Original Article

Pattern of antinuclear antibody and antiextractable nuclear antigen antibody test requisitions in Riyadh

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Abstract

Background: International guidelines for screening of systemic autoimmune rheumatic diseases (SARD) recommend antinuclear antibody (ANA) test as the first level test and antiextractable antigen (anti-ENA) along with anti-double-stranded DNA (anti-dsDNA) as second line tests following a reactive ANA test. This study was performed to assess adherence to international guidelines for investigation of SARD and to compare the requesting pattern of ANA and second level tests between rheumatology and nonrheumatology physicians in Riyadh. **Methodology:** This retrospective cross-sectional study comprising of 300 first time requests for investigation of SARD was performed in the immunology unit at King Khalid University Hospital (KKUH). Data were collected between April and May 2018. Information regarding the requesting physicians' specialty and the first time requested tests (ANA, anti-dsDNA, and anti-ENA) were extracted from the electronic medical records. Reasons for requisition of tests were also recorded. **Results:** Of the total requests, 159 (53%) requests included ANA as a single first level test, whereas the rest of the requests ($n = 141$, 47%) included ANA test in conjunction with second level tests for the investigation of SARD. From the department of rheumatology, 14 (29.8%) initial requests were for ANA test as the only first line investigation that was significantly lower than 145 (57.3%) similar requests from the rest of the departments ($P < 0.001$). **Conclusion:** ANA and second level tests requests by physicians particularly among rheumatologists lacked compliance to international guidelines. The current study strongly suggests the need for strict compliance to international guidelines for screening of systemic autoimmune disorders among physicians.

Keywords: ANA test, first level test in SARDs, SARDs, second level test in SARD

Introduction

Systemic autoimmune rheumatic diseases (SARD) comprise of various disorders such as systemic lupus erythematosus (SLE), systemic sclerosis (SSc), Sjögren's syndrome (SjS), rheumatoid arthritis (RA), and mixed connective tissue disease (MCTD).[1] These disorders are characterized by the presence of specific autoantibodies targeting specific auto-antigens.[2] Diagnosis of SARD is often challenging and depends upon factors like clinical history, physical examination, and serological testing for detection of specific autoantibodies.[3] Antinuclear antibody (ANA), antiextractable nuclear antigen (anti-ENA), and anti-double-stranded DNA (anti-dsDNA) tests are frequently requested for diagnosis of SARD. A number of international guidelines have been proposed for proper utilization of laboratory resources by avoiding unnecessary test requisitions.[1,4-7]

The presence of ANA is critical for establishing the diagnosis of SARD. ANA test is a sensitive test but lacks specificity and is usually performed as a first level screening test.[4,7-10] In case of positive ANA test, anti-dsDNA and anti-ENA tests are performed as second line investigations for confirmation of the diagnosis.[1,4-7] Anti-ENA test is highly specific and detects a group of autoantibodies comprising of anti-Sjögren's-syndrome-related

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antigen A (SSa), anti-Sjögren's-syndrome-related antigen B (SSb), anti-smooth muscle (Sm) antibody, anti-ribonucleoprotein (RNP) antibody, anti-Scl-70 antibody, and anti-Jo1 antibody.[10,11]

Based on the clinical presentation, the test requisitions for detection of specific antibodies are ordered for confirmation of diagnosis of SARD.[11] A number of studies have reported that the test requisitions for autoantibody detection in SARD are often ordered inappropriately and are a source of significant burden on laboratory resources.[2,8,12-18] A Canadian study performed in 2012 revealed that out of 18,475 ANA, 10,656 anti-ENA and 5,170 anti-dsDNA tests performed in a period of 3 years, less than 17% of each test yielded positive result.[14] A recent study performed in the Kingdom of Saudi Arabia reported 87% inappropriate ANA requisition by physicians in a tertiary care hospital in Riyadh.[2] Similarly, in the United States of America, 4--5% inappropriate anti-ENA requisitions by physicians for SLE patients have been reported.[18]

Strict adherence to international guidelines for SARD therefore appears to be important in cutting down the occurrence of inappropriate test requisitions and checking the avoidable burden on laboratory resources.[1,4-7] Additionally, pop-up educational messages associated with computerized orders,[19] automatic threshold-based laboratory restrictions with reflex cascade testing,[9] and adherence to disease-specific algorithms[14,20,21] have been suggested to cut down the inappropriate ANA, anti-dsDNA, and anti-ENA test requisitions. Previous studies have reported that implementation of simple algorithms for investigation of SARD have significantly reduced unnecessary healthcare expenses.[14,21]

This study was performed to assess the adherence of the physicians at King Khalid University Hospital to the international guidelines for investigation of SARD and to compare the requesting pattern of ANA and second level tests between rheumatology and non-rheumatology physicians.

Materials and Methods

This retrospective cross-sectional study was conducted in the immunology laboratory at King Khalid University Hospital, Riyadh, Kingdom of Saudi Arabia. A total number of 300 consecutive first time requests in the work list of the immunology unit for patients suspected to have SARD between April and May 2018 were included in the study. Information regarding the requesting physicians' specialty and the first time requested tests as the first level investigation whether ANA alone or ANA along with second level tests (anti-dsDNA or anti-ENA or both) were extracted from the electronic medical records. Reasons for tests requisition were also recorded. Records containing repeated requests for ANA or second level tests and requests for other types of autoantibodies tests were excluded. Furthermore, the physicians were grouped into rheumatologists and non-rheumatologists.

Autoantibody testing

ANA test was performed by indirect immunofluorescence assay on Hep-2 cells. The anti-dsDNA test and anti-ENA tests were performed by immunoenzymatic assays.

Statistical analysis

Data were analyzed using Statistical Package for the Social Sciences (SPSS) computer software version 21.0. The data were expressed in terms of frequencies and percentages. Chi-square test was used to compare the requesting pattern between rheumatology and non-rheumatology physicians. A P value of ≤ 0.05 at 95% confidence intervals was considered as statistically significant.

This study was approved by the Institutional Review Board at King Saud University-College of Medicine (April 8th, 2018 (No. E-18-3111)).

Results

Out of the 300 ANA test requisitions, 159 (53%) requests included ANA test alone, whereas the rest of the requests ($n = 141$, 47%) included ANA test in conjunction with second level tests for the investigation of SARD. There were 47 (15.7%) requests for ANA screening (ANA alone + ANA with other second level tests) from the department of rheumatology compared with 253 (84.3%) requests from the other departments. Figure 1 shows the departments which most frequently requested for ANA screening in the hospital during the study period. Majority of the requests originated from the departments of rheumatology, family medicine, neurology, dermatology, gynecology, and general medicine accounting for a total of 211 requests. The most common medical reasons noted in the requests sent to the immunology laboratory in descending order included joint pain followed by rheumatoid arthritis, abortion, neuropathy, and radiculopathy and urticaria. It was found that 31 requests were ordered without any clear reason [Figure 2].

The data for the proportions of the ANA requests as a single screening test from the departments which requested this test most frequently in the hospital is described in Table 1. Most of the ANA test requests originated from the departments of family medicine (37; 23.3%) followed by 32 (20.1%) from dermatology, 14 (8.8%) from rheumatology, 10 (6.3%) from general medicine, 9 (5.7%) from gynecology, and 8 (5.03%) from neurology. While the highest number of simultaneous ANA test along with second level tests as first level test were requested majorly by rheumatology department (33; 23.4%) followed by 32 (22.7%) from neurology department, 11 (7.8%) from gynecology department, 10 (7.1%) from family medicine department, 8 (5.7%) from general medicine department, and 7 (4.96%) from dermatology department as shown in Table 2.

Majority of simultaneous test requisitions comprising of second level tests was the combined request for ANA and

anti-dsDNA (66; 22%) followed by simultaneous ANA, anti-ENA, and anti-dsDNA requests (55; 18.3%) and there were only 20 (6.7%) requests for simultaneous ANA and anti-ENA tests [Table 3].

Furthermore, data was analyzed to assess and compare the adherence to published guidelines for SARD by rheumatologists and the rest of physicians', that is, non-rheumatologists from other departments [Figure 3]. From the department of rheumatology, 14 (29.8%) initial requests out of 47 were for ANA test as the only first line investigation that was significantly lower than 145 (57.3%) initial requests out of 253 from the rest of the departments ($P < 0.001$). Similarly, 33 (70.2%) requests for ANA as the initial screening test had simultaneous test requests for either anti-dsDNA or anti-ENA or both from the department of rheumatology which was significantly higher than 108 (42.7%) similar requests from all other departments ($P < 0.001$).

Discussion

Lack of adherence to recommended international guidelines for laboratory investigation of SARD by physicians was observed in the present study where 47% of ANA test requisitions were found to have simultaneous requests for second level autoantibody tests. The international guidelines recommend that ANA test should be requested as the first line investigation in the presence of high index of clinical suspicion for SARD.[1] In the event of non-reactive ANA test no additional investigation is recommended. The same has been suggested by the American College of Rheumatology.[22] The lack of adherence to guidelines

Table 1: Distribution of antinuclear antibody test requests from various departments in the hospital

Departments	ANA alone
Family medicine	37 (23.3%)
Dermatology	32 (20.1%)
Rheumatology	14 (8.8%)
General medicine	10 (6.3%)
Gynecology	9 (5.7%)
Neurology	8 (5.03%)

Table 2: Distribution of first line and second line test requests as an initial investigation of systemic auto-immune disorders from various departments in the hospital

Departments	ANA &Second level test
Rheumatology	33 (23.4%)
Neurology	32 (22.7%)
Gynecology	11 (7.8%)
Family medicine	10 (7.1%)
General medicine	8 (5.7%)
Dermatology	7 (4.96%)

Table 3: Frequency of second line test simultaneously requested with ANA as an initial investigation of systemic auto-immune disorders

Test	Frequency (%)
ANA + anti-dsDNA	66 (22.0%)
ANA + anti-ENA + anti-dsDNA	55 (18.3%)
ANA + anti-ENA	20 (6.7%)

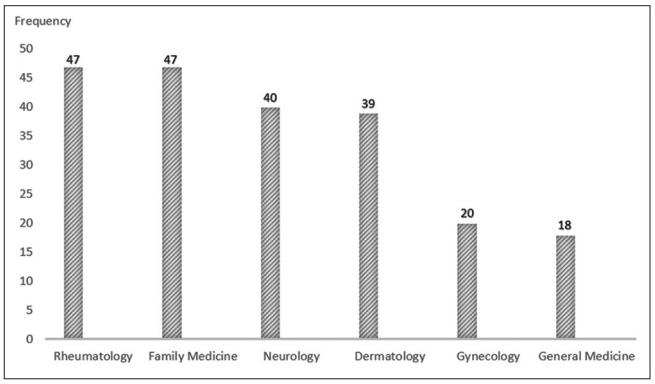

Figure 1: Departments which most frequently requested for ANA screening* in the hospital

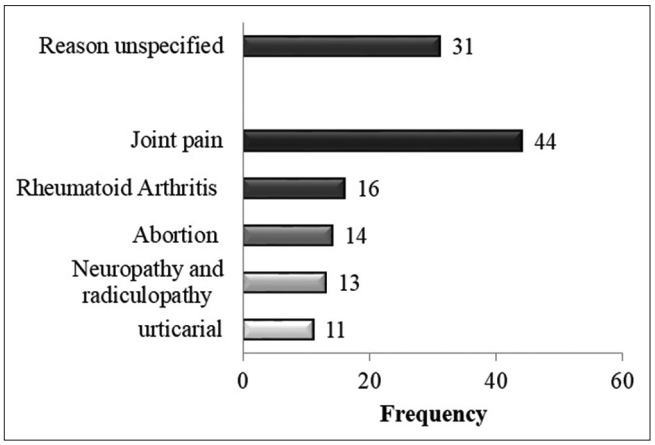

Figure 2: Most common medical reasons for placing requests

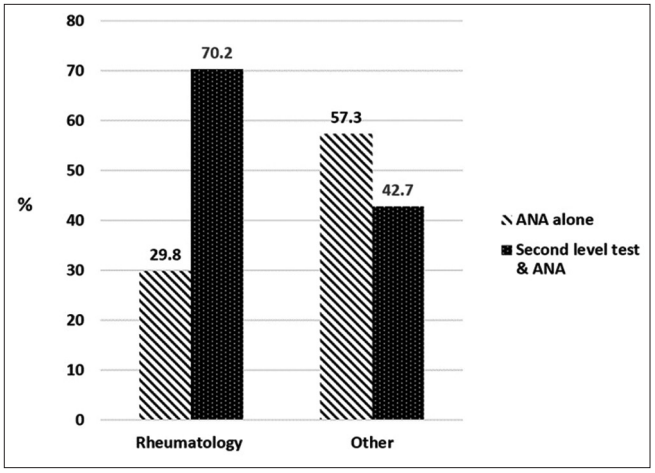

Figure 3: Comparison of the pattern of first-time test requests for investigation of systemic autoimmune disorders from the department of rheumatology and all other departments

appears to be an avoidable burden particularly in resource limited laboratories. A number of factors such as lack of knowledge on part of the requesting physician, non-compliance to international guidelines, and implementation of local laboratory practices to reduce the laboratory turnaround time for decision making have been implicated.[12,23] Previous literature has a sufficient evidence supporting that implementation of guidelines for recommending ANA as the single first line investigation significantly aids in reduction of unnecessary second level investigations without compromising the diagnostic efficacy of the test.[8] In addition, a large number of studies have highlighted the over usage of ANA and second level tests in clinical practice,[24-26] which is noted in this study as well.

In the present study, anti-dsDNA was the most frequent second level test requested simultaneously with ANA test as first level investigations. The same finding was reported in a study conducted in Colorado with 21.8% of such requests,[27] whereas anti-dsDNA is a useful diagnostic and monitoring test for SLE,[4,5] other anti-ENA tests are specific tests that aid in diagnosis of SLE, neonatal lupus, mixed connective tissue disease, Sjogren syndrome, polymyositis, and dermatomyositis.[7] Requesting physicians may use second level investigations for early diagnosis of systemic autoimmune disorders. These measures however do not seem to enhance the diagnostic efficiency. Introduction of protocols formulated following modification of request forms, implementation of guidelines, and highlighting economic advantages reduced the number of second-level tests, proved to be a highly cost-effective measure with enhancement in diagnostic efficacy.[28] Existence of a subset of patients with negative ANA and positive anti-dsDNA antibodies may justify the use of anti-dsDAN antibodies as first line investigation. The term ANA negative was initially coined in 1976.[29] ANA negative patients with SLE represent such group of patients with a reported prevalence of 8.9%.[30] Because of the technological advancement and sophistication achieved in ANA testing virtually eliminating false negative ANA test, the existence of ANA negative SLE has been questioned.[31]

Non-rheumatologists were requesting first level investigations at higher rates (57.3%) in accordance with the international guidelines compared with rheumatologists (29.8%). The probable reasons for such practice could be either higher compliance to standard guidelines by non-rheumatology physicians or tests requisitions by physicians were based on patient demands particularly in primary care as seen in previous studies.[32,33] Around 72% primary care physicians and specialists reported ordering unnecessary tests and 47% of these tests were at the patient's request.[34] The highest percentage of ANA as first line investigation requested by the department of family medicine in the present study could possibly be due to patient demand. However, this aspect has not been explored sufficiently in this study. Hence, this critical aspect should be explored in future as overuse and over-diagnosis has already reported to add to the burden on the healthcare expenditure particularly in resource limited laboratories.[35]

Compared to non-rheumatologists, rheumatology physicians requested significantly less number of ANA first level investigations (29.8%) and significantly higher number (70.2%) of initial ANA tests requests with concurrent second level tests in the present study. This percentage was remarkably higher than 51% ANA and second level tests requests as first level investigation reported in a separate study,[36] indicating that rheumatologists tend to order unjustified second level tests requests. On the contrary compared to rheumatologists, non-rheumatology physicians have also been shown to order significantly higher number of ENA antibody and anti-ds DNA tests as first level investigations along with ANA.[14] These observations indicate that both rheumatologists and non-rheumatology physicians tend to order unjustified test requests for the diagnosis of systemic autoimmune disorders.

The current study strongly suggests the need for strict compliance to international guidelines for screening of systemic autoimmune disorders among physicians. This calls for immediate need for training the physicians especially at primary care level. The number of family physicians along with primary care physicians definitely outnumber the proportion of specialized service providers, which is directly proportional to more number of undue tests prescribed by them. Hence, the basics and International guidelines needs to be reinforced at the primary care level so that undue investigations can be reduced thus reducing the out of pocket expenditure of patients.

Moreover, implementation of protocols or local laboratory practices after consultations with physicians and laboratory staff may be a useful approach to reduce unjustified ANA and second level tests requests and laboratory costs.

Conclusion

ANA and second level tests requests by physicians particularly among rheumatologists lacked compliance to international guidelines. Such practices not only contribute to avoidable work load on medical laboratories but also contribute significantly to the healthcare expenditure. Therefore, compliance to international guidelines for screening of systemic autoimmune disorders appears to be mandatory and it is important to create awareness among the clinicians for strict adherence to these guidelines. Better collaboration between physicians and laboratory staff may be an effective measure to cut down the number of inappropriate simultaneous ordering of multiple tests. This study has a limitation of not exploring the reasons behind such patterns. The future research can explore such option which can aid in better compliance behavior to the guidelines.

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Conflicts of interest

There are no conflicts of interest.

References

1. Stinton LM, Fritzler MJ. A clinical approach to autoantibody testing in systemic autoimmune rheumatic disorders. Autoimmun Rev 2007;7:77-84.
2. lmogren A, Shakoor Z, Hasanato RM, Algassim A, Al-Furaih NA, Al-Mudaiheem FA, *et al.* Autoantibody detection: Prevailing practices at a tertiary care hospital in Riyadh. Clin Lab 2014;60:671-5.
3. Stamouli M, Skliris A, Reppa D, Maganaki E, Totos G. Detection of antinuclear antibodies (ANA), antibodies to double stranded DNA (anti-dsDNA) and antibodies to extractable nuclear antigens (anti-ENA) in Greek patients. Clin Lab 2013;59:283-91.
4. Tozzoli R, Bizzaro N, Tonutti E, Villalta D, Bassetti D, Manoni F, *et al.* Guidelines for the laboratory use of autoantibody tests in the diagnosis and monitoring of autoimmune rheumatic diseases. Am J Clin Pathol 2002;117:316-24.
5. Kavanaugh A, Tomar R, Reveille J, Solomon DH, Homburger HA. Guidelines for clinical use of the antinuclear antibody test and tests for specific autoantibodies to nuclear antigens. American College of Pathologists. Arch Pathol Lab Med 2000;124:71-81.
6. Shojania K. Rheumatology: 2. What laboratory tests are needed? CMAJ 2000;162:1157-63.
7. Bizzaro N, Wiik A. Appropriateness in anti-nuclear antibody testing: From clinical request to strategic laboratory practice. Clin Exp Rheumatol 2004;22:349-55.
8. Tampoia M, Brescia V, Fontana A, Zucano A, Morrone LF, Pansini N. Application of a combined protocol for rational request and utilization of antibody assays improves clinical diagnostic efficacy in autoimmune rheumatic disease. Arch Pathol Lab Med 2007;131:112-6.
9. Bonaguri C, Melegari A, Dall'Aglio P, Ballabio A, Terenziani P, Russo A, *et al.* An italian multicenter study for application of a diagnostic algorithm in autoantibody testing. Ann N Y Acad Sci 2009;1173:124-9.
10. Habash-Bseiso DE, Yale SH, Glurich I, Goldberg JW. Serologic testing in connective tissue diseases. Clin Med Res 2005;3:190-3.
11. Colglazier CL, Sutej PG. Laboratory testing in the rheumatic diseases: A practical review. South Med J 2005;98:185-91.
12. Walraven C, Naylor CD. Do we know what inappropriate laboratory utilization is? A systematic review of laboratory clinical audits. JAMA 1998;280:550-8.
13. Plebani M. The clinical importance of laboratory reasoning. Clin Chim Acta. 1999;280:35-45.
14. Man A, Shojania K, Phoon C, Pal J, de Badyn MH, Pi D, *et al.* An evaluation of autoimmune antibody testing patterns in a Canadian health region and an evaluation of a laboratory algorithm aimed at reducing unnecessary testing. Clin Rheumatol 2013;32:601-8.
15. American College of Rheumatology Ad Hoc Committee on Immunologic Testing G. Guidelines for immunologic laboratory testing in the rheumatic diseases: An introduction. Arthritis Rheum 2002;47:429-33.
16. Vos PA, Bast EJ, Derksen RH. Cost-effective detection of non-antidouble-stranded DNA antinuclear antibody specificities in daily clinical practice. Rheumatology (Oxford) 2006;45:629-35.
17. Villalta D, Tozzoli R, Tonutti E, Bizzaro N. The laboratory approach to the diagnosis of autoimmune diseases: Is it time to change? Autoimmun Rev 2007;6:359-65.
18. Kugasia A, Sehgal N, Dollear M, Sequeira W, Block JA, Jolly M. Practice patterns in longitudinal lupus care provision: Patient and physician perspectives. Lupus 2017;26:1556-61.
19. Solomon DH, Shmerling RH, Schur PH, Lew R, Fiskio J, Bates DW. A computer based intervention to reduce unnecessary serologic testing. J Rheumatol 1999;26:2578-84.
20. Wiik A, Cervera R, Haass M, Kallenberg C, Khamashta M, Meroni PL, *et al.* European attempts to set guidelines for improving diagnostics of autoimmune rheumatic disorders. Lupus 2006;15:391-6.
21. Bonaguri C, Melegari A, Ballabio A, Parmeggiani M, Russo A, Battistelli L, *et al.* Italian multicentre study for application of a diagnostic algorithm in autoantibody testing for autoimmune rheumatic disease: Conclusive results. Autoimmun Rev 2011;11:1-5.
22. Yazdany J, Schmajuk G, Robbins M, Daikh D, Beall A, Yelin E, *et al.* Choosing wisely: The American College of Rheumatology's top 5 list of things physicians and patients should question. Arthritis Care Res (Hoboken) 2013;65:329-39.
23. Hindmarsh JT, Lyon AW. Strategies to promote rational clinical chemistry test utilization. Clin Biochem 1996;29:291-9.
24. Abeles AM, Abeles M. The clinical utility of a positive antinuclear antibody test result. Am J Med 2013;126:342-8.
25. Mohammadi SE, Shaik IH, Chevli P, Gonzalez-Ibarra F, Sarkar S, Acharya S, *et al.* Improper use of antinuclear antibody (ANA) test can result in misdiagnosis, increased patient anxiety, and wasted health care resources [abstract]. Arthritis Rheum 2014;66:S795.
26. Bulbin D, Meadows A, Kelsey S, Harrison H, Denio AE. Choosingsubserologies wisely: An opportunity for rheumatologic healthcare resource savings [abstract]. Arthritis Rheum 2014;66:S589.
27. Davis LA, Goldstein B, Tran V, Keniston A, Yazdany J, Hirsh J, *et al.* Applying choosing wisely: Antinuclear antibody (ANA) and sub-serology testing in a safety net hospital system. Open Rheumatol J 2015;9:82.
28. Mahler M, Andrade LE, Casiano CA, Malyavantham K, Fritzler MJ, *et al.* Anti-DFS70 antibodies: An update on our cur-rent understanding and their clinical usefulness. Expert Rev Clin Immunol 2019;15:241-50.
29. Koller SR, Johnston CL Jr, Moncure CW. Lupus erythematosuscell preparation antinuclear factor incongruity. A review of diagnostic tests for systemic lupus erythematosus. Am J Clin Pathol 1976;66:495-505
30. McHardy KC, Horne CH, Rennie J. Antinuclear antibody-negative systemic lupus erythematosus-how common? J Clin Pathol. 1982;35:1118-21.
31. Cross LS, Aslam A, Misbah SA. Antinuclear antibody-negative lupus as a distinct diagnostic entity--does it no longer exist? QJM.2004;97:303-8.
32. Fenton JJ, Franks P, Feldman MD, Jerant A, Henry SG,

Paterniti DA, *et al*. Impact ofpatient requests on provider-perceived visit difficulty in primary care. J Gen Intern Med 2015;30:214-20.

33. Brett AS, McCullough LB. Addressing requests by patients for nonbeneficial interventions. JAMA 2012;307:149-50.
34. Mason DJ. Choosing wisely: Changing clinicians, patients, or policies? JAMA 2015;313:657-8.
35. Chow SL, Thorne JC, Bell MJ, Ferrari R, Bagheri Z, Boyd T, *et al*. Choosing wisely: The Canadian Rheumatology Association's list of 5 items physicians and patients should question. J Rheumatol 2015;42:682-9.
36. Bulbin D, Meadows A, Denio AE, Kirchner HL, Kelsey S, Harrison H. Do rheumatologists (and other specialists) practice what we preach? a study of serology ordering patterns with attention to subserologies when the antinuclear antibody by enzyme linked immunosorbent assay is negative; and the clinical significance of these positive subserology results. 2013 ACR/ARHP. ACR Meeting Abstracts. Number: 847.