 ± 39.7	(n=22)** 20.7 ± 17.2 (n=38)*** 12.0 ± 16.6	**0.006** **0.0001**

**Cumulative corticosteroid dose in patients on corticosteroids at some point
***Cumulative corticosteroid dose all patients. (Assume = 0 in patients never on corticosteroid

Figure 3-1: Medication group remission outcomes



Supplementary Table 3-1: Patient demographics (p values reflect comparison to No Medication (NO MED) group

	NO MED (n=38)	**MED (n=34)**	**Matched controls (n=114)**	**Unmatched controls (n=114)**
Age at diagnosis (yrs)	36.1±15.2	27.9±11.7 p=0.01	36.6±14.9 p=0.87	29.7±14.6 p=0.02
Age at 1st visit (yrs)	38.5±15.0	33.8±11.6 p=0.14	38.7±14.5 p=0.97	33.4±13.9 p=0.05
Sex (Female)	32 (84.2%)	33 (97.1%) p=0.11	96 (84.2%) p=1.00	106 (93.0%) p=0.12
Race* Caucasian Black Asian Other	 32 (84%) 0 (0) 2 (5%) 4 (11%)	 25 (74%) 4 (12%) 4 (12%) 1 (3%) p=0.27	 82 (72%) 16 (14%) 10 (9%) 6 (5%) p=0.02	 83 (73%) 10 (9%) 10 (9%) 11 (10%) p=0.16
Disease duration at 1st visit (yrs)	2.4±5.8	5.9±7.0 p=0.02	2.1±4.5 p=0.71	3.7±6.0 p=0.23
Duration of clinic follow-up (yrs)	23.8±10.6	24.0±10.4 p=0.94	19.1±9.1 **p=0.009**	16.0±8.0 **p=0.0001**

p < 0.01 considered significant
*** Caucasian versus all others**

Supplementary Table 3-2: Clinical and laboratory characteristics at visit 2 years from clinic entry (if prior to remission; p values reflect comparison to No Medication (NO MED) group)

	NO MED (n=28)	MED (n=21)	p value	Matched controls (n=88)	p value	Unmatched controls (n=103)	p value
SLEDAI-2K	3.4±3.2	5.4±4.7	0.07	5.8±5.3	**0.005**	5.7±5.0	**0.004**
Corticosteroid (% use)	60.7%	95.2%	0.007	81.8%	0.02	79.6%	0.04
Antimalarial (% use)	35.7%	33.3%	0.86	50.0%	0.19	57.3%	0.04
Immunosuppressive (% use)	32.1%	42.9%	0.44	35.2%	0.76	42.7%	0.31
Musculoskeletal	28.6%	23.8%	0.71	27.3%	0.89	26.2%	0.80
Skin	82.1%	81.0%	1.00	88.6%	0.35	89.3%	0.33
Vasculitis	17.9%	19.1%	1.00	26.1%	0.37	24.3%	0.47
Renal	46.4%	47.6%	0.93	61.4%	0.16	67.0%	0.05
Central nervous system	25.0%	42.9%	0.19	40.9%	0.13	38.8%	0.18
Cardiac – SLE-related	17.9%	14.3%	1.00	26.1%	0.37	22.3%	0.61
Cardiac – atherosclerotic	10.7%	4.8%	0.63	3.4%	0.15	2.9%	0.11
Thrombotic	6.7%	12.5%	1.00	7.3%	1.00	14.6%	0.67
Pulmonary	0	19.1%	0.03	19.3%	0.01	23.3%	**0.005**
Leukopenia	14.3%	0	0.12	10.2%	0.51	10.7%	0.74
Thrombocytopenia	25.0%	14.3%	0.48	11.4%	0.12	14.6%	0.25
Coombs	39.3%	38.1%	0.93	37.5%	0.87	36.9%	0.82
Anti-Jo1	7.1%	9.5%	1.00	3.4%	0.59	1.9%	0.20
Anti-La	21.4%	14.3%	0.71	10.2%	0.19	6.8%	0.03
Anti-RNP	10.7%	14.3%	1.00	14.8%	0.76	22.3%	0.17
Anti-Ro	28.6%	23.8%	0.71	21.6%	0.45	17.5%	0.19
Anti-SCL70	17.9%	9.5%	0.68	6.8%	0.13	5.8%	0.06
Anti-Smith	14.3%	9.5%	0.69	10.2%	0.51	14.6%	1.00
ANCA	0	0	N/A	1.1%	1.00	1.9%	1.00
Anti-dsDNA (Farr)	42.9%	52.4%	0.51	64.8%	0.04	64.1%	0.04
ANA	92.9%	95.2%	1.00	95.5%	0.63	97.1%	0.29

p < 0.01 considered significant

Supplementary Table 3-3: Clinical and laboratory characteristics at visit 5 years from clinic entry (if prior to remission; p values reflect comparison to No Medication (NO MED) group)

	NO MED (n=20)	MED (n=15)	p value	Matched controls (n=59)	p value	Unmatched controls (n=104)	p value
SLEDAI-2K	3.1±4.2	4.1±4.6	0.47	5.1±3.7	0.04	4.5±4.2	0.15
Corticosteroid (% use)	60.0%	100%	0.006	91.5%	**0.003**	85.6%	0.01
Antimalarial (% use)	45.0%	40.0%	0.77	54.2%	0.47	74.0%	0.01
Immunosuppressive (% use)	35.0%	60.0%	0.14	39.0%	0.75	54.8%	0.10
Musculoskeletal	35.0%	26.7%	0.72	28.8%	0.60	37.5%	0.83
Skin	85.0%	100%	0.24	91.5%	0.41	92.3%	0.38
Vasculitis	40.0%	20.0%	0.28	39.0%	0.94	29.8%	0.37
Renal	45.0%	73.3%	0.09	80.0%	**0.003**	77.9%	**0.003**
Central nervous system	35.0%	60.0%	0.14	62.7%	0.03	51.0%	0.19
Cardiac – SLE-related	35.0%	20.0%	0.46	32.2%	0.82	29.8%	0.64
Cardiac – atherosclerotic	5.0%	20.0%	0.29	11.9%	0.67	6.7%	1.00
Thrombotic	8.3%	0	1.00	16.1%	0.66	17.9%	0.68
Pulmonary	10.0%	33.3%	0.11	25.4%	0.21	28.9%	0.08
Leukopenia	25.0%	0	0.06	13.6%	0.30	11.5%	0.15
Thrombocytopenia	30.0%	13.3%	0.42	17.0%	0.22	24.0%	0.57
Coombs	50.0%	40.0%	0.56	47.5%	0.84	53.9%	0.75
Anti-Jo1	10.0%	6.7%	1.00	6.8%	0.64	9.6%	1.00
Anti-La	20.0%	13.3%	0.68	17.0%	0.74	15.4%	0.74
Anti-RNP	25.0%	20.0%	1.00	17.0%	0.51	30.8%	0.61
Anti-Ro	25.0%	20.0%	1.00	27.1%	0.85	26.9%	0.86
Anti-SCL70	30.0%	6.7%	0.20	10.2%	0.06	14.4%	0.11
Anti-Smith	20.0%	13.3%	0.68	15.3%	0.73	20.2%	1.00
ANCA	10.0%	0	0.50	6.8%	0.64	12.5%	1.00
Anti-dsDNA (Farr)	65.0%	86.7%	0.24	83.1%	0.12	79.8%	0.15
ANA	95.0%	93.3%	1.00	96.6%	1.00	100%	0.16

p < 0.01 considered significant

Supplementary Table 3-4: Clinical and laboratory characteristics at visit 2 years from SLE diagnosis (if prior to remission; p values reflect comparison to No Medication (NO MED) group)

		NO MED (n=23)		MED (n=14)	p value		Matched controls (n=76)	p value		Unmatched controls (n=66)	p value
SLEDAI-2K		3.0±3.1		6.0±5.0	0.06		5.4±5.2	**0.008**		6.2±5.4	**0.001**
Corticosteroid (% use)		56.6%		100%	0.006		79.0%	0.03		71.2%	0.20
Antimalarial (% use)		47.8%		35.7%	0.47		48.7%	0.94		57.6%	0.42
Immunosuppressive (% use)		26.1%		28.6%	1.00		27.6%	0.88		30.3%	0.70
Musculoskeletal		34.8%		28.6%	1.00		26.3%	0.43		24.2%	0.33
Skin		87.0%		71.4%	0.39		84.2%	1.00		86.4%	1.00
Vasculitis		8.7%		21.4%	0.35		26.3%	0.07		21.2%	0.22
Renal		52.2%		42.9%	0.58		54.0%	0.88		57.6%	0.65
Central nervous system		26.1%		42.9%	0.47		35.5%	0.40		34.9%	0.44
Cardiac – SLE-related		13.0%		14.3%	1.00		23.7%	0.39		24.2%	0.38
Cardiac – atherosclerotic		13.0%		7.1%	1.00		5.3%	0.35		3.0%	0.11
Thrombotic		9.1%		20.0%	1.00		8.6%	1.00		9.4%	1.00
Pulmonary		0		28.6%	0.02		15.8%	0.06		24.2%	**0.009**
Leukopenia		13.0%		7.1	1.00		7.9%	0.43		9.1%	0.69
Thrombocytopenia		17.4%		21.4%	1.00		9.2%	0.28		12.1%	0.50
Coombs		34.8%		50.0%	0.36		32.9%	0.87		42.4%	0.52
Anti-Jo1		4.4%		14.3%	0.54		4.0%	1.00		1.5%	0.45
Anti-La		21.7%		21.4%	1.00		9.2%	0.14		7.6%	0.12
Anti-RNP		8.7%		14.3%	0.62		15.8%	0.51		27.3%	0.07
Anti-Ro		34.8%		35.7%	1.00		26.3%	0.43		15.2%	0.07
Anti-SCL70		8.7%		14.3%	0.62		6.6%	0.66		4.6%	0.60
Anti-Smith		8.7%		21.4%	0.35		7.9%	1.00		15.2%	0.72
ANCA		0		0	N/A		1.3%	1.00		4.6%	0.57
Anti-dsDNA (Farr)		43.5%		50.0%	0.70		65.8%	0.06		57.6%	0.24
ANA		95.7%		85.7%	0.54		89.5%	0.68		93.9%	1.00

p < 0.01 considered significant

Supplementary Table 3-5: Clinical and laboratory characteristics at visit 5 years from SLE diagnosis (if prior to remission; p values reflect comparison to No Medication (NO MED) group)

	NO MED (n=15)	MED (n=13)	p value	Matched controls (n=53)	p value	Unmatched controls (n=84)	p value
SLEDAI-2K	3.8±4.8	4.2±4.7	0.85	5.9±5.5	0.18	5.0±5.0	0.40
Corticosteroid (% use)	53.3%	100%	0.007	86.8%	**0.009**	78.6%	0.05
Antimalarial (% use)	40.0%	33.8%	0.71	52.8%	0.38	72.6%	0.02
Immunosuppressive (% use)	33.3%	53.9%	0.27	35.9%	0.86	41.7%	0.54
Musculoskeletal	26.7%	15.4%	0.65	28.3%	1.00	34.5%	0.55
Skin	86.7%	92.3%	1.00	90.6%	0.64	95.2%	0.22
Vasculitis	33.3%	23.3%	0.69	35.9%	0.86	27.4%	0.76
Renal	46.7%	69.2%	0.23	67.9%	0.13	65.5%	0.17
Central nervous system	46.7%	46.2%	0.98	52.8%	0.67	23.6%	0.99
Cardiac – SLE-related	33.3%	23.1%	0.69	28.3%	0.75	27.4%	0.76
Cardiac – atherosclerotic	6.7%	15.4%	0.58	11.3%	1.00	1.2%	0.28
Thrombotic	0	0	N/A	13.0%	0.55	15.4%	0.58
Pulmonary	6.7%	38.5%	0.07	26.4%	0.16	27.4%	0.11
Leukopenia	20.0%	0	0.23	15.1%	0.70	11.9%	0.41
Thrombocytopenia	33.3%	15.4%	0.40	18.9%	0.29	22.6%	0.51
Coombs	33.3%	46.2%	0.49	47.2%	0.34	50.0%	0.27
Anti-Jo1	13.3%	7.7%	1.00	5.7%	0.30	6.0%	0.29
Anti-La	20.0%	15.4%	1.00	13.2%	0.68	10.7%	0.39
Anti-RNP	20.0%	15.4%	1.00	18.9%	1.00	33.3%	0.38
Anti-Ro	33.3%	23.1%	0.69	26.4%	0.75	20.2%	0.31
Anti-SCL70	20.0%	7.7%	0.60	7.6%	0.18	11.9%	0.41
Anti-Smith	20.0%	23.1%	1.00	13.2%	0.68	20.2%	1.00
ANCA	13.3%	0	0.48	5.7%	0.30	8.3%	0.62
Anti-dsDNA (Farr)	66.7%	69.2%	1.00	75.5%	0.52	67.9%	1.00
ANA	93.3%	92.3%	1.00	90.6%	1.00	98.8%	0.28

p < 0.01 considered significant

Chapter 4: Damage accrual in serologically active clinically quiescent (SACQ) systemic lupus erythematosus (SLE)

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Abstract

Objectives: SACQ SLE patients' discordance presents a clinical dilemma: does active serology alone warrant treatment? We explore outcomes in patients with and without a prolonged SACQ period, comparing the rate of damage accrual by SLICC/ACR Damage Index (SDI), and incidences of renal damage and coronary artery disease (CAD), over a decade.

Methods: SACQ was defined as a ≥2-year sustained period without clinical activity, with persistent serologic activity (increased anti-dsDNA and/or hypocomplementemia); antimalarials were permissible, corticosteroids/immunosuppressives were not. SACQ patients were matched for relevant variables with SLE controls. Change in SDI, and incidences of CAD and renal damage were compared. Descriptive statistics were used; comparisons were made using t- and McNemar tests.

Results: 55 SACQ patients and 110 controls were identified. SDI at 3 years from the start of the SACQ period was 0.70±1.27 vs. 1.13±1.54 in controls ($p<0.0001$), and by 10 years was 1.26±1.68 vs 2.26±2.23 ($p=0.001$); intergroup difference in damage significantly increased over 10 years. Initially two (3.6%) SACQ patients had CAD vs. 7 (6.4%) controls ($p=0.32$), with 1 (1.8%) new case in SACQ patients vs 8 (7.3%) in controls over 10 years ($p=0.06$). Baseline serum creatinine did not differ between groups. By definition, SACQ patients had no baseline proteinuria,

versus 13 (12.3%) controls ($p<0.0001$). By year 10, two (3.6%) SACQ patients, vs 26 (23.6%) controls had renal damage ($p<0.0001$).

Conclusions: Patients with a prolonged SACQ period accrued less damage over a decade compared to matched controls, supporting management with active surveillance without treatment during a SACQ period.

Introduction

Systemic lupus erythematosus (SLE) is an autoimmune disease with protean manifestations. Integral to its pathophysiology is the presence of antibodies directed at self targets, the subsequent deposition of immune complexes, and resultant inflammation and organ damage.

Anti-double stranded DNA (anti-dsDNA) antibodies are recognized as highly specific diagnostic markers for SLE, found in 60% - 80% of patients(9,102,103), and have been included in the ACR classification criteria since 1982(104,105). Anti-dsDNA antibodies have been observed to be strongly correlated with SLE for over fifty years. In 1967 Koffler et al. observed deposition of DNA - anti-DNA immune complexes in the glomeruli of ten patients with nephritis of SLE, which supported the antigen-antibody complex hypothesis for renal injury in lupus(32).

Although not as specific for SLE as anti-dsDNA antibodies, serum hypocomplementemia has been regarded as a sensitive indicator of lupus activity(66,106,107) and has been found reliable in disease prognostication(108). The proposed pathophysiologic role for complement, i.e., consumption by immune complexes, may similarly be invoked in SLE.

Many SLE patients demonstrate concordance in levels of anti-dsDNA antibodies and/or complement with disease activity, in keeping with their presumed central role

in lupus pathophysiology. However, there is a small subset of SLE patients first described by Gladman et al. in 1979(57), who manifest prolonged, persistent serologic activity, as evidenced by elevated anti-dsDNA antibody levels and/or hypocomplementemia, despite clinical quiescence. Since ongoing disease activity is known to result in irreversible end-organ damage(27,109), the management of these SLE patients with a prolonged serologically active clinically quiescent (SACQ) period has been the subject of debate among rheumatologists: should the presence of active serology, in the absence of clinical manifestations of disease, be considered active disease, and does it warrant treatment with corticosteroids and/or immunosuppressive agents?

To answer the question of treatment during a SACQ period, one must address a more fundamental question: Does damage progress subclinically during a prolonged SACQ period? If it does, then treatment with corticosteroids and/or immunosuppressive medications is warranted; if it does not, then in being treated, the patient bears the risk of medication-related morbidity without benefit.

To address the question of subclinical progression, we explored outcomes in patients with SLE with and without a prolonged SACQ period through evolution in their SLICC/ACR Damage Index (SDI), a well-validated measure of damage accrual, the incidence of coronary artery disease (CAD); and the incidence of renal damage, over a decade. The SDI is the preferred method for measuring non-reversible damage related to disease activity and drug toxicity, and has been shown to be valid

and reproducible(26,27,109); CAD and renal damage have been consistently correlated with disease activity(9,110-113). As SLE treatment contributes significantly to morbidity and damage over time(27,28), we then subdivide damage into that definitely attributable to, possibly attributable to, and independent of corticosteroid use.

Methods

Setting: The University of Toronto Lupus Clinic at the Centre for Prognosis Studies in the Rheumatic Diseases, Toronto Western Hospital was established in 1970 to study clinical-laboratory correlations in SLE. All patients entered fulfill 4 or more of the 1971 or 1982 ACR classification criteria, or 3 criteria and a typical biopsy lesion of SLE. The Lupus Clinic is a tertiary care facility affiliated with the University of Toronto. It also serves as a primary and secondary care facility in downtown Toronto. The Clinic's patients range from those with acutely active disease of variable manifestations to patients with inactive disease on maintenance therapy to patients in complete remission, off all therapy(90). All patients sign informed consents to allow their clinical, serologic and genetic material to be studied and reported.

Patient Selection: SLE patients are followed with clinical and laboratory information collected using a standardized protocol at clinic visits, typically at 2 to 6-month

intervals, which occur regardless of disease activity. Patients registered in the Lupus Clinic database between July 1970 and April 2008 with visits no more than 18 months apart were identified. Serologically active clinically quiescent lupus patients, and lupus controls were selected from this population.

Definitions: SACQ was defined as at least a two-year period without clinical activity and with persistent serologic activity (SLE disease activity index 2000, SLEDAI-2K score = 2 or 4, from positive anti-dsDNA antibody and/or hypocomplementemia only, at each clinic visit). The patients could be taking antimalarials, but those on corticosteroids or immunosuppressive medications were excluded ensuring the clinicians' impression of clinical quiescence.

Disease flare was defined as any increase in SLEDAI-2K score not accounted for by either hypocomplementemia or anti-dsDNA, or the initiation of corticosteroid or immunosuppressive treatment.

Inception was defined by the first SACQ visit in a serologically active clinically quiescent patient's prolonged SACQ period, or by a visit matched for disease duration in the control group. In patients with more than one prolonged SACQ period, only the first was analyzed.

Damage was defined by SLICC/ACR Damage Index. Specifically, it was defined as a nonreversible change, not related to active inflammation, occurring since *diagnosis*

of lupus, ascertained by clinical assessment and present for at least *6 months* unless otherwise stated. Repeat episodes must occur at least 6 months apart to score 2. The same lesion cannot be scored twice. SDI was scored as per standardized protocol at yearly intervals.

Musculoskeletal or ocular damage was deemed definitely attributable to corticosteroid use; cardiovascular, peripheral vascular, diabetic, or neuropsychiatric damage was deemed possibly attributable to corticosteroid use; and renal, pulmonary, gastrointestinal, dermatologic, or gonadal damage and malignancy were deemed independent of corticosteroid use, as defined by Gladman et al(29).

CAD was defined as myocardial infarction, angina or sudden cardiac death, documented as per protocol questionnaire, at each clinic visit. As per standard definitions:
Myocardial infarction, defined as one of: definite ECG abnormalities, typical symptoms with probable ECG abnormalities and abnormal enzymes (>2 upper limit of normal); typical symptoms and abnormal enzymes. Angina, defined as severe pain or discomfort over the upper or lower sternum or anterior left chest and left arm, of short duration relieved by rest or vasodilators.

Renal damage was defined as creatinine doubling or as creatinine > 120 at ≥ two consecutive clinic visits and/or proteinuria greater than 3.5 gm/24 hr for ≥ 6 months.

Serologic studies: Anti-dsDNA antibodies were quantified by the Farr assay (normal ≤7 U/mL)(91). Serum complement components C3 and C4 were evaluated by nephelometry (normal range C3 0.9-1.8 g/L; C4 0.1-0.4 g/L, Siemens Healthcare Diagnostics Inc. Newark DE, 19714 USA)(92).

Control patients: SLE patients without a prolonged SACQ period, followed at the Lupus Clinic, were selected from the database, and matched to the SACQ patients for age (±5 years), sex (identical), disease duration (±1 year), decade of entry into the clinic (identical), and SDI at the beginning of the SACQ period (identical).

Serologically and clinically quiescent patients (SQCQ): A second comparator group of lupus patients, who were both serologically and clinically quiescent (SQCQ) for ≥ 2 years while not requiring corticosteroids or immunosuppressive medications, was also considered. Given the relatively low number of SQCQ patients within our cohort, these patients could not be similarly matched, and were thus compared descriptively only over the 10 year period from their remission inception.

Statistical Analysis: SACQ patients and SLE controls were matched in a 1:2 ratio, which permitted close matching on all relevant aforementioned clinical variables. SDI values, presence of CAD and presence of renal damage, were obtained for each clinic visit in both SACQ and SLE control patients. Results are presented using descriptive statistics. Comparisons were made using paired and unpaired t-tests and McNemar tests.

Results

Patients: 1,351 patients were registered in the database as of April 2008; of these, 924 met the criterion of having visits no more than 18 months apart. Fifty-five patients with a prolonged SACQ period, 110 SLE controls, and 92 SQCQ patients were identified. The baseline characteristics of the SACQ and control patients are highlighted in Table 4-1. The median SACQ period was 158 weeks. The median duration between SACQ clinic visits was 6 months (24).

Fewer SACQ patients used antimalarials (60 versus 77.3%) (p=0.004), corticosteroids (18.2% versus 76.4%) or immunosuppressives (5.5% versus 43.6%) (p<0.0001 for both) over the first five years from the start of the study. SACQ and control patients were matched for SDI at inception (0.56 ± 1.21). The mean absolute value of anti-dsDNA, C3 and C4 among SACQ patients was 27.5 ± 28.3 U/mL, 0.79 ± 0.21 g/L and 0.14 ± 0.06 g/L, respectively. The nature of serologic activity was similar in the SACQ and control groups (Table 4-2).

Outcomes

Overall damage: SDI was significantly lower at all times analyzed in the SACQ cohort compared to matched SLE controls (Table 4-3). SDI was higher overall in the unmatched SQCQ patients. However, these patients had a higher damage index at inception (1.08 vs 0.56)

Among SACQ patients, there was no correlation between the degree and/or type of serologic abnormality and damage accrual over the period studied.

SDI was then broken down according to corticosteroid effect. SDI items definitely corticosteroid-related (ocular, musculoskeletal) were lower in SACQ patients than controls at years 3, 5 and 7, but not at 10 years. The difference in damage possibly attributable to corticosteroid use (cardiovascular, peripheral vascular, diabetic, or neuropsychiatric damage) steadily increased in controls, and was significantly higher than in SACQ patients at 10 years. Damage independent of corticosteroid use (renal, pulmonary, gastrointestinal, dermatologic, or gonadal damage and malignancy) was significantly lower in the SACQ group versus controls at all time points analyzed. There was no difference in accrual of damage that was possibly attributable to corticosteroid use or damage independent of corticosteroid use between SACQ and SQCQ patients over the 10 years studied. (Table 4-4, Figure 4-1a-c).

Renal damage: Baseline serum creatinine did not differ between the two groups ($p=0.90$). By definition, baseline proteinuria was not found in any SACQ patient, while it was present in 13 (12.3%) controls ($p<0.0001$). There was a significantly lower incidence of renal damage in SACQ patients compared to controls at five and ten years from the start of the study (Table 4-5). There was no patient in either group who progressed to end-stage renal disease, requiring dialysis or renal transplant.

Coronary artery disease: Two (3.6%) SACQ patients had coronary artery disease prior to study start versus seven (6.4%) controls (p=0.32). There was a trend towards increased coronary artery disease over ten years in controls compared to SACQ patients (p=0.06) (Table 4-5).

Discussion

Damage in lupus is the sum of disease-(110-112,114-116) and treatment-related(29,106) effects. It stands to reason, then, that serologically active clinically quiescent patients, as we have defined them, are spared treatment-related damage for the duration of their SACQ periods. This is clearly corroborated by this study.

What had been unclear, however, was whether lupus progresses subclinically in these SACQ patients despite clinical quiescence given that: 1) elevated titres of anti-dsDNA antibodies(9,61,103,107,117-121) and hypocomplementemia(61,120,122) have been strongly correlated with disease activity; 2) in lupus, disease activity involves the activation of immune mediated inflammatory pathways and their deleterious effects; and 3) chronic inflammation has been definitively and causally linked to damage in multiple organ systems(110-114,116,123,124). The SACQ cohort constitutes patients with serology suggestive of immune mediated inflammation (and thus a propensity for damage accrual), without any clinical evidence of damage.

The discordant clinical/serologic profile of these patients has generated debate over the best course of management during a SACQ period. We had previously described the natural history of patients with a prolonged SACQ period, off all corticosteroids and immunosuppressives, and found that while nearly 60% of SACQ patients did flare, they did so after median 155 weeks, and with relatively minor disease manifestations(68). Furthermore, we found that we could not anticipate which of the SACQ patients would ultimately flare based on fluctuations in their anti-dsDNA antibodies or complement levels drawn at routine clinic visits preceding their flares(29). Similarly, Walz LeBlanc et al. reported that approximately half of their SACQ cohort flared, and that no predictive factors for flare could be elucidated(64). Based upon these findings, there would be no basis for a clinician to expose all SACQ patients (some of whom never flare) to the risk associated with corticosteroid and/or immunosuppressive therapy unless in doing so subclinical damage accrual during the SACQ period was prevented.

Tseng has shown that the addition of prophylactic, moderate-dose corticosteroids to prevent severe flares in patients with serologically active, but clinically stable SLE, could be beneficial(66). They found that there were fewer severe flares in the prednisone protocol-treated patients. These results, however, cannot be generalized to SACQ patients as defined by our study: many of Tseng's patients had active (albeit stable) disease, and were permitted to be on active corticosteroid and/or immunosuppressive therapy at inception. Thus these patients do not present the clinical conundrum of active serology in the absence of any clinical

manifestations of active disease, without the use of corticosteroids or immunosuppressives. Other studies have had some measure of success in anticipating SLE flare by following fluctuations in anti-dsDNA levels (interestingly, both increases and decreases), but do not address their role in this group of serologically discordant patients(63,65,125).

In spite of robust evidence for anti-dsDNA antibodies and hypocomplementemia being involved in lupus pathogenesis, this study revealed significantly less damage accrual in SACQ patients compared to matched lupus controls. This was confirmed through the use of the SDI score and through incidences of renal damage, and of coronary artery disease over a decade. This study also confirmed that there was no difference in disease-related damage accrual over a decade in SACQ compared to SQCQ patients, despite the former's persistently active serology.

The average disease duration at the start of our study was approximately 11 years. At that point, the SACQ and control groups were matched for SDI (0.56 ± 1.21). Furthermore, most of the damage at inception in our cohort was definitely or possibly attributable to corticosteroid use (Table 4). Swaak et al described outcomes of lupus patients, followed at ten European rheumatology centres, with disease duration of greater than ten years, who were also similar to the patients in our study with respect to age at diagnosis and percentage who were female. They documented the cumulative damage using the SDI attributable to both lupus itself and to treatment with corticosteroids(28). At 16 years' disease duration in Swaak's

cohort (the mean duration at study entry) the SDI attributable to SLE was 2.8, and SDI attributable to SLE and some (but not all) corticosteroid-related damage was 3.7. This is strikingly more damage than that accrued in our SACQ group at an equivalent disease duration.

In a study describing damage accrual in a Nordic lupus cohort, the median last observed SDI through 11.9 years of follow-up (i.e. approximately equal to disease duration at *inception* in our cohort) was 1.26 (range 0 – 8)(109). Even more germane to this study is that when damage accrual was studied in an inception cohort with disease duration of at least 15 years at our centre in 2003, it was found that the mean SDI at ten years' disease duration was 1.2(29). Thus, our SACQ patients and matched controls had relatively little damage 11 years into their disease course. At 3 years into the study, which was the median length of the SACQ period in our cohort, the average SDI in SACQ patients was only 0.70 ± 1.27 versus 1.13 ± 1.54 ($p<0.0001$) in controls, most of which was attributable, or possibly attributable, to corticosteroid use. Thus over the duration of the average SACQ period, the SACQ patients did not accrue a clinically significant amount of disease-related damage, and accrued significantly less damage than controls. This significant difference was amplified through the ten years of follow up studied. By ten years from inception (that is, approximately 21 years from SLE diagnosis), the average SDI in the SACQ group was only 1.26 ± 1.68 (most of which was corticosteroid-attributable; Table 4), versus 2.26 ± 2.23 in controls ($p=0.001$). In Gladman's 2003 inception cohort, by contrast, the mean SDI at 15 years was 1.9(29). This reveals not only that damage

does not accrue subclinically through the SACQ period, but that SACQ patients have a relatively benign course beyond their SACQ years, even when compared to lupus patients with relatively mild disease. This difference cannot be attributed to antimalarial therapy as more control subjects than SACQ patients took antimalarials.

In further subdividing damage according to corticosteroid-related effects, we observed that there was a significant difference in damage between SACQ and control patients early on when SACQ patients, by definition, were not being exposed to corticosteroids. As illustrated in Table 4, the difference remained through the first seven years, but the difference was not statistically significant at year ten, at which point a number of SACQ patients had been treated with corticosteroids beyond their SACQ periods. This is similarly reflected when comparing the SACQ to SQCQ patients in their corticosteroid-attributable damage accrual. Gladman et al(29) found that the musculoskeletal system (avascular necrosis and osteoporosis) was the most commonly damaged organ system in their cohort, suggesting that limiting corticosteroid exposure to the lowest dose required to treat disease is important to minimize damage.

In damage classified as possibly related to corticosteroid use, which includes cardiovascular disease, we observe that while there was no significant difference in damage from inception to year 10 in the SACQ group, the damage in the control group more than tripled, representing a significant difference between the groups. While there was no significant difference in incidence of coronary artery disease

between the groups, a trend towards a higher incidence in the control group could be observed. Given that the development of coronary artery disease is an indolent process occurring over decades, and that the systemic inflammation characteristic of active lupus has been observed to accelerate vascular risk above and beyond conventional CAD risk factors(110-112,114,115,126), one would expect that the gap would continue to widen between groups with further years of observation. Indeed this would be an important area of follow up in this cohort.

There was significantly less corticosteroid-independent damage in SACQ patients compared to controls throughout the time period studied. Importantly, there was a very significant difference in renal damage between SACQ patients and controls at five and ten years from study inception. Serum anti-dsDNA antibody levels, especially when measured by Farr assay, have been correlated with nephritis, with progression to end-stage renal disease, and with increased damage and reduced survival(9,127). Furthermore, in comparing damage accrual over 10 years from remission inception in SACQ versus SQCQ patients, there was no difference in the rate of corticosteroid-independent damage accrual at any point, lending further support to the notion that active serology, alone, does not lead to subclinical damage accrual during a SACQ period.

While the SACQ cohort was carefully and relevantly matched to serologically and clinically active controls, we acknowledge that the inability to similarly match SACQ to SQCQ patients, owing to limited numbers in the latter group, was a study

limitation. We thus cannot be assured that the observed associations are not attributable to inherent differences between these cases and controls. We look forward to the opportunity to test this hypothesis more robustly as further SQCQ patients are accrued into our prospective cohort.

Over time with increasing sophistication in assay techniques and a growing body of knowledge of anti-dsDNA antibodies and their features, it is clear that not all anti-dsDNA antibodies are created equal. In 2006 Prasad et al found that the presence of anti-dsDNA antibodies, as measured at two consecutive visits, was not predictive of damage at five years(128), which is certainly consistent with our findings in SACQ patients. The relative pathogenicity of anti-dsDNA antibody isotypes is now appreciated, with anti-dsDNA IgG, specifically IgG2 and IgG3, found to be most pathogenic, perhaps owing to a propensity to activate complement or engage Fc receptors(9,102,119,129,130). We feel a particular strength of this study lies in the use of the Farr assay to detect anti-dsDNA antibodies, as it has been found to preferentially bind high-avidity antibodies, and to be best correlated with global disease activity, and renal and vasculitic involvement(42,131). Thus further characterization of anti-dsDNA antibodies by isotype, and search for alternate biomarkers such as anti-alpha-actinin and anti-nucleosome antibodies may yield novel methods for identifying and/or predicting flare in SACQ patients, and, perhaps, in all lupus patients(56,67,132-134).

Conclusions

SLE patients with a prolonged SACQ period accrue significantly less damage over a decade compared to matched SLE controls, and appear to have comparable disease-related damage to SQCQ patients. This supports the practice of active surveillance without treatment with corticosteroids or immunosuppressives during the SACQ period. Further investigation into the utility of alternate biomarkers to predict disease activity is warranted in these patients.

Table 4-1: Baseline characteristics of SACQ and control patients (reported as mean ± SD)

Characteristics	SACQ patients (n=55)	SLE Controls (n=110)
Female	48 (87%)	96 (87%)
Age at SLE diagnosis (years)	28.7 ± 15.0	27.6 ± 12.3
Age at study start (years)	39.9 ± 16.6	38.7 ± 14.6
Disease duration at study start (years)	11.2 ± 9.4	11.1 ± 9.1
Length of follow up from study start to last clinic visit (years)	9.1 ± 6.5	10.3 ± 6.2
SLEDAI-2K at study start	2.54 ± 0.98	5.05 ± 5.37
Adjusted mean SLEDAI (AMS) over study period	2.97 ± 0.97	4.78 ± 3.10
SDI at study start	0.56 ± 1.21	0.56 ± 1.21

Table 4-2: Serologic abnormalities

Serologic abnormality	SACQ (n=55)	Control* (n=110)	Serologically active control (n=67)	p value**
↓ complement	18 (32.7%)	21 (19.1%)	21 (31.3%)	0.87
↑ anti-dsDNA	21 (38.2%)	25 (22.7%)	25 (37.3%)	0.92
Both	16 (29.0%)	21 (19.1%)	21 (31.3%)	0.79
□□↓ C3	21 (38.2%)	28 (25.5%)	28 (41.8%)	0.69
□□↓ C4	23 (41.8%)	24 (21.8%)	24 (35.8%)	0.50

* 43 controls remained serologically inactive over the study period
** SACQ versus serologically active controls

Table 4-3: SDI at inception, 3, 5, 7 and 10 years

SDI	SACQ patients	SLE Controls	Differences	SQCQ patients	p value* (SACQ vs. controls)	p value** (SACQ vs. SQCQ)
Study start	0.56 ± 1.21	0.56 ± 1.21	0	(n=92) 1.08 ± 1.45	n/a	0.03
Year 3	(n=54) 0.70 ± 1.27	(n=109) 1.13 ± 1.54	(n=107) -0.50 ± 1.04	(n=92) 1.33 ± 1.52	<0.0001	0.01
Year 5	(n=45) 0.89 ± 1.37	(n=105) 1.36 ± 1.66	(n=86) -0.69 ± 1.19	(n=76) 1.32 ± 1.43	<0.0001	0.11
Year 7	(n=36) 0.94 ± 1.28	(n=83) 1.71 ± 1.86	(n=60) -0.75 ± 1.40	(n=51) 1.51 ± 1.57	0.0001	0.08
Year 10	(n=23) 1.26 ± 1.68	(n=53) 2.26 ± 2.23	(n=35) -1.14 ± 1.88	(n=36) 1.67 ± 1.62	0.001	0.36

*** Paired t-test**
**** Unpaired t-test**

Table 4-4: SDI breakdown by corticosteroid effect

SDI	SACQ patients	SLE Controls	Difference	SQCQ patients	p ¶ (SACQ vs. control	p ¶¶ (SACQ vs. SQCQ)
	Damage attributable to corticosteroid use*					
Study start	0.26 ± 0.64	0.27 ± 0.74	-0.018 ± 0.52	0.58 ± 1.09	0.72	0.03
Year 3	(n=54) 0.31 ± 0.67	(n=109) 0.61 ± 1.04	(n=107) -0.32 ± 0.81	0.74 ± 1.19	<0.0001	0.007
Year 5	(n=45) 0.40 ± 0.72	(n=105) 0.70 ± 1.08	(n=86) -0.40 ± 0.88	0.72 ± 1.15	<0.0001	0.06
Year 7	(n=36) 0.53 ± 0.84	(n=83) 0.90 ± 1.18	(n=60) -0.33 ± 0.95	1.00 ± 1.39	0.009	0.05
Year 10	(n=23) 0.83 ± 1.34	(n=53) 1.23 ± 1.38	(n=35) -0.43 ± 1.61	1.14 ±1.64	0.13	0.45
	Damage possibly attributable to corticosteroid use**					
Study start	0.22 ± 0.63	0.15 ± 0.59	0.06 ± 0.43	0.32 ± 0.61	0.13	0.36
Year 3	(n=54) 0.28 ± 0.68	(n=109) 0.24 ± 0.56	(n=107) 0 ± 0.66	0.37 ± 0.66	1.00	0.42
Year 5	(n=45) 0.33 ± 0.74	(n=105) 0.31 ± 0.68	(n=86) -0.03 ± 0.77	0.34 ± 0.62	0.68	0.94
Year 7	(n=36) 0.31 ± 0.62	(n=83) 0.43 ± 0.84	(n=60) -0.18 ± 0.85	0.29 ± 0.54	0.10	0.93
Year 10	(n=23) 0.26 ± 0.45	(n=53) 0.55 ± 1.03	(n=35) -0.37 ± 1.03	0.19 ± 0.48	0.04	0.56
	Corticosteroid-independent damage***					
Study start	0.09 ± 0.29	0.14 ± 0.34	-0.05 ± 0.31	0.18 ± 0.49	0.13	0.15
Year 3	(n=54) 0.11 ± 0.32	(n=109) 0.28 ± 0.56	(n=107) -0.18 ± 0.56	0.22 ± 0.51	0.002	0.12
Year 5	(n=45) 0.16 ± 0.37	(n=105) 0.35 ± 0.57	(n=86) -0.26 ± 0.64	0.25 ± 0.54	0.0003	0.26
Year 7	(n=36) 0.11 ± 0.32	(n=83) 0.37 ± 0.58	(n=60) -0.23 ± 0.59	0.22 ± 0.42	0.003	0.21
Year 10	(n=23) 0.17 ± 0.39	(n=53) 0.49 ± 0.70	(n=35) -0.34 ± 0.80	0.33 ± 0.48	0.02	0.19

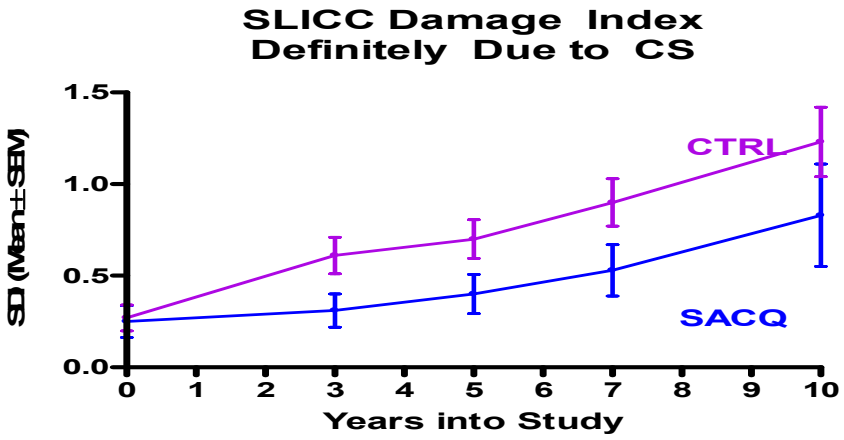
* Ocular or musculoskeletal damage
** Cardiovascular, peripheral vascular, diabetic, or neuropsychiatric damage
*** Renal, pulmonary, gastrointestinal, dermatologic, or gonadal damage and malignancy
¶ paired t-test
¶¶ unpaired t-test

Table 4-5: Renal damage and coronary artery disease (CAD) at five and ten years:

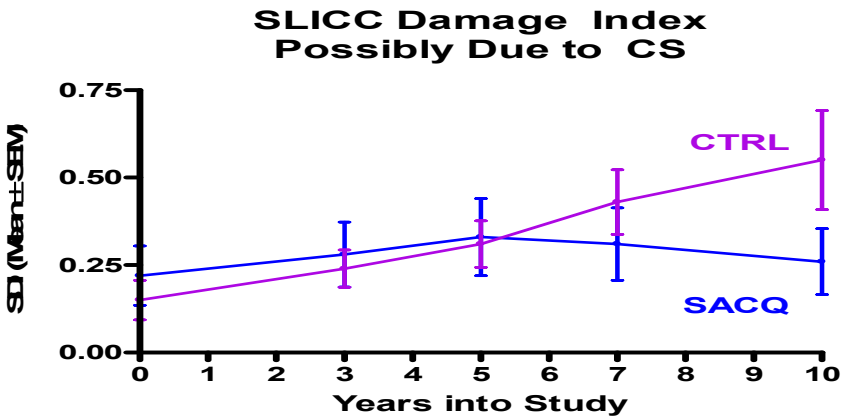
	Incidence	SACQ patients	SLE controls	p value
Renal damage	**Study start**	0 (0%)	13 (12.3%)	<0.0001
	5 years	1 (1.8%)	17 (15.5%)	0.0006
	10 years	2 (3.6%)	26 (23.6%)	<0.0001
CAD	**Study start**	2 (3.6%)	7 (6.4%)	0.32
	5 years	1 (1.8%)	6 (5.5%)	0.16
	10 years	1 (1.8%)	8 (7.3%)	0.06

Figure 4-1(a-c): SDI breakdown by corticosteroid (CS) effect

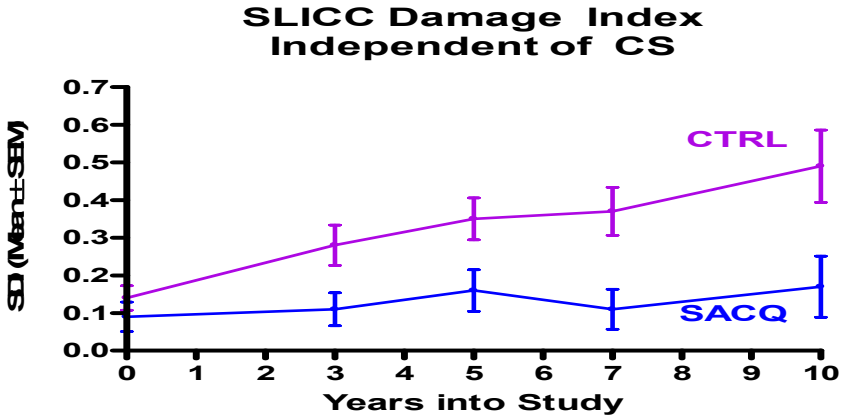
a)



b)



c)



Chapter 5: Do differences in anti-dsDNA and anti-chromatin antibody isotype predict flare among patients with serologically active clinically quiescent (SACQ) systemic lupus erythematosus (SLE)?

This work has been submitted for publication

Abstract

Purpose: SACQ SLE patients are clinically quiescent despite serologic activity. Studies suggest that anti-chromatin (-nucleosome) antibodies are more sensitive than anti-dsDNA to detect active SLE, and correlate with time to flare after a SACQ period. We investigate whether levels of anti-dsDNA and anti-chromatin isotypes, measured during a SACQ period, differed in SACQ patients who remained SACQ versus those who flared.

***Methods*:** Serum samples of SACQ patients were categorized by disease activity (SACQ vs flare). Levels of IgM, IgA, IgG, and IgG1-4 anti-dsDNA and anti-chromatin were measured by ELISA. SACQ was defined as ≥2 years with SLEDAI-2K=2 or 4 from serologic activity, during which patients could be taking antimalarials, but not corticosteroids or immunosuppressives. Flare was defined as clinical SLEDAI-2K ≥1 and/or treatment initiation. Nonparametric statistics were used, and generalized estimating equations were applied to account for multiple samples in the same patient.

***Results*:** Thirty-eight samples (from 23 patients) that corresponded to prolonged SACQ were analyzed. Fifteen of the 38 (39%) SACQ samples corresponded to patients whose SACQ period ended in flare. When the sample drawn latest in a SACQ period was analyzed there was no difference between anti-chromatin or anti-dsDNA isotype or IgG sub-class levels between patients who flared and those who remained SACQ. With all samples analyzed, anti-chromatin IgG2 and anti-dsDNA IgG were higher in SACQ patients who remained SACQ.

Conclusions: In this pilot study neither anti-chromatin nor anti-dsDNA isotype

or IgG sub-class levels were clearly predictive of flare in SACQ patients. Alternate biomarkers must be sought.

Introduction

Systemic lupus erythematosus (SLE) is a chronic autoimmune disease characterized by exacerbations and remissions in affected organ systems. In many patients with SLE, serum levels of anti-double stranded DNA antibodies (anti-dsDNA) and/or complement correlate with disease course, and can thus be of utility in predicting disease flare; however, in other patients, clinical and serologic courses are discordant(9). One such group of patients, termed serologically active clinically quiescent (SACQ), manifests persistent elevations in anti-dsDNA antibodies and/or hypocomplementemia in the absence of clinical manifestations of SLE, over a protracted period of time(57).

These patients present a clinical conundrum: how does the treating physician reconcile and manage such discordance? In SACQ patients, of whom nearly 60% ultimately flare(68), a serologic predictor of exacerbation would provide the treating physician, faced with the early signs and symptoms of flare, with another tool in the diagnostic armamentarium. Not only might severe flare be averted by early treatment, but the physician could be confident in the decision to follow a discordant patient, off all immunosuppressive medications – and thus free from their risks and side effects.

Thus for SACQ patients, in whom routine measurement of fluctuation in anti-dsDNA and/or complement levels is of no utility in monitoring disease activity(68), alternate biomarkers must be sought. It has been suggested that not all anti-dsDNA are equally pathogenic, with anti-dsDNA of high avidity, of IgG isotype, and complement-fixing IgG sub-class best correlating with disease activity and renal involvement(33,38-41,43-45,102). As a corollary, IgM isotype and non-complement-fixing IgG sub-class best correlate with disease quiescence. Furthermore, antibodies to chromatin (anti-chromatin), the native nuclear form of DNA and histone proteins (or its repeating element, the nucleosome), have been shown by some to be of higher sensitivity and specificity than anti-dsDNA for SLE disease activity, especially when the latter is not present, or proves discordant(50,51,53-56,135-137).

In this study we investigate the role of anti-dsDNA and anti-chromatin isotypes and IgG sub-classes in distinguishing between SACQ patients who ultimately flare and those who do not.

Methods

Patient selection*:* The University of Toronto Lupus Clinic at the Centre for Prognosis Studies in the Rheumatic Diseases, Toronto Western Hospital was established in 1970 to study clinical-laboratory correlations in SLE. All patients entered fulfill 4 or more of the 1971 or 1982 ACR classification criteria, or 3

criteria and a typical renal biopsy lesion of SLE. The Clinic's patients range from those with acutely active disease of variable manifestations to patients with inactive disease on maintenance therapy to patients in complete remission, off all therapy(90). All patients sign informed consents to allow their clinical, serologic and genetic material to be studied and reported.

SLE patients are followed with clinical and laboratory information collected using a standardized protocol at clinic visits, typically at 2 to 6-month intervals, which occur regardless of disease activity. Patients registered in the Lupus Clinic database between July 1970 and April 2008 with visits no more than 18 months apart were identified. SACQ lupus patients were selected from this population.

Definitions: **SACQ** was defined as at least a two-year period without clinical activity and with persistent serologic activity (SLE disease activity index 2000, SLEDAI-2K score = 2 or 4, from positive anti-dsDNA antibody and/or hypocomplementemia only, at each clinic visit). The patients could be taking antimalarials, but those on corticosteroids or immunosuppressive medications were excluded, ensuring the clinicians' impression of clinical quiescence.

Disease flare was defined by any increase in clinical SLEDAI-2K score at a clinic visit not accounted for by either hypocomplementemia or anti-dsDNA, or by the initiation of corticosteroid or immunosuppressive treatment.

***Laboratory analyses**:* Archived serum samples of patients with a prolonged SACQ period, stored at -80^{o} C, and which had not undergone a previous freeze-thaw cycle, were retrieved and sorted by disease activity: during a prolonged SACQ period or during disease flare. Serum levels of IgM, IgA, IgG, IgG1, IgG2, IgG3, and IgG4 anti-dsDNA and anti-chromatin antibodies were measured by ELISA. Briefly, H1-stripped, mainly mono- and dinucleosome, chromatin was prepared from the human cell line, MOLT4(138). ELISA plates (Immunolon 2HB, VWR) were coated overnight with dsDNA (40 µg/ml) or chromatin (8 µg/ml) diluted in PBS at 4^{o}C. Serum was diluted 1/100 for measurement of IgM, IgA, and IgG, or 1/50 for IgG1-4. Bound antibodies were detected using alkaline-phosphatase conjugated goat anti-human IgM, IgA, or IgG antibody (1:1000 dilution, all from Cedarlane) or biotin conjugated mouse anti-human IgG1-4 antibody (1:500 dilution, all from Cedarlane except IgG2 from Caltag) followed by detection with alkaline-phosphatase conjugated streptavidin (1:1000 dilution). Absorbance was read at 405 nm. Human sera with known anti-dsDNA or anti-nucleosome antibody levels were utilized to allow for inter-plate standardization, therefore the data are expressed as normalized OD values.

Statistical analysis: P-values were reported using Wilcoxon rank sum tests and generalized estimating equations (GEEs). The use of multiple samples from one patient was adjusted for through the use of a repeated measure model with an exchangeable correlation structure.

Results

Thirty-eight serum samples, from 23 patients, drawn during a prolonged SACQ period, were analyzed. Fifteen of the 38 (39%) samples corresponded to patients whose SACQ period eventually ended in flare. Patient demographics did not differ between those who remained SACQ and those who ultimately flared (Table 5-1). Among those who flared, disease activity manifested mainly as arthritis or mucocutaneous involvement; one patient had serositis, another hemolytic anemia necessitating corticosteroid use, and none had major organ manifestations.

Anti-chromatin and anti-dsDNA IgM and IgG levels among SACQ patients were not significantly lower than those seen in an unselected SLE cohort, and with the exception of IgM anti-chromatin (for which only a small number of controls were tested) that were significantly elevated as compared to healthy controls (Table 5-2).

When only the last sample drawn during a SACQ period was analyzed (9/23 (39%) of whom flared), there was no difference between anti-chromatin or anti-dsDNA isotype or IgG sub-class levels between patients who flared and those who remained SACQ (Table 5-3). When all samples were included using a GEE, anti-chromatin IgG2 and anti-dsDNA total IgG levels were significantly higher in SACQ patients who remained quiescent ($p<0.0001$ for both) (Table 5-4). There was no difference in anti-chromatin or anti-dsDNA IgM/IgG ratio in

those SACQ patients who ultimately flared versus those who did not (Tables 5-3 and 5-4).

While otherwise not attaining statistical significance after accounting for multiple comparisons, there appeared to be a trend toward higher anti-dsDNA and anti-chromatin immunoglobulin levels in patients who remained SACQ compared to those who ultimately flared. There was no correlation between the levels of anti-dsDNA or anti-chromatin immunoglobulin isotype or IgG sub-class and time to flare. The mean time to flare (or most recent SACQ visit) from last sample analyzed was 2.1 and 2.2 years in SACQ patients who remained SACQ and those who flared, respectively (ranges 1.0 – 3.8 years and 0.7 – 6.9 years, respectively).

There was no correlation between levels of autoantibodies and outcome after adjusting for length of time between sample and outcome dates (data not shown). A time to event analysis corroborated these negative findings.

There were only five patients in a convenience sample of patients in whom serologic samples were available both during SACQ periods and during flares. The nature of disease activity in flare in these five patients is outlined in Supplementary Table 5-1. Given the small sample size, these data were analyzed only qualitatively. (In the cases where > 1 sample was available for a disease state, the mean autoantibody level was recorded.) There did not appear

to be any trend in fluctuations in autoantibody levels between SACQ and flare in any of these patients (Supplementary Table 5-2).

Discussion

SACQ SLE patients represent a unique phenotype with clinical and serologic discordance. Previously, we have shown that among this unique patient subset, reflecting 6% of our SLE cohort, there are no clinical predictors of flare. Similarly, fluctuations in anti-dsDNA and/or complement levels in SACQ patients during a SACQ period were not predictive of disease flare(68). We have also learned that SLE-related organ damage does not accrue subclinically in this group during a prolonged SACQ period, supporting the practice of close clinical monitoring, without the use of prophylactic corticosteroid or immunosuppressive medications(23).

In this small pilot study we determine that predicting clinical outcomes by serologic changes remains an elusive goal among SACQ patients. In analyzing only the latest sample available during each patient's SACQ period, we found that neither anti-dsDNA nor anti-chromatin isotypes or IgG subclass differ significantly in SACQ patients who remain SACQ versus those who ultimately flare. However, in applying a GEE model, which adjusts for within-patient and between-patient variability, we found that anti-chromatin IgG2 and anti-dsDNA total IgG were significantly higher in patients who remained SACQ compared to those who eventually flared. We acknowledge the significant latency between

sample collection and time to flare in some patients as a significant limitation of this study: it appears, however, that those with the highest levels were no more likely to flare, adjusting for time to event.

The significant findings in these analyses are hypothesis-generating, and must be confirmed in studies with larger sample size. They diverge from the classic SLE paradigm, supported by numerous studies, which suggest higher levels of IgG, especially of the complement-fixing sub-classes IgG1 and IgG3, are predictive of active disease and that the IgM isotype is protective(40,44,139,140).

Previously, increased levels of IgG as compared to IgM anti-dsDNA antibodies have been shown to be associated with active disease. For instance, Kessel and colleagues found that the SLEDAI was increased in patients whose anti-dsDNA IgG titres were higher than anti-dsDNA IgM titres(44). Forger et al. studied anti-dsDNA IgG/IgM ratio in over 200 SLE patients, and found this ratio was a significant parameter distinguishing patients with and without nephritis(102). It has been theorized that IgM may be protective due to its ability to downregulate autoreactive B-cells, resulting in decreased pathogenic IgG production(44). Alternatively, IgM may act by binding circulating antigens, activating complement, and accelerating immune complex clearance. In support of this concept, administration of anti-dsDNA IgM to lupus-prone mice resulted in attenuated renal pathology and improved survival(140).

By contrast, we found a trend toward relatively higher IgG and lower IgM in SACQ patients who remained SACQ compared to those who flared (Tables 5-1 and 5-2). While these results did not achieve statistical significance, the consistency of this trend across both anti-chromatin and anti-dsDNA, and regardless of whether one or multiple samples were analyzed, suggests that "protective" serology is not the driver of SACQ status.

Anti-dsDNA IgG of high avidity is directly implicated in SLE activity(102). Other studies have emphasized IgG1 and IgG3's complement-fixing, pathogenic role as complement, in turn, promotes recruitment and activation of proinflammatory effector molecules driving SLE activity(40) (141). Bijl and others longitudinally analyzed IgG anti-dsDNA and anti-nucleohistone antibodies in SLE patients before and at the moment of renal or extrarenal relapse, and found IgG2 anti-nucleohistone and IgG1 anti-dsDNA were increased just prior to renal relapse(45). They theorized that both played a pathogenic role in lupus nephritis. To build further, Flierman and Daha hypothesized that lupus nephritis activity may be driven by interactions between anti-nucleosome antibody complexes desposited in the glomerular basement membrane and their recognition by C1q. They proposed that this C1q binding results in the creation of a neoepitope that is recognized by anti-C1q antibodies, further triggering the classical complement pathway(142). These theories place a rise in autoantibody levels and

subsequent complement activation in a central role in the etiology of disease flare.

By contrast, we found that anti-chromatin IgG2 and anti-dsDNA total IgG were significantly higher in SACQ patients who remained SACQ compared to those who flared. These findings are particularly striking in patients who, by definition, have evidence of complement activation and should thus be expected to manifest disease activity. They suggest that there is a fundamental difference in SACQ patients' response to the presence of (pathogenic) immune complexes and resultant hypocomplementemia, rather than their quiescence stemming from a dearth of antigen/antibody. Further longitudinal analysis, with larger sample size, would be required to determine whether this signature is unique to and predictive of SACQ patients.

Anti-nucleosome antibodies are among the first detected in the sera of patients with SLE. They are thought to be pathogenic, as evidenced by their presence in glomerular deposits and eluates of patients with lupus nephritis(50,143,144). In fact, it is only through epitope spreading that nucleosome-specific T cells stimulate B-cells to produce anti-dsDNA and anti-histone antibodies(50). By contrast, although anti-dsDNA is a hallmark of SLE, it has been found to be poorly immunogenic: in their native state, strands of dsDNA are always complexed to histones, and it is these complexes of chromatin, rather than dsDNA, itself, that are thought to be the drivers of the immune response(145).

Thus it follows that anti-chromatin (nucleosome) antibodies would be more sensitive and specific for SLE, as they are more closely linked to its pathophysiology.

There is general consensus that anti-nucleosome antibodies are both sensitive and highly specific for SLE diagnosis and disease activity(50-53). A meta-analysis by Bizzaro and colleagues determined that in selected studies anti-nucleosome – but not anti-dsDNA – antibodies were associated with disease activity ($p<0.0001$), although neither autoantibody correlated with kidney involvement(50). Suleiman and colleagues found anti-nucleosome antibodies 98% sensitive and 86% specific for detecting active SLE, versus 61% and 84% for anti-dsDNA sensitivity and specificity, respectively(53).

Several studies have investigated the role of anti-nucleosome antibodies in renal disease. Most found anti-nucleosome antibodies elevated in the setting of lupus nephritis(51,54,55), with some finding them of improved sensitivity compared to anti-dsDNA(51,56). However in this study we did not find that anti-chromatin was any more instructive than anti-dsDNA in predicting clinical outcome among SACQ patients. Since we have confirmed that renal involvement is no less likely to occur over the disease course prior to a SACQ period compared to non-SACQ patients, and that there were no differences in other organ-specific disease manifestations, this lack of benefit cannot be attributed to differences in SACQ patients' clinical features(68).

Ng and colleagues(67) studied the role of anti-nucleosome antibodies in “SACQ” patients, and, contrary to our study, found that their presence and titre was significantly correlated with time to first flare after a SACQ period. These patients, however, were defined quite differently than ours, as those with a BILAG score < 6, and without any mention of a requirement of successful weaning from corticosteroids and/or immunosuppressive medications. Thus, our patients represent a subset unique from those studied by Ng and colleagues’, whose patients had low – but not absent - disease activity, which may have merely been suppressed by ongoing treatment rather than reflecting true SACQ remission, as we had defined it. We did not find that fluctuations in anti-chromatin were instructive in predicting flare in our stringently-defined SACQ patients, further supporting the notion that SACQ status does not occur as a result of protective serology.

This was a small pilot study investigating the role of anti-dsDNA and anti-chromatin isotypes and IgG sub-classes in predicting flare among SACQ SLE patients. As such, the study was not powered to detect small differences between groups. To highlight this point, we performed a power calculation on the results obtained for the anti-dsDNA IgM/IgG ratio, and found that 110 patients per group would be required to achieve 80% power. As such, a multicentred approach would be required to generate the appropriate number of patients to adequately power such a study.

Conclusions

The comparable levels of antibodies we observed in patients with a sustained SACQ period compared to those who flare suggest that these patients' unique clinical phenotype stems from an altered response to immune complexes that warrants further investigation. In this patient cohort, alternate biomarkers must be sought to aid in clinical decision-making.

Table 5-1: Patient demographics

	Flare patients (n=9)	SACQ patients (n=14)	p value
Sex - female	9 (100%)	11 (78.6%)	0.25
Age at sample date	43.7 ± 15.3 years	47.7 ± 20.5 years	0.73
Disease duration at sample date	20.1 ± 14.4 years	13.7 ± 9.3 years	0.37
Steroids ever	5 (55.6%)	9 (64.3%)	1.00
Steroid - cumulative dose at sample date	19.5 ± 15.7 gm	11.1 ± 7.3 gm	0.37
Antimalarials ever	8 (88.9%)	11 (78.6%)	1.00
Immunosuppressives ever	1 (11.1%)	2 (14.3%)	1.00

Table 5-2: Comparison of IgM and IgG anti-chromatin and anti-dsDNA levels in SACQ patients with unselected SLE patients and healthy controls

	SACQ (n=23)	SLE control (n=21)	Healthy control (n=49)	p value SACQ vs. healthy controls
Anti-chromatin IgM	0.52 ± 0.67 (0 – 2.2)	0.39 ± 0.53 (0 – 1.76)	0.15 ± 0.02 (0.12 – 0.17) (n=3)	0.52
Anti-chromatin IgG	0.59 ± 0.57 (0.07 – 2.21)	0.40 ± 0.39 (0.04 – 1.44)	0.08 ± 0.04 (0.01 – 0.20)	< 0.0001
Anti-dsDNA IgM	0.38 ± 0.26 (0 – 0.94)	0.13 ± 0.07 (0 – 0.30)	0.02 ± 0.10 (0.07 – 0.49)	<0.0001
Anti-dsDNA IgG	0.40 ± 0.62 (0.03 – 3.04)	0.22 ± 0.19 (0 – 0.65)	0.10 ± 0.04 (0.04 – 0.28)	0.002

Table 5-3: Mean anti-chromatin and anti-dsDNA levels during SACQ in patients who flared versus those who remained SACQ (1 sample/pt)

	Anti-chromatin			Anti-dsDNA		
	Flare (n=9 patients)	**SACQ (n=14 patients)**	**Wilcoxon p value**	**Flare (n=9 patients)**	**SACQ (n=14 patients)**	**Wilcoxon p value**
IgA	0.077 ± 0.117	0.071 ± 0.085	0.93	0.032 ± 0.030	0.073 ± 0.087	0.34
IgM	0.664 ± 0.909	0.430 ± 0.480	0.83	0.434 ± 0.220	0.338 ± 0.287	0.12
IgG	0.423 ± 0.314	0.691 ± 0.682	0.60	0.297 ± 0.139	0.474 ± 0.788	0.48
IgG1	0.133 ± 0.112	0.153 ± 0.112	0.60	0.078 ± 0.065	0.141 ± 0.106	0.17
IgG2	0.031 ± 0.026	0.069 ± 0.089	0.56	0.026 ± 0.033	0.022 ± 0.023	0.78
IgG3	0.095 ± 0.080	0.075 ± 0.070	0.60	0.049 ± 0.054	0.058 ± 0.060	0.85
IgG4	0.132 ± 0.253	0.046 ± 0.110	0.50	0.046 ± 0.058	0.035 ± 0.054	0.66
IgM/IgG	1.26 ± 1.66	1.79 ± 2.87	0.60	1.46 ± 0.97	3.76 ± 7.73	1.00

Table 5-4: Mean anti-chromatin and anti-dsDNA levels during SACQ in patients who flared versus those who remained SACQ (all samples)

	Anti-chromatin			Anti-dsDNA		
	Flare (n=15 samples)	**SACQ (n=23 samples)**	**GEE p value**	**Flare (n=15 samples)**	**SACQ (n=23 samples)**	**GEE p value**
IgA	0.057 ± 0.097	0.063 ± 0.091	0.98	0.028 ± 0.025	0.081 ± 0.095	0.02
IgM	0.460 ± 0.758	0.328 ± 0.409	0.55	0.413 ± 0.281	0.267 ± 0.252	0.64
IgG	0.361 ± 0.277	0.835 ± 0.700	0.05	0.334 ± 0.176	0.510 ± 0.701	<0.0001
IgG1	0.122 ± 0.093	0.160 ± 0.122	0.40	0.083 ± 0.063	0.142 ± 0.120	0.03
IgG2	0.043 ± 0.035	0.099 ± 0.116	<0.0001	0.029 ± 0.032	0.020 ± 0.022	0.41
IgG3	0.089 ± 0.071	0.084 ± 0.063	0.71	0.049 ± 0.052	0.063 ± 0.068	0.48
IgG4	0.103 ± 0.202	0.037 ± 0.095	0.24	0.028 ± 0.049	0.040 ± 0.056	0.53
IgM/IgG	1.01 ± 1.35	1.17 ± 2.35	0.63	1.52 ± 1.15	2.44 ± 6.18	0.40

Supplementary Table 5-1: Clinical characteristics of flare in patients for whom both SACQ and flare samples were available

	Clinical characteristics	Anti-dsDNA	Low C3/C4
Patient 1	leukopenia	+	+
Patient 2	arthritis	+	+
Patient 3	rash	+	+
Patient 4	rash	+	+
Patient 5	alopecia, arthritis, leukopenia, rash	+	+

Supplementary Table 5-2: Comparison of autoantibody levels taken in the same patient during SACQ vs during flare

	Patient 1		Patient 2		Patient 3		Patient 4		Patient 5	
	SACQ	Flare	SACQ	Flare	SACQ	Flare	SACQ	Flare	SACQ	Flare
Anti-chromatin										
IgA	0.000	0.110	0.004	0.046	0.149	***0.130***	0.066	***0.058***	0.018	0.059
IgM	0.000	0.314	0.155	***0.000***	0.000	0.198	0.117	0.350	0.048	0.254
IgG	0.308	1.474	0.561	***0.279***	0.774	***0.723***	0.602	0.709	1.237	***1.094***
IgG1	0.152	***0.146***	0.108	***0.006***	0.206	0.320	0.202	***0.182***	0.117	0.180
IgG2	0.016	0.212	0.052	***0.000***	0.080	***0.054***	0.064	0.102	0.110	0.274
IgG3	0.087	0.128	0.037	0.090	0.005	0.180	0.159	0.169	0.108	0.161
IgG4	0.000	0.316	0.000	0.058	0.000	0.292	0.000	0.142	0.000	0.040
Anti-dsDNA										
IgA	0.004	0.065	0.055	***0.046***	0.022	0.058	0.030	0.048	0.044	0.083
IgM	0.421	0.436	0.264	***0.224***	0.000	0.139	0.762	***0.502***	0.084	0.230
IgG	0.206	0.613	0.185	***0.151***	0.317	***0.264***	0.536	***0.201***	0.357	0.750
IgG1	0.045	0.125	0.096	***0.048***	0.180	***0.041***	0.100	***0.068***	0.120	***0.070***
IgG2	0.014	0.032	0.008	***0.000***	0.000	***0.000***	0.517	***0.000***	0.026	***0.007***
IgG3	0.060	***0.010***	0.057	***0.000***	0.000	0.022	0.132	***0.000***	0.113	***0.016***
IgG4	0.060	***0.038***	0.008	0.066	0.147	***0.000***	0.000	0.095	0.081	***0.043***

*** *Bolded, italicized, underlined* cells indicate decreased level in flare state**

Chapter 6: The interferon-α signature in patients with serologically active clinically quiescent systemic lupus erythematosus

Abstract

Objectives: Clinical/serologic discordance may illuminate SLE pathophysiology: peripheral IFN-α production is blunted in some autoantibody-producing, clinically quiescent SLE mice despite abundant IFN-α-producing plasmacytoid dendritic cells (pDCs). SACQ patients, who, like these mice, exhibit clinical/serologic discordance, may provide unique insights. We thus measured 5 IFN-associated genes and IFN-associated cyto/chemokines in SACQ patients, compared to serologically and clinically active (SACA) and serologically and clinically quiescent (SQCQ) patients.

Methods: We defined SACQ and SQCQ as ≥2-year periods without clinical activity, with/without persistent serologic activity, respectively, by SLE Disease Activity Index 2000; antimalarials were permissible, corticosteroids/immunosuppressives were not. SACA was defined as disease activity compelling immunosuppression. Levels of *OAS1*, *IFIT1*, *MX1*, *LY6E* and *ISG15* was measured by *q*RT-PCR. A composite gene score was developed. Plasma cyto/chemokines were measured by Luminex panel. Non-parametric univariate and logistic regression analyses were conducted.

Results: Twenty-two, 27 and 43 SACQ, SQCQ and SACA patients, respectively, were analysed. There were no differences in gene expression, or in cyto/chemokine levels between SACQ and SQCQ. The SACQ IFN gene score was significantly lower than that of SACA (p=0.003). Levels of GM-CSF, IL-6, IL-10, IP-10, MCP-1 and TNF-α were significantly lower in SACQ than

SACA. Logistic regression analysis revealed that anti-La antibody positivity, and low levels of MCP-1 and *LY6E* were associated with SACQ status.

Conclusions*:* The SACQ interferon signature mirrors that of patients who are in complete remission. Anti-La antibody positivity and low levels of MCP-1 and *LY6E* were associated with SACQ status. The presence of this combination of factors may reinforce the clinical impression of disease quiescence in SACQ patients.

Introduction

Interferon-α (IFN-α) is a cytokine that, in health, is released in response to infection to assist in its appropriate containment. However when present in excess, or in the absence of an infectious stimulus, IFN-α can exert deleterious, proinflammatory effects on the host organism. Indeed, plasmacytoid dendritic cells (pDCs) produce copious IFN-α in the setting of active systemic lupus erythematosus (SLE), a prototypic autoimmune disease, through classic IFN-α activation by immune complexes: pDCs driven, in part, by unrelenting and inappropriate stimulation of Toll-like receptors (TLRs) 7 and 9 by self-nucleic acid immune complexes, such as anti-double-stranded DNA antibodies(71,74,77,146) stimulate the production of further proinflammatory cytokines and activation of immune effector cells, such as T-cells, B-cell, macrophages and natural killer cells, which then orchestrate the autoimmune barrage that characterizes SLE(71,146). This culminates in the further development of antibodies directed against self targets, classically nuclear elements such as anti-double-stranded DNA (anti-dsDNA), and the subsequent activation of complement(71,146). IFN-α is thus thought to play a prominent, causal role in the pathogenesis of SLE. This is perhaps most elegantly demonstrated in patients treated with IFN-α for malignancy or chronic infection who develop lupus-like illnesses, which promptly abate on IFN-α discontinuation(147-149).

TLR-independent pathways of IFN activation are also being elucidated(150,151). There is mounting evidence that these, too, may play a role in lupus

pathophysiology. For instance, neutrophils stimulate the production of IFN-α through stimulation by chromatin, a known lupus-associated autoantigen(151);

Thus interferon-α and interferon-related genes proximately drive the production of pathogenic autoantibodies and/or the activation of complement. Elevations in anti-dsDNA antibodies and/or hypocomplementemia, in turn, are often closely correlated with SLE disease activity, and are consequently included as the serologic components of the SLE Disease Activity Index 2000 (SLEDAI-2K), a well-validated tool for monitoring disease activity over time(152). A unique subset of SLE patients, however, evolves from active disease to durable clinical quiescence despite the presence of persistent elevations in anti-dsDNA antibodies and/or hypocomplementemia (the latter, in turn, imputing the persistence of immune complexes) (57). This phenotype, termed serologically active clinically quiescent (SACQ), which conservatively represents 6% of lupus patients in one cohort studied(68), is thus remarkable for its discordance between its pathogenic serology and concurrently benign clinical picture.

These patients present a management dilemma for the treating physician, who is left to reconcile serology suggestive of impending disease flare, on the one hand, with disease quiescence, on the other. While the physician strives to avert flare, this can only be accomplished at the expense of the significant toxicity and morbidity associated with the use of corticosteroids and/or immunosuppressive medications. In this case, then, the treatment may be worse than the disease,

especially since we know that, while 60% of SACQ patients ultimately flare, they do so only after a mean of 3 years of disease quiescence off corticosteroids and immunosuppressive medications(68). To date, attempts to predict which of these patients ultimately flare on the basis of clinical features, fluctuations in anti-dsDNA and/or complement levels, or on the basis of immunoglobulin isotypes and idiotypes have proven unsuccessful. In doing so, we have also shown that SACQ status does not evolve as a result of a dearth of immune complexes driving the IFN response(68,101). Furthermore, we have learned that disease-associated organ damage does not accrue subclinically during a SACQ period, thus abrogating the role for prophylactic treatment with corticosteroids or immunosuppressive medications(23).

In light of these challenges, a reliable biomarker of disease activity in SACQ patients would be a welcome tool in the physician's arsenal. Given their centrality in proinflammatory autoimmune activation, it is not surprising that IFN-α and IFN-regulated gene transcripts, or the so-called "interferon signature," have been the foci of considerable efforts to better track disease activity and elucidate pathogenesis(78-80,82-86,153-157). Similarly, fluctuations in IFN-associated cytokine and chemokine expression have been shown to correlate with SLE disease activity and specific organ manifestations, and thus they have been sought as potential biomarkers for this complex, classically relapsing-remitting disease(84,158-163). Such biomarkers would be of particular benefit in such

cases as SACQ when conventional biomarkers of disease activity, such as anti-dsDNA and hypocomplementemia, fail.

In search of a useful SACQ biomarker, we investigated the level of expression of five interferon-associated genes in SACQ patients, compared to SLE patients who are serologically and clinically quiescent (SQCQ), and patients who are serologically and clinically active (SACA). We then compared the concentrations of 19 interferon-associated cytokines and chemokines in SACQ, SQCQ and SACA patients.

Methods

Setting: The University of Toronto Lupus Clinic at the Centre for Prognosis Studies in the Rheumatic Diseases, Toronto Western Hospital was established in 1970 to study clinical-laboratory correlations in SLE. All patients entered fulfill 4 or more of the 1971 or 1982 ACR classification criteria, or 3 criteria and a typical biopsy lesion of SLE. The Lupus Clinic is a tertiary care facility affiliated with the University of Toronto. It also serves as a primary and secondary care facility in downtown Toronto. The Clinic's patients range from those with acutely active disease of variable manifestations to patients with inactive disease on maintenance therapy to patients in complete remission, off all therapy(90). All patients sign informed consents to allow their clinical, serologic and genetic material to be studied and reported.

Patient selection: SLE patients are followed with clinical and laboratory information collected using a standardized protocol at clinic visits, typically at 2 to 6-month intervals, which occur regardless of disease activity. Patients registered in the Lupus Clinic database between July 1970 and January 2012 with visits no more than 18 months apart were identified. SACQ, SQCQ, and SACA lupus patients were selected from this population.

Patient charts were screened prior to clinic visits, and those meeting criteria for SACQ and SQCQ (see below) were identified *a priori.* If they were deemed clinically quiescent by the treating clinician at that visit, study blood samples were drawn, as specified by protocol. Patients were ultimately included in the study once their serologic status at the time of the visit was confirmed and deemed congruent with their past clinical course (i.e., either SACQ or SQCQ). Clinically active patients were identified were identified by the treating rheumatologist at the time of visit, from whom study blood samples were drawn, and included in the study once serologic activity at the time of visit was confirmed. Alternately, some SACA samples were obtained from banked blood, with SACA status confirmed on the basis of chart/lab review at the visit coincident with sample date.

Definitions: SACQ was defined as at least a two-year period without clinical activity and with persistent serologic activity (SLE disease activity index 2000(152) (SLEDAI-2K) score = 2 or 4, from positive anti-dsDNA antibody and/or hypocomplementemia only, at each clinic visit). The patients could be taking

antimalarials, but those on corticosteroids or immunosuppressive medications were excluded ensuring the clinicians' impression of clinical quiescence.

SQCQ was defined as at least a two-year period without clinical and serologic activity (SLEDAI-2K score = 0). As in SACQ, the patients could be taking antimalarials, but those on corticosteroids or immunosuppressive medications were excluded ensuring the clinicians' impression of clinical quiescence.

SACA was defined as disease activity requiring the use of corticosteroids and/or immunosuppressive medications.

Organ damage was measured using the SLICC/ACR Damage Index(26,27).

Gene expression analyses:

As previously described (83), "total RNA was isolated from blood archived in PAXgene tubes using the PAXgene Blood RNA Kit (Qiagen, Basel, Switzerland) with the following modifications to improve RNA yield and quality: addition of RNAse inhibitor, an off-column DNAse I digestion and final ethanol precipitation. A first-strand complementary DNA was produced using a High Capacity cDNA Reverse Transcription Kit (Applied Biosystems, Foster City, California, USA). Quantitative real-time PCR amplification was performed using a custom TaqMan Low Density Array (Applied Biosystems) with genes printed in duplicate. Normalisation and quantification of the PCR signals was performed by

comparing the cycle threshold value of the gene of interest with GAPDH. Identification numbers for the five genes tested w56631_g1 (*IFIT1*), Hs00182073_m1 (*MX1*), Hs00192713_m1 (*ISG15*), Hs00158942_m1 (*LY6E*) and Hs00242943_m1 (*OAS1*)."

An IFN score, reflecting the sum of these five genes, was derived for each subject(80,83) and used in the analyses.

Cytokine and chemokine analyses: Plasma cytokine and chemokine concentrations were measureded using a 65-plex Luminex Cytokine panel (Eve Technologies, Calgary, Alberta, Canada). The following 19 cytokines, deemed most germane to our study, were selected *a priori* for analysis: GM-CSF, IFN-α, IFN-γ, IL-1α, IL-1β, IL-2, IL-6, IL-10, IL-12 (p40 and p70), IL-17, IL-21, IL-23, IP-10, MCP-1, MCP-2, RANTES, TNF-α, and TRAIL.

In some cases, values were extrapolated beyond standard range, meaning that the sample's signals were outside the curve, yet the mathematical formula of the curve was still able to calculate a concentration from the sample's signal for the given range. Values that were out of range below the curve's lower limit were assigned a value of 0.

Serological testing: Anti-dsDNA was measured by Farr assay, and C3 and C4 were measured by nephelometry in the hospital laboratory.

Statistical analyses: The Mann-Whitney U test was used for comparisons of gene and cyto/chemokine expression between SACQ and SACA, SACQ and SQCQ and SQCQ and SACA. Given multiple comparisons, a Bonferroni correction was applied to these analyses, and p values < 0.003 were considered significant(164). For demographic data, two-sample t-tests were used. For analysis of correlations between variables, a multivariate logistic regression model was built. Given the small sample size with considerable data spread, data were transformed to percentiles to maintain rank but decrease sensitivity to extreme values.

Results

Patient demographics:

Age at diagnosis did not differ between SACQ and SACA, or SACQ and SQCQ patients. SACQ patients were older (43.7 ± 13.7 vs 28.7 ± 9.4, p < 0.0001) and had longer disease duration (18.5 ± 12.5 vs 7.4 ± 7.1 years, p=0.0005) at study start than did SACA patients. SACQ and SACA patients presented similarly at first clinic visit, with no difference in SLEDAI-2K score. Damage accrual to study start, as measured by the SLICC/ACR damage index did not differ between the three groups, however this was not adjusted for disease duration. Anti-Ro (82% vs 46%, p=0.007) and anti-La (50% vs 13%, p=0.002) antibodies were significantly more prevalent in SACQ than in SACA patients (Table 6-1). Anti-

RNP antibodies were significantly more prevalent in SACA than in SACQ (74% vs 36%, p=0.004). There were no between-group differences in prevalence of anti-Smith, anti-Jo-1, ANCA, APLA, or LE cells.

There were no differences in SLE clinical manifestations between groups. There was a trend toward fewer SACQ patients using corticosteroids (68% vs 88%, p=0.09) and significantly fewer using immunosuppressive medications (32% vs 67%, p=0.006) over the course of their disease than did SACA patients. The majority of patients had used antimalarials over their disease course, with no difference in frequency between groups (74% in SQCQ, 82% in SACQ and 84% in SACA patients).

Gene expression:

Twenty-two, 27 and 43 SACQ, SQCQ and SACA patients were included in the gene expression analyses, respectively. The SACQ IFN score was significantly lower than that of SACA (p=0.003) (Figure 6-1). When the genes were studied individually, only *LY6E* expression was significantly lower in SACQ than in SACA patients (p=0.002) after applying the specified Bonferroni correction. Gene expression did not differ between SACQ and SQCQ patients. The IFN score remained consistent over time in the SACA or SQCQ groups, but significantly declined in SACQ patients (p = 0.02) (Figure 6-2).

We then grouped IFN score by autoantibody profile and found no difference in its level, for all SLE patients studied, between those with or without anti-Ro and/or anti-La antibodies, with or without anti-Sm and/or anti-RNP antibodies, and those with or without anti-dsDNA and/or hypocomplementemia. When subdivided by clinical status, the IFN signature was significantly higher in SACQ patients who were anti-Ro/anti-La antibody positive than those who were negative for these autoantibodies; still, the absolute values were consistent with those with SQCQ status, and lower than those with SACA status.

Cytokine and chemokine concentrations:

Twenty-five, 28 and 48 SACQ, SQCQ and SACA patients were included in the cytokine and chemokine concentration analyses, respectively. Of the 19 cytokines and chemokines analyzed, the concentrations of six were significantly lower in SACQ compared to SACA patients after Bonferroni correction was applied. These were GM-CSF, IL-6, IL-10, IP-10, MCP-1 and TNF-α (Figure 6-3). There was a trend toward a lower concentration of IFN-α in the SACQ group compared to SACA ($p=0.006$), however this did not meet the predetermined threshold for statistical significance for this data set. Of the cytokines and chemokines that significantly differed between SACQ and SACA groups, only IL-10 levels correlated with IFN score, overall, ($r=0.310$; $p=0.002$), and in SACQ patients, specifically ($r=0.535$; $p=0.01$).

Logistic regression analyses:

Guided by the findings of the univariate analyses, multivariable models were tested to determine associations with SACQ status through stepwise logistic regression. We determined that anti-La antibody positivity, and low levels of MCP-1 and *LY6E* were associated with SACQ status (Table 6-2).

Post hoc analyses:

We performed exploratory analyses on further IFN-associated cytokines. Of IL-15, MIP-1A, MCP-3, BCA-1 (CXCL13), and MIP-1B. Of these, only BCA-1 was significantly lower in SACQ than SACA patients ($p = 0.0002$).

To determine whether those SACQ patients with higher IFN signatures were more likely to flare, we performed a chart review of those with IFN signature in the upper quartile, over an average of 5 clinic visits and 1.5 years. All of the patients remained SACQ at their most recent clinic visit. There was no divergence in CRP values, or any historical documentation suggestive of acute infection that would provide an alternate explanation for transiently high IFN expression.

Discussion:

Beyond its centrality to SLE pathophysiology, there were two experiments that inspired our exploration of the significance of the IFN-α signature and associated cytokines and chemokines in SACQ SLE.

The first, by Kwok and colleagues, hypothesized that in SLE, persistent stimulation of TLR9 by self nucleic acid results in pDC dysfunction(88). They found that, contrary to prevailing theories of pathogenesis, the number of pDCs and quantity of peripheral IFN-α were decreased in SLE patients. In fact, they found that stimulation of SLE patients' peripheral blood mononuclear cells (PBMCs) with TLR9 ligands led to decreased IFN-α production compared to healthy controls. And yet, when healthy controls' PBMCs were exposed to SLE serum, the IFN-α response was robust; seemingly paradoxically the serum which generated the most robust response actually bore the lowest autologous IFN-α response. Kwok and colleagues then tested whether repeated TLR9 stimulation could lead to tolerance and resultant blunting of IFN-α response. Healthy control pDCs were incubated with TLR9 ligands, washed, and then restimulated after 0, 24 or 48 hours. They found the IFN-α response was blunted in the time 0 reexposure group, but became progressively more robust at 24 and 48 hours, supporting their theory.

The second experiment by Pau and colleagues similarly suggests a pathophysiologic link between decreased IFN-α production in the setting of continual TLR stimulation in a clinically quiescent murine model of SLE(87).

They produced a lupus-prone mouse with a phenotype resembling that of SACQ patients, with autoantibody positivity and low disease activity. These mice displayed marked pDC expansion and, in spite of this, decreased splenic IFNα gene expression, even following TLR stimulation in vitro. Pau and colleagues wondered if TLR tolerance, similar to that seen in Kwok et al's experiment, was the result of chronic exposure to nuclear antigen-containing immune complexes. Following repeated stimulation with a TLR ligand, they found markedly decreased IFNα secretion, suggesting that pDC expansion and increased autoantibody production need not be associated with increased IFNα production and a resultant active SLE phenotype.

We wondered how SACQ patients maintain their quiescent phenotype in spite of evidence of pathogenic immune activation in the form of anti-dsDNA positivity and/or hypocomplementemia. While the observations made in this study cannot directly substantiate the importance of any specific mechanism in SACQ, they are certainly supportive of altered response to typically disease-promoting stimuli. Specifically, we describe a patient cohort with robust pathogenic autoantibody generation with a concurrently blunted IFN signature, associated with low levels of proinflammatory cytokines and chemokines, resembling those of patients who are both serologically and clinically quiescent. Even the SACQ patients with the highest IFN scores did not subsequently flare after a mean 1.5 years of further SACQ status. This conflicts with studies which cite anti-dsDNA as an IFN-α inducer(165). Another unique finding in these patients was a

significant decline in the IFN signature over time, in spite of the persistent presence of autoantibodies (especially anti-dsDNA antibodies) and/or hypocomplementemia. This differs from patients who were SQCQ or SACA, in whom the IFN score remained stable. This finding, in SACQ patients only, supports a theory of tolerance with persistent TLR stimulation, and thus profers a mechanism for SACQ status, to be tested in future studies.

It is interesting to note that anti-Ro and anti-La antibodies were significantly more prevalent in SACQ than in SACA patients, although their presence across groups did not correlate with an increased IFN score. Among SACQ patients, however, anti-Ro and/or anti-La antibody positivity correlated with a higher IFN score, but with absolute value was comparable to SQCQ patients. These findings are divergent from other studies which had found that increased IFN-associated gene expression was actually associated with the presence of these autoantibodies(157,166,167), but perhaps consistent with the notion of TLR tolerance in SACQ patients. Thus one might speculate that anti-Ro and anti-La antibodies could be potential TLR tolerizers in SACQ patients. By contrast, and consistent with previous studies, anti-RNP antibodies were associated with SACA status, and thus with an elevated IFN signature(157,158). Another group's observation worthy of further pursuit was the finding of a significantly higher prevalence of anti-IFN-α antibodies in SACQ patients compared to non-SACQ patients(168), as this could be a potential mechanism for SACQ patients' clinical quiescence. Still, this would not explain all of our findings, such as lower

levels of other pro-inflammatory (but not IFN-associated) cytokines and chemokines.

We observed decreased levels of IFN-associated IP-10 and MCP-1 and associated proinflammatory IL-6, IL-10 and TNF-α, comparable to levels seen in patients who were both clinically and serologically quiescent. These cytokines have consistently been observed to be elevated in the setting of increased IFN gene expression and active disease states (158,169-173), thus their relative quiescence in SACQ compared to SACA patients is consistent with these patients' clinical phenotypes. Furthermore, that these cytokines and chemokines were all low in the setting of robust autoantibody positivity in SACQ lends further support for differences occurring proximally in SACQ patients' IFN-associated, proinflammatory cascade. We also observed significantly lower levels of GM-CSF in SACQ patients compared to SACA patients, which is of interest, considering its purportedly prominent role in TLR-independent activation of pDCs(174). Thus in SACQ patients it would appear that pDC activation, and subsequent release of IFN-mediated proinflammatory factors may be suppressed.

This is further supported by the significantly lower levels of IFN-associated gene expression that declined over time observed in SACQ, compared to SACA, patients, suggestive of progressive blunting of the IFN response in the presence of persistent autoantibody generation.

We sought to determine whether a composite associated with SACQ status could be determined, which could serve as a prediction tool for the clinician. In our model, we determined that low levels of MCP-1 and *LY6E* were associated with SACQ status, as was anti-La antibody positivity. Our alternate model similarly revealed a strong association with low levels of MCP-1 and SACQ status, as well as lack of anti-RNP antibodies and low levels of proinflammatory IL-10. The prominence of MCP-1 in both models supports its role as an important proinflammatory chemokine in SLE with the ability to serve as a predictor of active disease(158,161,170,173). Levels of *LY6E* have been shown to correlate with renal disease and SLE activity(82,83). Correlations between antibodies, IL-10 and active disease are cited in detail, above. While none of these markers are routinely measured in the clinical setting, there may be utility in their measurement as an adjunct to confirming SACQ status.

That disease duration and age were highly significant in the univariate analyses is not surprising, given the consistent observation that SLE disease activity wanes over time(2,3). We thus did not feel that these variables would contribute useful information to our multivariable model. The decision to omit these variables from the multivariable models was legitimated by analyses revealing that, within each SACQ, SQCQ and SACA group there was no correlation between age or disease duration and gene expression, cytokine or chemokine levels over time.

The primary goal of this study was hypothesis generation, and we feel that our findings have prompted new and researchable questions. We do, however, acknowledge an important limitation in our ignorance of the signficance of temporality of gene/cyto/chemoine fluctuations and resultant clinical manifestation. Since these fluctuations may be fleeting, highly variable, and likely interactive, and since the study of novel biomarkers in SLE clinical prediction is a fledgling science, there is much to be learned about the timing of sample retrieval relative to its anticipated/purported clinical effect. We thus may have inadvertently missed the window of opportunity to identify an important and defining biomarker in SACQ. This, of course can be borne out in future studies as more is learned in the field.

Conclusions:

The SACQ interferon signature and cytokine/chemokine profile closely resembles those of patients who are in complete remission. Anti-La antibody positivity and low levels of MCP-1 and *LY6E* were associated with SACQ status in this small pilot study. The presence of this combination of factors may serve to reinforce the clinical impression of disease quiescence in SACQ patients.

Table 6-1: Patient demographics*

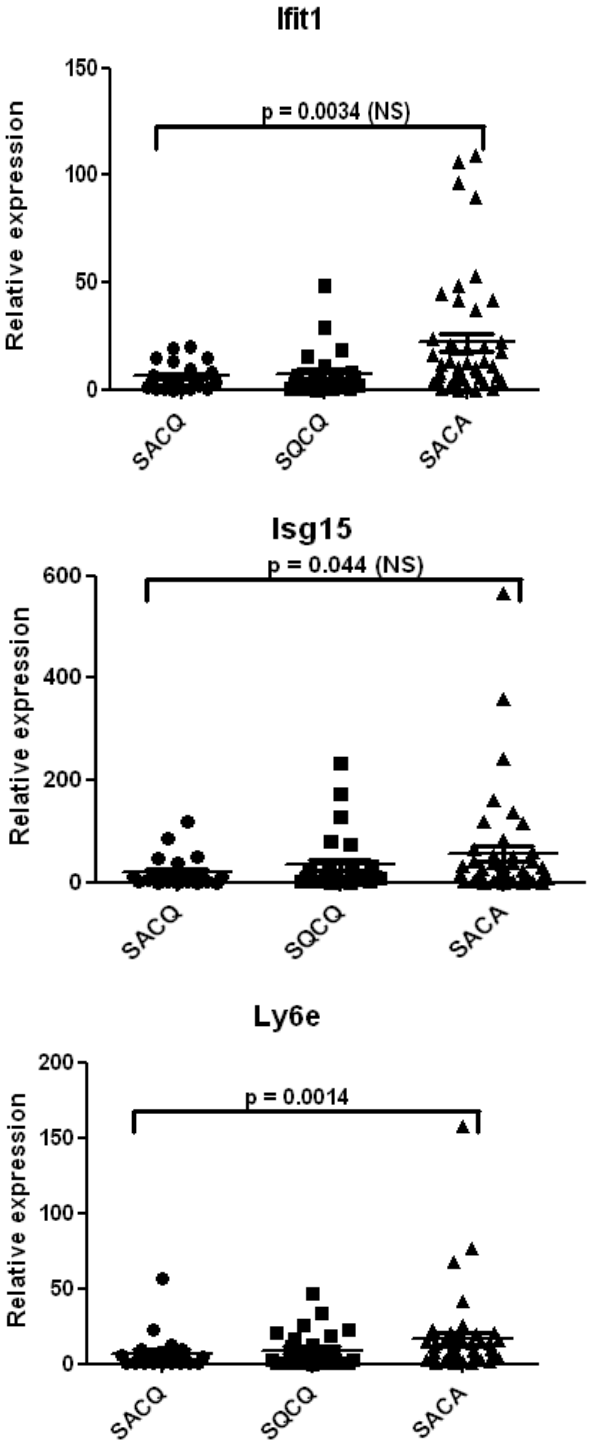
	SACQ (n=22)	SQCQ (n=27)	SACA (n=43)		p SACQ vs SQCQ	p SACQ vs SACA
Sex (% female)	19 (86.4)	26 (96.3)	37 (86.1)		0.31	1
Age at diagnosis	25.2±9.9	30.8±12.0	21.6±9.8		0.08	0.17
Age (study start)	43.7±13.7	55.0±12.3	28.7±9.4		0.004	<0.0001
Disease duration (study start)	18.5±12.5	24.2±11.1	7.12±7.0		0.1	0.0005
SLICC Damage Index (study start)	(n=22) 1.05±1.68	(n=27) 1.70±1.91	(n=39) 0.95±1.4		0.21	0.81
Adjusted mean SLEDAI (study start)	3.57±1.37	1.89±1.34	12.85±7.56		<0.0001	<0.0001
SLEDAI-2K at 1st visit	10.77±10.93	6.30±5.50	12.67±8.70		0.09	0.45
Race Caucasian Black Asian Other	 12 (54.6) 0 (0) 4 (18.2) 6 (27.3)	 22 (81.5) 4 (14.8) 1 (3.7) 0 (0)	 18 (41.9) 12 (27.9) 10 (23.3) 3 (7.0)		0.04 (Caucasian vs others)	0.33 (Caucasian vs others)
ANA (% positive)	22 (100)	25 (92.6)	40 (100)		0.49	N/A
Anti-Sm (% positive)	6 (27.3)	10 (37.0)	17 (43.6)		0.47	0.21
Anti-dsDNA (Farr) (% positive)	20 (90.9)	19 (70.4)	40 (93.0)		0.15	1
Anti-Ro (% positive)	18 (81.8)	16 (59.3)	18 (46.2)		0.09	0.007
Anti-La (% positive)	11 (50.0)	9 (33.3)	5 (12.8)		0.24	0.002
Anti-RNP (% positive)	8 (36.4)	7 (25.9)	29 (74.4)		0.43	0.004

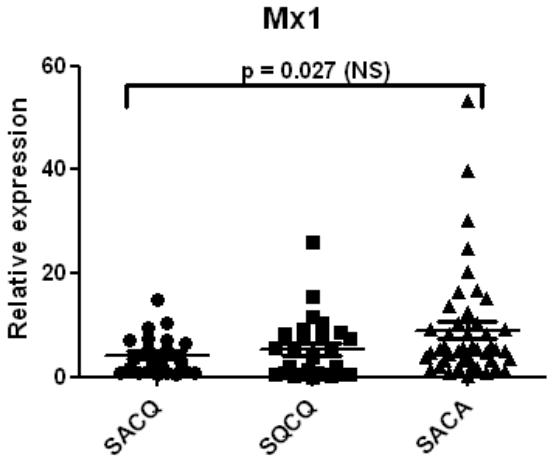
* All durations measured in years

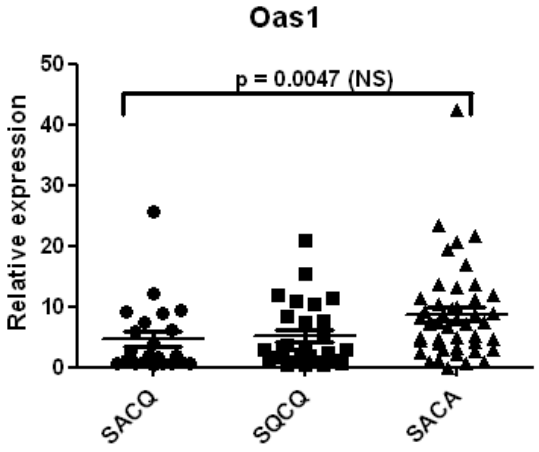
Table 6-2: Associations with SACQ status by logistic regression analysis

	OR (95% CI)	P value
Anti-La positivity	168.1 (4.85, >999)	0.005
MCP-1 high	0.87 (0.80, 0.96)	0.003
Ly6e high	0.90 (0.85, 0.97)	0.004

Figure 6-1: Relative gene expression




Mx1
p = 0.027 (NS)
Relative expression
60
40
20
0
SACQ
SQCQ
SACA


Oas1
p = 0.0047 (NS)
Relative expression
50
40
30
20
10
0
SACQ
SQCQ
SACA

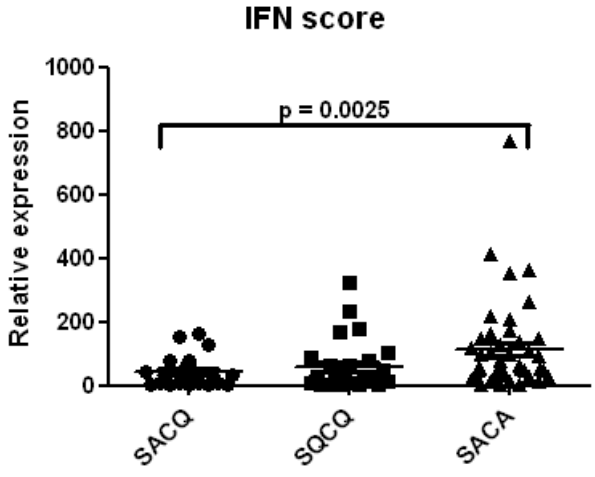

IFN score
p = 0.0025
Relative expression
1000
800
600
400
200
0
SACQ
SQCQ
SACA

Figure 6-2: IFN score over time

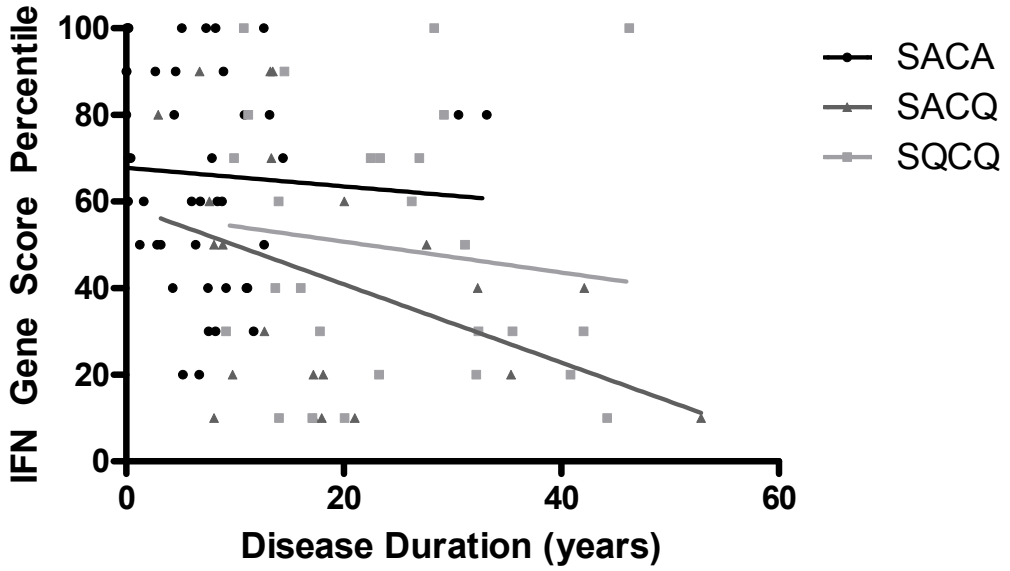
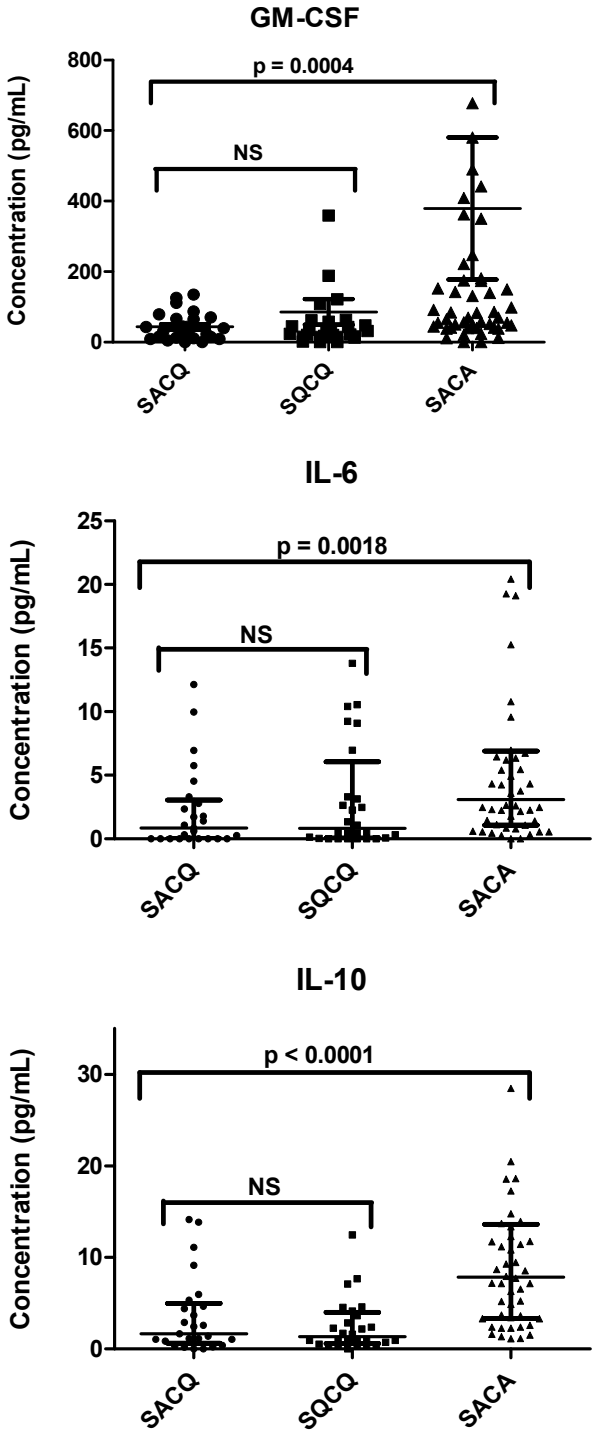
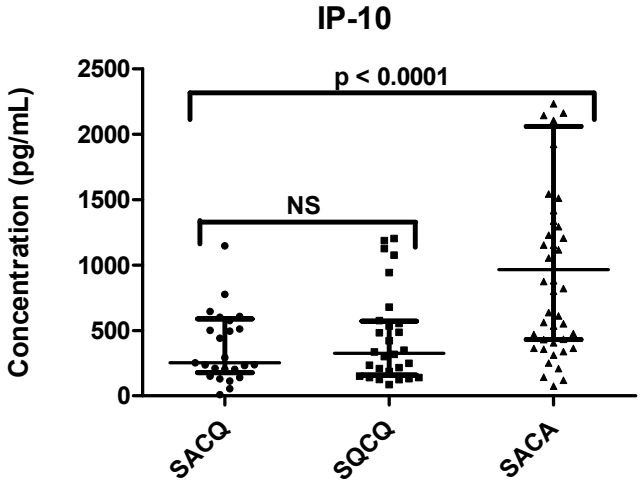
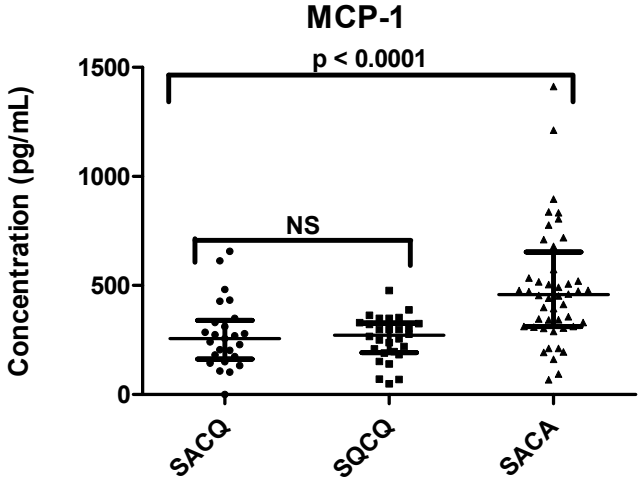
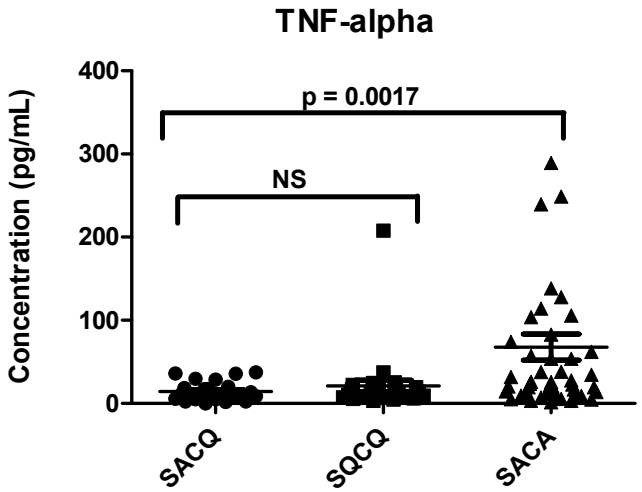


Figure 6-3: Cytokine and chemokine concentrations




IP-10
Concentration (pg/mL)
2500
2000
1500
1000
500
0
p < 0.0001
NS
SACQ
SQCQ
SACA


MCP-1
Concentration (pg/mL)
1500
1000
500
0
p < 0.0001
NS
SACQ
SQCQ
SACA


TNF-alpha
Concentration (pg/mL)
400
300
200
100
0
p = 0.0017
NS
SACQ
SQCQ
SACA

Chapter 7: General Discussion, Future Directions and Conclusions

Why study remission?

Remission, whether spontaneous, or induced by treatment, represents a state of reprieve from the signs and symptoms of an incurable disease, in this case, SLE. That remissions occur, rarely, in SLE is widely accepted(3,16,57,68,69). Why they occur, however, is not understood, but reflects a pathophysiologic shift which, if harnessed, could represent the "holy grail" of SLE treatment. Of particular interest are SACQ SLE patients, whose clinical remissions occur despite the presence of autoantibodies which are known to be pathogenic and/or hypocomplementemia, which reflects immune activation.

Describing remission

We took a stepwise approach to the study of remission and the patients in whom it occurs. First, in Chapter 3, we described prolonged remission, precisely and stringently defining it. We felt this was of paramount importance to ensure homogeneity of the cohort and thus avert some of the shortcomings of the studies with broadly defined remission states, described at length in Chapter 1. It was through carefully defining this group that we could confirm in our case-control analysis that those patients who had achieved prolonged remission differed phenotypically from matched SLE controls in terms of their disease activity in the years preceeding quiescence, and, as a result, the likelihood of requiring corticosteroids and immunosuppressive medications. Still, these differences were not sufficiently specific to identify patients who would evolve to

a remitted state *a priori*, and thus did not lend themselves to use in clinical prediction. We were the first to identify a unique subset of SLE patients, whose disease to date, with more than two decades of follow-up in some cases, has run an atypically monophasic course. These patients are of particular interest, as they have seemingly subverted the mechanism(s) which results in relapse in the vast majority of SLE patients.

We then subdivided these patients into those with and without pathogenic serologic activity, as the former group's clinical-serologic discordance presents a clinical conundrum, and likely stems from unique pathophysiologic mechanisms underlying their unique disease state. Finally, we separated those in a clinically quiescent state under the coverage of medications from those who achieved quiescence without, which allowed for the identification of patients who were in true remission versus those whose disease was merely suppressed by ongoing pharmacotherapy. These two groups are likely unique pathophysiologically, thus past studies which combined them may have inadvertently obfuscated important results which could be borne out by their segregation.

Confirming quiescence

Thus in Chapter 3 we carefully defined two related clinical phenotypes: patients in prolonged remission with or without concomitant pathogenic serology. At the bedside, patients belonging to each of these groups were identical, but their serologic profiles differed significantly. How could this serologic activity be so

closely linked to – or even be the driver of - disease activity in many SLE patients and not have any deleterious effect on these seemingly remitted patients? The next step, then, and the subject of Chapter 4, was to ensure that SACQ patients were, indeed, in a remitted state, and that their disease course was not insidiously progressive, falling under the radar of bedside clinical surveillance, but accruing damage over the long term. We thus compared SACQ patients to SQCQ and SACA patients and measured damage accrual over a decade. This study corroborated our initial clinical impression of complete disease quiescence during a SACQ period, with no evidence of disease-related damage accruing over time.

From these initial two chapters, we carefully confirmed the existence of a unique and contradictory cohort, whose discordance, at the patient level, poses a management dilemma for the physician, who must reconcile a clinically quiescent state, on the one hand, with serology suggestive of impending flare, on the other. On an academic level, the existence of these patients and their discrepant clinical/serologic profiles illuminates an important divergence from the classic SLE pathophysiologic paradigm of pathogenic immune complex deposition driving disease activity. Thus, at both a patient and population level, there are compelling reasons to discover predictors of flare in SACQ patients: at an individual level, severe relapse could be prevented by early treatment; for SLE patients, in general, studying SACQ may elucidate unique pathophysiologic mechanisms which could inform treatment targets.

The pursuit of novel biomarkers

Armed with the knowledge that conventional biomarkers were insufficient predictors of flare in SACQ patients, we sought novel methods to detect fluctuations in disease activity in this group. In Chapter 5 we explored the predictive utility of anti-dsDNA and anti-chromatin antibody isotypes. Their potential relevance as biomarkers in SACQ was founded upon numerous past studies citing their relative specificity over conventional measures in SLE, in general, and driven by a dearth of literature exploring their utility in remitted patients. Studying subsets of anti-dsDNA and related anti-chromatin antibodies was an intuitive step in confirming that the discordance of anti-dsDNA in SACQ patients, overall, did not belie an association with disease activity if one were to delve deeper and subdivide by isotype. While we found no association between levels of either of these autoantibody isotypes and disease activity in SACQ patients, and thus did not elucidate a novel biomarker, we felt that this negative study was critical as it suggested that differences in immunoglobulin expression did not appear to be the drivers of the SACQ phenotype.

We thus sought an alternate, biologically-plausible mechanism for SACQ clinical-serologic discordance. That these patients were clinically well, presence of pathogenic autoantibodies and evidence of immune activation notwithstanding, suggested that a central player in SLE-associated inflammation was being circumvented. We postulated that IFN-α production, driven by TLR stimulation

by self nucleic acids, was a candidate. Our hypothesis was bolstered by past studies revealing blunting of the IFN response in the face of persistent TLR stimulation, which could account for the discordance of SACQ patients. Our finding of SACQ patients' IFN signature, cytokines and chemokines mirroring those of SQCQ patients, and diverging significantly from SACA patients, supports the theory of IFN blunting in these patients, to be borne out in future studies. That anti-Ro and anti-La antibodies were significantly more prevalent in SACQ than SACA patients, and that they have been shown to induce IFN similarly supports this theory.

Major Contributions

SLE patients with clinical-serologic discordance present a clinical conundrum for the treating physician – which of these parameters should guide management? This has resulted in disparity in approach among clinicians, who can be separated into two factions: those guided by clinical quiescence(67,68), and those compelled to treat on the basis of serologic activity(65,66). While every physician acknowledges that the "art" of medicine allows for differences in approach between clinicians to the same patient, this dichotomy has very significant ramifications for the SACQ cohort. On the one hand, SLE flare is associated with significant morbidity, progressive damage accrual, and even mortality; ongoing, frivolous immunosuppression may be similarly deleterious, on the other. To our knowledge, this body of work represents the most exhaustive study of this unique patient subset.

A strength of this work is in its meticulous definition and prospective, fastidious surveillance of patients in remitted states. It thus provides compelling evidence to suggest that prolonged SACQ periods, spanning years, do occur and, as such, serologic activity in these patients should not be interpretted as a sign of impending flare. We have identified a novel subset of SLE patients with a monophasic clinical course, debunking the dogma that SLE will necessarily relapse. Furthermore, we have proven that remission in these patients is not undermined by subclinical, insidious damage accrual, which further reinforces our position that these patients require close clinical monitoring, without ongoing coverage with corticosteroids and/or immunosuppressive medications. The onus thus rests with the treating physician to identify patients exhibiting this discordance, and to manage them expectantly.

One might argue that the impact of this thesis is limited by the rarity of prolonged, discordant remission in SLE, and that its findings are thus only applicable to few: it is true that our most stringently-defined SACQ patients represent less than 2% of our SLE cohort. However, accepting the published estimated global SLE prevalence of 52 per 100,000 population(175), our findings, if adopted, could directly impact the management of at least 62,000 SACQ patients worldwide. This, in turn, could translate to considerable reduction in needless treatment-associated morbidity, at the individual level, and have concomitant health economic effects.

Generation of pathogenic autoantibodies is a hallmark of SLE, thought central to the disease process, and is thus a disease classification criterion(105,176). However, as outlined in Chapter 5, we found that, in SACQ patients, increased levels of anti-dsDNA and anti-chromatin immunoglobulin levels (which are classicaly considered pathogenic) were not predictive of flare, and were actually numerically (but not statistically significantly) higher in those patients who remained quiescent. This hypothesis-generating finding suggests that, in these patients, the effects of these pathogenic antibodies are counteracted, and has never been reported prior.

The study of differences in cytokine/chemokine and IFN-associated gene expression between SACQ, SQCQ and SACA patients contributes to a growing body of literature exploring the utility of novel biomarkers in SLE prognostication, at large. Most notable is our finding of suppression of the IFN signature in SACQ patients, despite an abundance of IFN-driving substrate in their robust autoantibody profile. This finding is surprising, and thus hypothesis-generating, with the potential for generalizability beyond SACQ patients. Specifically it implies altered function and/or blockade of the IFN pathway in these patients, with a resultant clinically quiescent phenotype. It directs future investigation elucidating the nature of this discrepancy, and may yield important insights leading to drugable targets.

Limitations

Prolonged remission is rare in SLE, and its prospective study is dependent upon clinical encounters with these quiescent patients which, on average, occur every six months in our clinic(68). A major issue which arises in studying uncommon states is that of being underpowered to detect subtle but important differences between groups. This can be addressed in future studies by lengthening our recruitment window or by collaboration with other centres.

While it is pragmatic to see patients who are clinically well less frequently, as we have in the case of SACQ patients, the latency between visits increases the likelihood that unmeasured/undocumented fluctuations in serologic and/or clinical status may have evolved. If this were to have occurred, these patients may have been misclassified as "SACQ," thus detracting from the homogeneity of the sample and, potentially, the findings unique to this group. Of course, meticulous attention was paid to a patient's intervisit history, which was of utmost importance in ascertaining continued clinical quiescence.

Given significant interethnic differences in SLE severity and phenotype, coupled with inconsistent approaches to its management globally, the generalizability of the findings of a single-centre study to SLE patients, at large, must be considered. Specifically, the impact of studying a predominantly Caucasian patient cohort such as ours must be balanced with the knowledge that Black, Asian and Hispanic patients, phenotypically, have more severe disease, which is

likely reflective of both genetic and environmental differences between groups(20). The generalizability of findings could be confirmed – or improved – by pursuing a multicentre, international study wherein our previous findings could be reproduced. This approach would also benefit our sample size and improve our statistical power.

Future Directions

Despite extensive research to date, SLE genetics and pathophysiology remain only partially elucidated. This may, in large part, be owing to its protean manifestations, near-limitless presentations and considerable variability in severity and phenotype across ethnicities. In fact, the clinical entity referred to as “SLE” may actually represent the clinical endpoint of several unique pathophysiologic pathways, unified by a propensity to autoimmunity. It is in this context that we study the exceptional patients who evolve to a state of prolonged clinical quiescence: if these patients can be compared to those whose disease runs a more typical course, the differences discovered between groups may illuminate important drivers of active disease. Furthermore, SACQ patients, themselves, present a management dilemma for the treating physician, who must balance their clinical-serologic discordance in bedside decision-making. Reliable, objective biomarkers of impending disease activity in this cohort would be of benefit in averting disease flare and, ultimately, treatment- and disease-associated organ damage.

Thus SACQ patients are of particular interest, as described at length in previous chapters, because of their surprising clinical-serologic discordance, which refutes the classic lupus paradigm of loss of self-tolerance resulting in production of pathogenic autoantibodies and resultant end-organ dysfunction and, ultimately, damage. Our work to date addresses the clinical correlates of patients who evolve to SACQ status, their damage accrual over time, and the early exploration of alternate biomarkers which may assist in clinical decision making. SACQ patients, however, represent a subset whose unique characteristics deserve further investigation which may lead to a heightened appreciation of SLE pathophysiology. There are thus several research questions which arise directly from our work to date:

Does SACQ clinical quiescence stem from a lack of autoantigen?

SACQ patients unequivocally produce copious, consistent autoantibodies. While anti-dsDNA antibodies are presumably complexed to nucleic acid autoantigen, one needs to confirm that their presence is not simply reflective of excess production of antibody. If this were the case, a state of clinical quiescence would be unsurprising, as it is immune complex deposition - and not the presence of excess immunoglobulin - which is thought to drive SLE.

To pursue this, we plan to isolate IgG from SACQ patients. If the IgG is complexed, the same IFN response should be generated when reassayed, as it

is driven by the presence of immune complexes. If antigen exists in SACQ serum, there should not be an excess of unbound anti-dsDNA antibodies.

Does the SACQ anti-dsDNA and anti-chromatin antibody profile evolve with disease activity within the same patient over time?

The study outlined in Chapter 5 relied upon the availability of archived serum samples of SACQ patients, drawn either during a SACQ period or during disease flare. Serial samples, drawn longitudinally, over both of these disease states for the same patient were not available. Fluctuations in levels and subtypes of immunoglobulins within individual SACQ patients may be instructive in determining patterns in and predictors of flare, both in this patient subset, and SLE patients, more generally. For example, and as described in Chapter 5, high levels of IgM may be protective in SLE(44,140). Anti-chromatin antibodies may be more sensitive and specific in flare prognostication in some SLE patients(50,53). Thus longitudinal analysis of these autoantibodies may elucidate useful biomarkers, which may be implemented in daily practice, for patients in whom fluctuations in anti-dsDNA and/or complement levels are not concordant with disease activity.

Does the SACQ IFN signature and cytokine/chemokine profile evolve with disease activity within the same patient over time?

The study outlined in Chapter 6 could be extended to include serial prospectively-collected samples in SACQ, SQCQ and SACA patients, in order to

determine relative stability or, alternately, potentially instructive fluctuations in the IFN signature and/or cytokine/chemokine profile over time. While, on the one hand, there is conflicting evidence in the literature pertaining to the stability of the IFN signature over time(82,83), we found that in SACQ patients, there was a significant decrease. We speculated that this might stem from progressive TLR tolerance, which could drive these patients' phenotype. It would be instructive to determine whether the IFN signature increases in the context of flare in SACQ patients.

Similarly, levels of proinflammatory cytokines and chemokines were significantly lower in clinically quiescent patients than in active patients in our study. We did not, however, have the opportunity to follow fluctuations therein over time. They may prove instructive in anticipating flare in SACQ patients, and would thus be of considerable clinical utility.

Is TLR tolerance the driver of the SACQ phenotype?

The study outlined in Chapter 6 describes the IFN signature, and proinflammatory cytokine and chemokine levels in SACQ patients compared to those who are SQCQ and SACA. The mechanism behind the significantly lower levels of these components in SACQ patients compared to SACA patients, despite active pathogenic serology in the former group has not yet been elucidated. A biologically plausible mechanism for SACQ patients' clinical phenotype is that of TLR tolerance, as outlined in Pau and colleagues and Kwok

and colleagues(87,88), but the theory has not been tested in this patient population.

A potential method for pursuing this theory would be to reproduce Kwok and colleagues' experiment in SACQ patients. First, we would stimulate SACQ patients' PBMCs with TLR9 ligands and measure the IFN response. If TLR tolerance were at the heart of the SACQ phenotype, we would expect a blunted IFN response. We would then expose healthy control PBMCs to SACQ SLE patients' serum to compare IFN response, which we would anticipate would be robust, given the abundance of TLR ligands in SACQ serum.

A corollary to this experiment would be the measurement of peripheral pDCs in SACQ patients compared to SLE controls to explore whether a dearth of IFN-producing cells is culprit for the discordant quiescence in SACQ patients. Further analyses of pDC function could then be explored.

Could personalized medicine hold the key to averting flare in SLE patients?

The pursuit of novel biomarkers is tempered by both fiscal constraints and by the limits of our understanding of the significance of derangements in an exponentially expanding catalogue of cytokines, chemokines and genes. If one were to hypothetically overcome the former issue, however, there might be significant knowledge to be derived from prospectively collecting and studying proinflammatory markers in fluctuating disease states, in all SLE patients

throughout their disease course. This pursuit may hold the key to identifying pathophysiologic drivers of disease activity leading to the clinical phenotype of SLE. Eventually, this could lead to successful anticipation and early treatment of flare, or perhaps illuminate drugable targets tailored to the individual patient.

Conclusions

Prolonged clinical remission is a rare and desirable outcome in SLE and, in some patients, may occur despite ongoing and robust serologic activity. These remissions are not associated with subclinical damage accrual and can thus be managed safely with close clinical observation, without prophylactic or anticipatory treatment with corticosteroids and/or immunosuppressive medications. The pathophysiologic milieu which permits for serologically discordant remission is associated with a low IFN signature and proinflammatory cytokine and chemokine expression, and does not appear to be driven by a dearth of immunoglobulins/autoantibodies. Future studies exploring pathways proximal to these biomarkers may elucidate important regulatory steps, which could hold the key to inducing remissions in SLE patients, and are thus worthy of pursuit.

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