PEER REVIEW HISTORY

BMJ Open publishes all reviews undertaken for accepted manuscripts. Reviewers are asked to complete a checklist review form (see an example) and are provided with free text boxes to elaborate on their assessment. These free text comments are reproduced below. Some articles will have been accepted based in part or entirely on reviews undertaken for other BMJ Group journals. These will be reproduced where possible.

ARTICLE DETAILS

TITLE (PROVISIONAL)	Associations between antinuclear antibody staining patterns and clinical features of systemic lupus erythematosus: analysis of a regional Swedish register
AUTHORS	Sjöwall, Christopher; Frodlund, Martina; Dahlström, Örjan; Kastbom, Alf; Skogh, Thomas

VERSION 1 - REVIEW

REVIEWER	Schur, Peter Brigham and Womens Hospital-Harvard Med School, Medicine
REVIEW RETURNED	09-Aug-2013

THE STUDY	1. When one is doing multiple comparisons and thus calculating p values, one needs to correct for type I error (that the differences could have been due to chance) by using a Bonferroni or equivalent correction, such as multiplying all the p values by the number of comparisons in which p values were shown to get a corrected p value--your tables show 26 p values--thus if you multiply all your p values by 26, the only noncorrected p values that will remain "significant" is a uncorrected p of <0.001 2. Some critical references omitted
RESULTS & CONCLUSIONS	There are a number of papers etc omitted as follows: 1. No correlation with ANA titer and clinical activity (DH Solomon et al Arth Rheum 2002; 47:434-44 2. Specific solid phase immunoassays have largely replaced immunodiffusion tests and looking at patterns: L Cook Clin Inmmunol Immunopath 1998; 88:211-20 3. Homogeneous=diffuse pattern best recognizes the DNA-histone complex (nucleosome) (and this Ab responsible for the LE cell phenomenon) 4. The peripheral (also called rim) pattern is due to anti-dsDNA, and is not seen well on Hep-2 cells, but is well seen on mouse/rat liver sections 5. The Danish group have described about 20 speckled patterns--major Ab associated with speckled include anti-Sm, RNP, Ro/SSA, La/SSB, Scl-70, centromere, PCNA 6. M Reichlin et al described Ro and La in SLE patients, and a few years later E Tan described SSA and SSB in patients with Sjogren's--years later they were convinced to exchange reagents, and lo and behold Ro=SSA and La=SSB--so no now we routinely say Ro/SSA and La/SSB 7. For a ACR sponsored review of the significance of anti-Sm and anti-RNP by immunodiffusion and ELISA (EIA) see: Arth & Rheum 2004; 51:1030-44--which gives better data than cited by the authoirs 8. For the ACR sponsored review of anti-DNA see ARTH RHEUM 2002; 47:546-555 9. Solid phase assays (ELISA, EIA) have virtually replaced

	immunodiffusion for detection of anti-DNA, ENA etc, in virtually all labs in the USA--more sensitive , easier to do, better QC, automated--see above #7 and #8 10. Jill Buyon has written more recent ref that you cited#28 11. For publication purposes we usually only include SLE patients who have 4 or more ACR criteria--having less just means they might have SLE (according to the ACR Classification criteria) 12. ANA patterns may vary over time and at different dilutions 13. Most USA labs use a titer of 1:40 as a cut off--SLE patients who were positive at 1:200 (or greater) who turn negative, may thus in the USA still be positive if they have a titer betyween 1:40 and 1:200 14. Under results you mention "immunologic disorder"--please define 15. Results: the frequency of renal disease and anti-DNA in your cohort is lower than most other published chorts--why--is there something unique about your group of patients--usually renal is at least 50%, and anti-DNA about 75% 16. Discussion: Friou not Holman and Kunkel described the IF ANA in SLE : with different cell substrates (liver, kidney, Hep-2, other cell lines) see different patterns 17. No association of photosensitivity with Ro: Paz ML, Gonzalez Maglio DH, Pino M, Ferrari A, Weill FS, Nasswetter G. Leoni J. Anti-ribonucleoprotein autoantibodies in patients with systemic autoimmune diseases. Relation with cutaneous photosensitivity. Clinical Rheumatology 2011; 30:209-16. Boey ML, Peebles CL, Tsay G, et al. Clinical and autoantibody correlations in Orientals with systemic lupus erythematosus. Ann Rheum Dis 1988; 47:918-23. Wang CL, OOI L, Wang F: Prevalence and clinical significance of antibodies to ribonucleoproteins in systemic lupus erythematosus in Malaysia. Br Soc Rheumatol 1996, 35:2; 129-32 Mok CC, Lau CS, Chan TM, Wong RWS: Clinical characteristics and outcome of southern Chinese males with systemic lupus erythematosus. Lupus 1999,8: 188-96. Christian N, Smikle MF, DeCeulaer K, Daniels L, Walravens MJ, Barton EN. Antinuclear antibodies and HLA class II alleles in Jamaican patients with systemic lupus erythematosus. West Indian Med J 2007; 56: 130-3.

REVIEWER	Catharina Eriksson, MD. Department of Clinical Immunology/Clinical Microbiology, Umeå University No competing interests.
REVIEW RETURNED	15-Aug-2013

THE STUDY	1)Page 8, Patients and methods: The authors describe their definition of cut-off level for IF-ANA as >95th percentile for female blood donors, and conclude that this has a very high sensitivity for SLE. There are indeed 24 men with SLE in the study. The authors has to better describe the cut-off level for and the sensitivity for SLE by the described criteria even for the male patients. 2)Page 9, Indirect IF microscopy: Line 38 - was the secondary antibody also for anti-dsDNA FITC conjugated gamma-chain specific anti-human ÍgG? Was the cut-off level for anti-dsDNA also refering to female blood donors? 3) Page 9, Anti-ENA antibodies: SSA should be defined with respect to Ro-60 and Ro-52, and if possible report these separately.

	4) Routine laboratory analyses, page 10: Lupus anticoagulant was analysed, but not anti-cardiolipin antibodes. Why? A comment should be added.
RESULTS & CONCLUSIONS	1)A couple of more questions should be of interest to know: did the disease duration, or the number of nuclear antibodies have any impact on the clinical feature? 2)Table 1b: If possible - anti-SSA/Ro could be presented in Ro60 and Ro52 separately. 3)In the Introduction the auhtors refer to a study showing that homogenous ANA pattern is the most common in healthy individuals. There are indeed several publications showing that fine speckled pattern is the most common in healthy persons. The authors has to comment on this subject.

REVIEWER	Kevin Nickerson, PhD Research Assistant Professor Department of Immunology University of Pittsburgh
REVIEW RETURNED	16-Aug-2013

- The reviewer completed the checklist but made no further comments.

REVIEWER	Shinu John, Ph. D Postdoctoral Fellow Department of Immunobiology/Laboratory Medicine Yale University School of Medicine USA
REVIEW RETURNED	19-Aug-2013

THE STUDY	Were any of the patients with discrepant staining patterns excluded? The authors have adequately described the status of the patients used in the study but do not comment on exclusion criteria, if any
RESULTS & CONCLUSIONS	Do the various drug treatments alter the ANA patterns? Since a significant number of patients were on some sort of disease modifying drug, the table should indicate the category of drug and the predominant ANA pattern. Where mixed ANA patterns are observed, sera should be further dluted and IF assys performed to assess the most dominant pattern Please include representative IF images of other ANA (oANA) pattern. Were these patients on any "disease modifying drug"? It would be helpful to have RF and Blys/BAFF titers of these patients.

VERSION 1 – AUTHOR RESPONSE

Reviewer: Peter H Schur MD, Brigham and Womens Hospital-Harvard Med School, Medicine

1. When one is doing multiple comparisons and thus calculating p values, one needs to correct for type I error (that the differences could have been due to chance) by using a Bonferroni or equivalent correction, such as multiplying all the p values by the number of comparisons in which p values were shown to get a corrected p value--your tables show 26 p values--thus if you multiply all your p values by 26, the only noncorrected p values that will remain "significant" is a uncorrected p of <0.001

Reply: We completely agree with the reviewer that results should be associated with appropriate correction (Bonferroni is one such very conservative correction) when interpreting the p-values in Table 1a and 1b. There are many different ways to apply such a correction, which should be based on how many independent sets of tests that have been performed and then attached with a proper family-wise significance level for each such set (e.g. Proschan MA, Waclawiw MA. Control Clin Trials 2000;21:527-39). We had a discussion in the author-group as to whether we should decide upon a division of tests into different sets, and also on a given correction method within each such set with a given family-wise significance level. Opinions vary greatly regarding how to divide the tests and regarding the method of choice when applying the correction. According to our opinion, it is most honest to report the exact p-values for each test, regardless of statistical significance. We also think that the exploratory nature of this study motivates reporting the exact results of each test (hopefully they will generate other studies to confirm or reject the findings presented in this manuscript). It should thereby be an easy task for every reader, if she or he prefers, to adjust the significance level by her/his own preferred method (for example by the Bonferroni method mentioned by the reviewer). However, judged from the reviewer's comment, we realize that this was not evident from the text in the first draft of the manuscript. We have therefore included a comment that hopefully clarifies this concern:
Page 12, line 8-9:
Removed sentence: P-values <0.05 were considered significant
Replaced with: For each statistical test, exact p-values (non-adjusted) are reported.
Page 17, line 12-13:
Inserted sentence: No corrections for multiple comparisons were made, but by reporting the exact p-values this can easily be done with a preferred method [43].
Page 24:
43. Proschan MA, Waclawiw MA. Practical guidelines for multiplicity adjustment in clinical trials. Control Clin Trials 2000;21:527-539.

Some critical references omitted. There are a number of papers etc. omitted as follows:
1. No correlation with ANA titer and clinical activity (DH Solomon et al Arth Rheum 2002; 47:434-44
Reply: This paper was indeed already cited in the manuscript draft (ref. 9). In the revised version we have also included information on the lack of association between ANA titer and clinical SLE activity (page 6, line 2-3).

2. Specific solid phase immunoassays have largely replaced immunodiffusion tests and looking at patterns: L Cook Clin Immunol Immunopath 1998; 88:211-20
Reply: We are aware of this, but cannot see the implications for our study, where two antigen-specific methods were used: double radial immunodiffusion (DRID) and/or line blot. Contrasting to other methods, DRID identifies a subgroup of antigen-precipitating antibodies, which in some in-stances have higher diagnostic specificity (albeit lower sensitivity) than ELISA and other solid-phase assays. We (and other Swedish laboratories) still claim that total replacement of DRID by solid phase assays is not advisable. We advocate that some anti-ENA specificities captured by ELISA should be confirmed with 'gold standard' methods such as immunodiffusion. We and others have discussed this matter in more detail elsewhere (Dahle C, et al. J Autoimmun 2004;22:241-48).

3. Homogeneous=diffuse pattern best recognizes the DNA-histone complex (nucleosome) (and this Ab responsible for the LE cell phenomenon)
Reply: We cannot see that this remark implies additional work or revision of the manuscript. Of course, we are well aware of the fact that classical homogenous nuclear IF-staining reflects occurrence of antibodies targeting DNA-histone complexes or distinct epitopes on dsDNA or histone proteins alone, but in some cases also to other DNA-associated proteins (see also our response to point 16).

4. The peripheral (also called rim) pattern is due to anti-dsDNA, and is not seen well on Hep-2 cells, but is well seen on mouse/rat liver sections
Reply: We are also aware of the ”rim” (or rim-homogenous) hepatocyte nuclear staining pattern reflecting anti-dsDNA on cryostat sections of rat and mouse liver. However, as whole fixed HEp2 cells have replaced rat liver cryostat sections for routine purposes, we do not think that the rim pattern is a matter to discuss in the present study.

5. The Danish group have described about 20 speckled patterns--major Ab associated with speckled include anti-Sm, RNP, Ro/SSA, La/SSB, Scl-70, centromere, PCNA
Reply: Indeed, a multitude of speckled ANA patterns (as well as variants of nucleolar, nuclear dot, and nuclear membrane staining patterns) have been described. In the present study we found it adequate to concentrate on four main patterns:
(1) homogenous/nucleosomal, (2) speckled/nucleoplasmic, (3) mixed homogenous/speckled, and (4) nucleolar. In addition, we describe two small pattern groups (centromeric and 'other ANA') that were not considered large enough for statistical comparisons.

6. M Reichlin et al described Ro and La in SLE patients, and a few years later E Tan described SSA and SSB in patients with Sjogren's--years later they were convinced to exchange reagents, and lo and behold Ro=SSA and La=SSB--so no now we routinely say Ro/SSA and La/SSB
Reply: In accordance with the reviewer’s suggestion, we have changed the nomenclature to Ro/SSA and La/SSB all through the manuscript.

7. For a ACR sponsored review of the significance of anti-Sm and anti-RNP by immunodiffusion and ELISA (EIA) see: Arth & Rheum 2004; 51:1030-44--which gives better data than cited by the authors
Reply: This paper has been added to the reference list.

8. For the ACR sponsored review of anti-DNA see ARTH RHEUM 2002; 47:546-555
Reply: This paper has been added to the reference list.

9. Solid phase assays (ELISA, EIA) have virtually replaced immunodiffusion for detection of anti-DNA, ENA etc, in virtually all labs in the USA--more sensitive, easier to do, better QC, automated--see above #7 and #8
Reply: Please see our response under point 2.

10. Jill Buyon has written more recent ref that you cited#28
Reply: This reference has been replaced by a more recent publication by Dr Buyon.

11. For publication purposes we usually only include SLE patients who have 4 or more ACR criteria--having less just means they might have SLE (according to the ACR Classification criteria)
Reply: The 1982 American College of Rheumatology criteria for SLE (ACR-82) were not intended as diagnostic criteria, but as criteria for the classification of SLE. This means that the clinician may well diagnose a patient with SLE, although the ACR-82 criteria are not met. Michelle Petri and co-workers in the Systemic Lupus International Collaborating Clinics (SLICC) group recently demonstrated that the ACR-82 criteria have a low sensitivity, but a rather high specificity, for SLE (Arthritis Rheum 2012;64:2677-86).
The present study was done to reflect SLE in a real life clinical setting, where 20% of the SLE patients did not fulfill ≥4/11 the ACR-82 classification criteria – NB the presence of anti-phospholipid antibodies (aPL) analyzed by ELISA or as a positive “lupus anticoagulant” test are not included in the “immunologic disorder” criterion according to the validated ACR-82 criteria set. Nevertheless, aPL is often applied as a classification criterion with reference to the non-validated update of ACR-82 suggested by Dr Hochberg in a short letter to Arthritis & Rheumatism in 1997 (Hochberg MC. Arthritis Rheum 1997;40:1725). In the revised version of the manuscript, we clearly state that 31 of the 44

(70%) “Fries patients” had positive aPL tests and would thus have been classified as SLE according to the “1997 update”. Nevertheless, we have refrained from applying the 1997 update, and chosen to rely on the validated ACR-82 classification criteria. In addition, we respectfully wish to stick to the “Fries’ criteria”, which we (and others, e.g. Vikerfors A, et al. Rheumatology 2013;52:501-9) find helpful as a “clinical check-up” for the clinical diagnosis of SLE.

12. ANA patterns may vary over time and at different dilutions
Reply: We completely agree with this comment. As mentioned in the Discussion, we found in a previous investigation based on South Swedish SLE patients who had all been judged IF-ANA positive at the time-point of diagnosis that a considerable proportion (24%) lost their ANA positivity over time (Sjöwall C, et al. J Rheumatol 2008;35:1994-2000). This finding is also in line with the results from a recent clinical trial for belimumab in SLE (Wallace DJ, et al. Arthritis Rheum 2009;61:1168-78). However, the present study had a cross-sectional design and aimed to compare the IF-ANA staining patterns from the time-point of SLE onset with clinical and laboratory features (see Patients & Methods, page 10).

13. Most USA labs use a titer of 1:40 as a cut off--SLE patients who were positive at 1:200 (or greater) who turn negative, may thus in the USA still be positive if they have a titer between 1:40 and 1:200
Reply: According to the 1982 ACR criteria, a positive ANA test is defined as an abnormal titer of ANA judged by immunofluorescence microscopy (or equivalent method). At Swedish laboratories accredited by SWEDAC for ANA diagnostics (ISO 15189), an abnormal ANA titer corresponds to ≥95th percentile among healthy female blood donors, meaning that up to 5% healthy women may be ANA-positive. The actual corresponding cut-off titer varies between laboratories depending on the microscope equipment including light source, filter combinations, lens, source and dilution of the fluorochrome-labelled secondary antibodies, etc! As stated in the Methods section, the cut-off titer (≥95th percentile) for positive IF-ANA at our laboratory was 1:200, whereas at a serum dilution of 1:40, 45% of both healthy women and men have a “positive” IF-ANA test (Sjöwall C, et al. J Rheumatol 2008;35:1994-2000). Thus, a cut-off titer of 1:40 would be absurd in laboratories with modern equipment!

14. Under results you mention "immunologic disorder"--please define
Reply: In the Results section of the revised version of the manuscript it has been clarified that 'Immunologic disorder' refers to the definition of the 10th criterion according to the 1982 ACR SLE clas-sification criteria (Tan E, et al. Arthritis Rheum 1982;25:1271-77). Thus, 'immunologic disorder' is met by at least one of the following:
a) Positive LE cell preparation
OR
b) Anti-DNA: antibody to native DNA in abnormal titer
OR
c) Anti-Sm: presence of antibody to Sm nuclear antigen
OR
d) False positive serologic test for syphilis known to be positive for at least 6 months and con-firmed by Treponema pallidum immobilization or fluorescent treponemal antibody absorption test.

15. Results: the frequency of renal disease and anti-DNA in your cohort is lower than most other published cohorts--why--is there something unique about your group of patients--usually renal is at least 50%, and anti-DNA about 75%
Reply: One plausible explanation for this is that ethnicity is strongly linked to the risk of nephritis (Hopkinson ND, et al. Ann Rheum Dis 2000;59:116-19) with a lower frequency of renal involvement in white population. Caucasians constitute 90-95% of patients in Swedish SLE cohorts (93% in this study), and the frequencies of nephritis and anti-dsDNA antibodies do not differ much between Swedish SLE centers (unpublished data from Linköping, Stockholm, Lund, Uppsala and Umeå). For ex-

ample, the percentage of newly diagnosed SLE patients with renal involvement in the late 1980's was less than 20% in southern Sweden (Ståhl-Hallengren C, et al. J Rheumatol 2000;27:685-91).
Another likely explanation is the selection of patients. In our region, the Linköping rheumatology unit is the only clinic that diagnoses and treats SLE patients. Thus, we can conclude that about 95% of the expected SLE cases in the catchment area of Linköping and ≥98% of all known SLE cases were included in this study. Consequently, this means that our clinic takes care of the whole spectrum of SLE – from uncomplicated cases with quiescent disease and skin/joint involvement to severely ill individuals with full-blown multisystem disease.
The slightly lower frequency of anti-dsDNA antibody positive individuals observed in our cohort is probably related to the choice of method. The Clinical immunology unit at our hospital has (so far) refrained from the use of other methods for anti-dsDNA antibody detection than the Crithidia test (mainly because of its high specificity for SLE compared to solid-phase assays). In addition, gamma-chain specific FITC-conjugate is always used in order to only detect IgG antibodies (although not demanded by the ACR-82 criteria!).

16. Discussion: Friou not Holman and Kunkel described the IF ANA in SLE : with different cell sub-strates (liver, kidney, Hep-2, other cell lines) see different patterns
Reply: As a matter of fact, in 1957 Halsted Holman & Henry Kunkel published a beautiful experimen-tal study where they described that the "LE-cell" phenomenon reflected antinuclear antibodies giving rise to a homogenous nuclear staining pattern of phagocytized ANA-opsonized leukocyte nuclei. They also showed that the autoantibodies had affinity for a nucleoprotein associated with DNA (Hol-man HR, Kunkel HG. Science 1957;126:162-63). Although not representing an orthodox means of ANA-diagnostics, it is undoubtedly an ingenious example of IF-microscopical ANA identification.

17. No association of photosensitivity with Ro:
Paz ML, Gonzalez Maglio DH, Pino M, Ferrari A, Weill FS, Nasswetter G. Leoni J. Anti-ribonucleoprotein autoantibodies in patients with systemic autoimmune diseases. Relation with cuta-neous photosensitivity. Clinical Rheumatology 2011; 30:209-16.
Boey ML, Peebles CL, Tsay G, et al. Clinical and autoantibody correlations in Orientals with systemic lupus erythematosus. Ann Rheum Dis 1988; 47:918-23.
Wang CL, OOI L, Wang F: Prevalence and clinical significance of antibodies to ribonucleoproteins in systemic lupus erythematosus in Malaysia. Br Soc Rheumatol 1996, 35:2; 129-32
Mok CC, Lau CS, Chan TM, Wong RWS: Clinical characteristics and outcome of southern Chinese males with systemic lupus erythematosus. Lupus 1999,8: 188-96.
Christian N, Smikle MF, DeCeulaer K, Daniels L, Walravens MJ, Barton EN. Antinuclear antibodies and HLA class II alleles in Jamaican patients with systemic lupus erythematosus. West Indian Med J 2007; 56: 130-3.
Reply: We agree with the reviewer that this question may be controversial. However, when we scru-tinized these papers lack of association between photosensitivity and anti-Ro/SSA was evident only in 3 cases (Paz ML, et al. Clin Rheumatol 2011; Boey ML, et al. Ann Rheum Dis 1988; Wang CL, et al. Br J Rheumatol 1996). Thus, these papers were included in the reference list along with the fol-lowing paper: Sutej PG, et al. Photosensitivity and anti-Ro (SS-A) antibodies in black patients with systemic lupus erythematosus (SLE). Br J Rheumatol 1989;28:321-324.

Reviewer: Catharina Eriksson MD, Department of Clinical Immunology/Clinical Microbiology, Umeå University, S-901 87 Umeå, Sweden.

1) Page 8, Patients and methods: The authors describe their definition of cut-off level for IF-ANA as >95th percentile for female blood donors, and conclude that this has a very high sensitivity for SLE. There are indeed 24 men with SLE in the study. The authors have to better describe the cut-off level for and the sensitivity for SLE by the described criteria even for the male patients.

Reply: This remark is well taken. However, as mentioned in Patients & Methods (page 10) "IF-ANA staining patterns, anti-ENA reactivity and dsDNA antibodies were analyzed on a routine basis at the Clinical immunology laboratory, Linköping university hospital and were extracted from medical records. In many patients IF-ANA analysis was performed at several occasions over time, but discrepant staining patterns were achieved in less than 5% of these cases. Herein, IF-ANA staining pattern from the time-point most adjacent to SLE onset was used for comparisons with clinical and labora-tory features". Although there is indeed support for the use of different cut-offs for men and women, it is not realistic to perform this on a routine basis. In addition, the vast majority of the SLE cases had ANA titers considerably above 1:200 including all of the 24 men. All three of the ANA negative cases were women.

2) Page 9, Indirect IF microscopy: Line 38 - was the secondary antibody also for anti-dsDNA FITC conjugated gamma-chain specific anti-human IgG? Was the cut-off level for anti-dsDNA also referring to female blood donors?
Reply: The secondary FITC-conjugated antibody used in the anti-dsDNA antibody assay was indeed also gamma-chain specific (information now provided in the revised version). The cut-off level for anti-dsDNA referred to 50 female and 50 male healthy blood donors (none of them anti-dsDNA anti-body positive).

3) Page 9, Anti-ENA antibodies: SSA should be defined with respect to Ro-60 and Ro-52, and if possible report these separately.
Reply: Since this study is based on historical data extracted from medical records (see reply to first comment) from the time-point of SLE onset, complete anti-ENA antibody profiles were unfortunately not available in a majority of the patients.

4) Routine laboratory analyses, page 10: Lupus anticoagulant was analysed, but not anti-cardiolipin antibodies. Why? A comment should be added.
Reply: Again, the study is based on historical IF-ANA data from the time-point of SLE onset. While the lupus anticoagulant test (dilute Russell's viper venom test) has been very stable over the years, this has unfortunately not been the case with the anti-cardiolipin antibody assay. In fact, since the 1990's several different assays with different cut-offs have been used at the Clinical immunology unit. Samples that previously were judged as low anti-cardiolipin antibody positive are now negative with the present automated method. Thus, good reproducibility was not reassured with the anti-cardiolipin assay and this is the reason why we chose to exclude these data.

1) A couple of more questions should be of interest to know: did the disease duration, or the number of nuclear antibodies have any impact on the clinical feature?
Reply: Since this study is based on historical autoantibody data from the time-point of SLE onset, it does not make sense to compare the antibody patterns/nuclear antibodies with disease duration. However, as suggested by the reviewer, we made additional analyses of the number of nuclear antibodies in comparison with IF-ANA patterns (shown in table below). The maximum number of concomitant anti-nuclear antibody fine-specificities recorded was 5 (anti-dsDNA, anti-Sm, anti-Ro/SSA, Anti-La/SSB and anti-snRNP).

	0	1	2	3	4
H	32	52	24	11	0
S	11	21	11	4	2
N	8	7	2	1	1
H/S	6	6	7	5	0

Patients with 2, 3 or 4 ANA-fine-specificities (none had 5!) were put in one group (three groups were composed; 0, 1 or >1 nuclear antibodies) and proportions of different staining patterns were tested. There were, however, no significant differences in proportions of the number of antibodies over different staining patterns (H, S, N or H/S), $p=0.29$. This information is provided in the revised version of

the manuscript (page 13-14).

2) Table 1b: If possible - anti-SSA/Ro could be presented in Ro60 and Ro52 separately.
Reply: Since this study is based on historical data from the time-point of SLE onset, complete anti-ENA antibody profiles were unfortunately not available in a majority of the patients.

3) In the Introduction the authors refer to a study showing that homogenous ANA pattern is the most common in healthy individuals. There are indeed several publications showing that fine speckled pattern is the most common in healthy persons. The authors have to comment on this subject.
Reply: This objection is indeed relevant and well taken. The type of (very) fine-speckled pattern, which has been described in healthy individuals, also reacts with chromatin, which is evident in dividing HEp-2 cells. Therefore, we have actually labelled this pattern as "homogenous ANA". This staining pattern was not seen in the SLE material. We have now modified the text in the Introduction section (page 6).

Reviewer: Shinu John Ph. D, Department of Immunobiology/Laboratory Medicine, Yale University School of Medicine, USA

Were any of the patients with discrepant staining patterns excluded? The authors have adequately described the status of the patients used in the study but do not comment on exclusion criteria, if any
Reply: All SLE patients within the county of Östergötland meeting either the 1982 American College of Rheumatology criteria for SLE (ACR-82) or the "Fries' criteria" (defined in Patients & Methods) were offered to take part of this structured follow-up programme. Less than 5% of suitable patients have denied participation for different reasons. The ethical permission did not allow us to use data from patients that refrained from participation in the follow-up programme. No other exclusion criteria were used.

Do the various drug treatments alter the ANA patterns? Since a significant number of patients were on some sort of disease modifying drug, the table should indicate the category of drug and the predominant ANA pattern.
Reply: To answer the question raised by the reviewer, one would need a longitudinal study. The present study had a cross-sectional design and aimed to compare the IF-ANA staining patterns from the time-point of SLE onset with clinical and laboratory features. Consequently, the vast majority of patients did not have any disease-modifying drug (but possibly glucocorticoids) when the ANA test was performed. Provided data on disease-modifying drugs refer to what the patients were prescribed once the SLE diagnosis was confirmed.
Our experience, however, is that a considerable proportion (24%) of SLE patients lose their ANA positivity over time (Sjöwall C, et al. J Rheumatol 2008;35:1994-2000). In the present study, patients that remained ANA positive over time mainly displayed a constant pattern: "In many patients, IF-ANA analysis was performed at several occasions over time but discrepant staining patterns were achieved in less than 5% of these cases" (page 10).

Where mixed ANA patterns are observed, sera should be further diluted and IF assays performed to assess the most dominant pattern
Reply: As mentioned in Patients & Methods (page 10) "IF-ANA staining patterns, anti-ENA reactivity and dsDNA antibodies were analyzed on a routine basis at the Clinical immunology laboratory, Linköping university hospital and were extracted from medical records." Since further dilution of serum samples with mixed ANA patterns is not part of the clinical routine at our Clinical immunology laboratory, we have no possibilities to perform this.

Please include representative IF images of other ANA (oANA) pattern. Were these patients on any

"disease modifying drug"?
Reply: The 5 SLE patients in the “oANA group” consisted of 1 case with a pattern very rarely seen in SLE patients (nuclear dots) and in 4 cases the Clinical immunology laboratory was unable to recover documentation of the IF-ANA patterns, but had classified the samples as “IF-ANA positive”. 4 of these 5 individuals were prescribed at least one disease-modifying drug. This information is now given in the text (page 13).

It would be helpful to have RF and Blys/BAFF titers of these patients
Reply: Unfortunately, neither RF nor Blys/BAFF levels were routinely measured at inclusion in our cohort. We are not familiar with whether or not RF and Blys/BAFF levels are associated with certain SLE phenotypes or specific IF-ANA patterns.

VERSION 2 – REVIEW

REVIEWER	Peter H Schur Harvard Medical School, USA Brigham and Women's Hosp;ital, USA
REVIEW RETURNED	06-Sep-2013

- The reviewer completed the checklist but made no further comments.