Clinical Utility of Specialized Immunologic Testing in Rheumatology in a Secondary Level Hospital in Mexico

Israel David Campos-González,[a] Martha Eva Viveros,[b] and Mario H. Cardiel[a,c,d]

[a]Unidad de Investigación, Hospital General Dr. Miguel Silva, Morelia, Michoacán, México
[b]Laboratorio de Análisis Clínicos, Hospital General Dr. Miguel Silva, Morelia, Michoacán, México
[c]Servicio de Medicina Interna, Hospital General Dr. Miguel Silva, Morelia, Michoacán, México
[d]Servicio de Reumatología, Hospital General Dr. Miguel Silva, Morelia, Michoacán, México

Introduction: Laboratory tests have an important role in rheumatology for evaluation, diagnosis, and follow up in several diseases. Specialized tests such as antinuclear antibodies (ANA), anti single, or double stranded DNA antibodies (anti-DNA), and anticardiolipin antibodies (ACL) are frequently used and its diagnostic performance is well known in tertiary care centers. Our setting is a secondary care center that implemented these tests 2 years ago. After 1 year of implementation, we decided to evaluate the frequency of use, who orders these tests, and their diagnostic properties for the diagnosis of systemic lupus erythematosus (SLE) and antiphospholipid syndrome (APLS).
Patienst and method: All patients with clinical charts and a request for these tests were evaluated from September 1, 2005 to June 30, 2006. These evaluations were done prospectively by a single, trained evaluator following a standardized format looking at pretest clinical information such as pretest diagnosis, physician's level of training, service, and posttest results as well as therapeutic changes after results. Statistical analysis: descriptive statistics and 2 by 2 tables to estimate diagnostic performance of most common indications.
Results: Two hundred and eighty-six requests were reviewed and only 157 were evaluated. Rheumatology and Internal Medicine services sent 63 and 31 requests for these tests respectively. Diagnostic properties of ANA for SLE were sensitivity (sen) 70%, specificity (spec): 92%, positive predictive value (PPV): 81%, negative predictive value (NPP): 86%, positive likelihood ratio (PLR): 8.73, and negative likelihood ratio (NLR): 0.33. Anti-double stranded DNA, Sen: 78%, spec: 50%, PPV: 80%, NPP: 46%, PLR: 1.56, NLR: 0.44; ACACL y Sen: 78%, spec: 92%, PPV: 78%, NPV 92%, PLR: 10, NLR 0,24.
Conclusions: These specialized tests are not frequently used in our setting. Their diagnostic properties are not as accurate as those published in medical literature. Guidelines are needed in our hospital to improve their diagnostic performance.

Key words: Immunologic testing. Antinuclear antibodies. Anticardiolipin antibodies. Anti-DNA antibodies.

Correspondence: Dr. I.D. Campos-González.
Isidro Huarte esq. Samuel Ramos, s/n. Centro. Código postal 58000.
Morelia. Michoacán. México.
E-mail: israeldavid_cg@hotmail.com; mhcardiel@hotmail.com

Manuscript received November 3, 2006; accepted for publication March 20, 2007.

Utilidad clínica de las pruebas inmunológicas especializadas en reumatología en un hospital de segundo nivel de atención en México

Introducción: El laboratorio en reumatología tiene un papel importante en la evaluación, el diagnóstico y el seguimiento de diversos padecimientos. Los anticuerpos antinucleares (ANA), los anticuerpos anti-ADN de cadena sencilla o doble (ss o dsAnti-ADN) y anticuerpos anticardiolipínicos (AcACL) se usan frecuentemente y su utilidad diagnóstica es bien conocida en centros de atención de tercer nivel. Nuestro hospital es un centro de atención de segundo nivel que implementó hace 2 años estas pruebas. Después de 1 año de su introducción, decidimos evaluar la frecuencia en su uso, quién solicita estas pruebas, su utilidad diagnóstica en lupus eritematoso generalizado (LEG) y síndrome antifosfolipídico (SAF).
Pacientes y método: Se evaluó a todos los pacientes con cuadro clínico de estas enfermedades y solicitud de estas pruebas del 1 de septiembre de 2005 al 30 de junio de 2006. De manera prospectiva, los analizó un evaluador con un formato estandarizado que contenía información clínica, diagnóstico inicial, datos del médico solicitante, servicio, diagnóstico tras resultado y los cambios en la terapéutica. Análisis estadístico: se utilizó estadística

descriptiva y tablas de 2 × 2 para evaluar la utilidad diagnóstica en las indicaciones más comunes.
Resultados: De un total de 286 solicitudes recibidas, s e analizaron 157. Reumatología y medicina interna enviaron 63 y 31 solicitudes respectivamente. Con respecto a los ANA en LEG, se calculó la sensibilidad (70%); la especificidad (92%); el valor predictivo positivo (VPP) (81%); el valor predictivo negativo (VPN) (86%); la razón de verosimilitud positiva (RVsP) (8,73) y la razón de verosimilitud negativa (RVsN) (0,33). En relación con los anti-ADN en LEG, la sensibilidad (78%); la especificidad (50%); el VPP (80%); el VPN (46%); la RVsP (1,56) y la RVsN (0,44). Respecto a los AcACL en SAF, la sensibilidad (78%); la especificidad (92%), el VPP (78%), el VPN (92%), la RVsP (10) y la RVsN (0,24).
Conclusiones: En nuestro hospital hay poca frecuencia en la solicitud de estos estudios. La sensibilidad y la especificidad parecen no estar acordes con lo publicado. Es necesaria la elaboración de lineamientos que en nuestro medio regulen la solicitud de estudios especializados en reumatología y aumenten su utilidad clínica.

Palabras clave: Pruebas inmunológicas. Anticuerpos antinucleares. Anticuerpos anticardiolipínicos. Anticuerpos anti-ADN.

Introduction

Autoimmune rheumatic diseases are more important every day. They represent a daily challenge that is not limited to the physician in specialty hospitals and institutions. Most of these patients are seen in general, secondary-levels hospitals, and primary care physicians. Since the description of LE cells[1] until the identification of specific against different cell structures, rheumatology, as other areas of medicine, has been considerably benefited with the appearance of laboratory studies specialized in immunology.
Although guidelines have been published for the use of these resources in specialized centers, such as the ones mentioned by Kavanaugh et al[2] in 1999 or the ones cited by Solomon et al[6] in 2002 and more recently the guidelines published by the American College of Rheumatology in 2004,[7] there is no applicable norm in our country for the request of such tests in secondary level hospitals. In a previous retrospective study from our center, we analyzed the use of antinuclear antibodies (AAN), anti-DNA antibodies, and anticardiolipin antibodies (ACL) and found that the frequency of their use and their interpretation does not agree with what is published.[8] Therefore, in a prospective manner we carried out the present study with the objectives of establishing the cumulative frequency of the request for these studies during the period between September 2005 and June 2006, identifying the profile of the physicians and departments requesting them and knowing the sensitivity, specificity, predictive values and the trustworthiness of these tests in the context of our hospital. The secondary objectives were to identify the most frequent diagnosis established before requesting the test, evaluating if the test result affects the presumptive diagnosis and if the test result has any influence over the therapeutic decisions that are made.

Patients and Method

The study was carried out in the Hospital General Dr. Miguel Silva de la Secretaría de Salud of the state of Michoacán, México, in the Dr. Mario Alvizouri Muñoz Research Unit and the Immunology area of the Clinical Laboratory. This hospital is a secondary level hospital that belongs to the Mexican Health Secretariat and has 120 beds distributed in 6 basic departments: internal medicine, trauma and orthopedics, general surgery, gynecology and obstetrics, emergencies and intensive care; it also has a subspecialty outpatient clinic, among which rheumatology is counted, which attends approximately 400 patients a month; approximately 150 patients have systemic lupus erythematosus (SLE) and 30 have a diagnosis of primary antiphospholipid syndrome (APS). The protocol was reviewed and approved by the ethics committee.

Study design: Descriptive and observational.

Case identification. We included patients that were attended both in the outpatient clinic as in the hospitalization area for whom serum determination of AAN, anti-DNA, IgG, and IgM ACL had been requested at the clinical laboratory of our hospital and that had a clinical file at our institution. The information was then registered into a formulary specifically elaborated for this study. The results were analyzed by a single person who everyday reviewed the laboratory notebook and obtained the patients' name, the name of the department that requested the study and the type of study requested. Once the result was delivered, the clinical files were independently analyzed and the information included in the registry so that the diagnostic accuracy and the therapeutic conducts could also be analyzed.

Review of the Clinical File

The clinical files of patients that has been submitted for sampling were reviewed.
Only patients with no prior determinations by other laboratories were included to avoid overestimating the

tests' diagnostic usefulness. General and demographic data was evaluated in every one of the files, as well as comorbidities and concurrent treatment, presumptive diagnosis, department that requested the tests, description of the results in the clinical file, and the impact of testing on diagnosis and treatment. In the first formulary both the profile of the requesting physician as well as the department it originated in were evaluated, the referral diagnosis, initial treatment, date of the next visit, follow-up of requests of the same kind in the same patient, and the cost of the study were studied.
In the second one we evaluated whether the results of the study were registered in the clinical file, if the initial diagnosis was confirmed, if the possibility of seronegative diseases or false positives was mentioned, the repercussion on treatment and if the studies were used as follow-up markers. An analysis of patients that fulfilled the modified 1997 ACR clinical criteria for SLE[21] was included as well as patients who complied with the criteria of Sapporo for APS.[22]

Laboratory Studies

Biologic samples. A blood sample was obtained from the patients through aseptic venipuncture using a vacuum tube without anticoagulant. Samples were later centrifuged during 15 minutes and serum was separated, undergoing analysis on the same day.

Antinuclear Antibodies

The detection of ANA was made by means of the indirect immunofluorescence technique (IIF) on HEp-2 cells (ImmunoConcepts Fluorescent ANA Test System). Briefly, the serum of the patients was diluted in a 1:40 proportion with phosphate regulator (PBS) at pH 7.2. The diluted serums were incubated at room temperature during 30 min on slides with HEp-2 cells fixed to their surface. After the incubation, the slides, under continuous agitation, were washed with PBS during 10 min and a polyclonal antibody against human immunoglobulins marked with fluorescein (FITC-1) was added, and was incubated with them during 30 min more. A lavage with 20 mililiters of PBS for 10 min was carried out, adding 1 drop of Evans blue. Finally, the slides were studied at 100 increase in a standard fluorescence microscope. If the test was positive to the initial dilution 1:40, successive dilutions 1:80, 1:160, 1:320 took place, etc, until the dilution in which fluorescence was observed, registering it. With each group of samples a positive and negative control were also mounted. The analysis was made following the instructions of the manufacturer.

Anti-DNA, Anticardiolipin, and Extractable Nuclear Antigen Antibodies

We studied double stranded anti-DNA antibodies and extractable nuclear antigen antibodies (anti-ENA) by means of ELISA using Biosystems reactants (Barcelona, Spain); ACL of the IgG and IgM isotypes, by means of a technique dependent on ELISA reactive to β_2-glucoprotein of the same manufacturer. The anti-ENA ELISA, also of Biosystems, is a polyspecific sifting test that detects antibodies against Ro (SS-A), La (SS-B), RNP, Jo, and SCL-70. All the ELISA analyses were made with an identical procedure: serologic samples were diluted 1:50 (for ACL) and 1:100 for anti-DNA, and anti-ENA using a liquid extender provided by the manufacturer. A total of 100 μL diluted samples were placed in the ELISA plate wells and they were incubated during 30 min. Four lavages of 10 s each with 200 μL of washing solution (provided by the manufacturer) were done, adding 100 μL of antibody anti-IgG human afterwards. The plates were incubated 30 min and 4 more lavages took place. A chromogen was later added, incubated for 15 min and finally the reaction was stopped adding a sulfuric acid solution. The development of color was quantified by means of StatFax 3000 reader and the concentration of antibodies was extrapolated on a curve made from 6 positive controls of well-known concentration provided by the manufacturer. In each lot of samples both positive and negative controls were included.

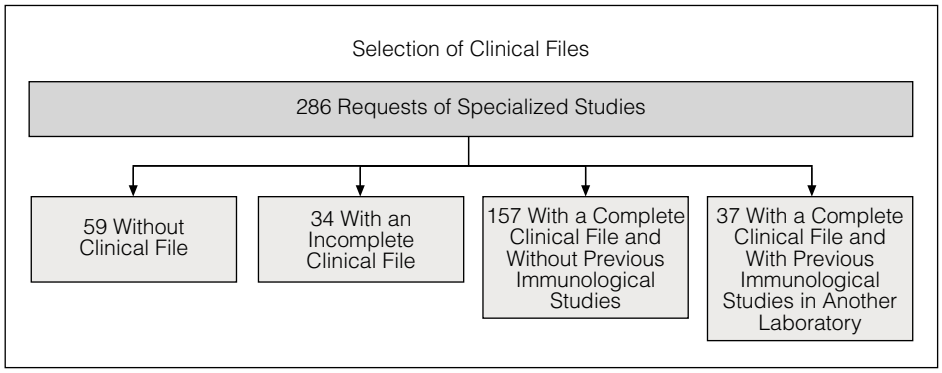

Figure 1. *Selection of clinical files included in this study.*

Statistical Analysis

Descriptive statistic were used to know the frequency, the type of indication, the profile of the physician, the department, and the patient. It evaluated the pertinence of the request, its diagnostic utility and the effect on treatment. This last one was measured by changes in therapeutic decisions after knowing the result. The sensitivity, specificity, positive and negative predictive values, the truthfullness, punctual values, and the 95% confidence intervals were analyzed in 2×2 tables.

Results

During the period of the September 1, 2005 to June 30, 2006, the clinical laboratory of the Hospital General Dr. Miguel Silva received a total of 286 requests in which for some of the following immunological tests: ANA, anti-DNA, IgG, and IgM ACL, anti-ENA antibodies, or C3-C4complement. Of the total, 59 corresponded to patients who did not have a clinical file; 34 corresponded to patients with incomplete an clinical file, 37 corresponded to patients with a clinical file with previous reports of such studies in another laboratory, and finally 157 corresponded to patients with a complete clinical file and without previous immunological studies, that were those analyzed (Figure 1). Of the 157 files reviewed, 31 corresponded to men, and 126 to women (19.75% and 80.25%, respectively). The mean age of all the patients was 35.26 (range, 14-68) years. The requests for studies were made by the following services: rheumatology, 63 (40.12%); internal medicine, 31 (19.74%); gynecology, 19 (12.10%); nephrology, 15 (9.55%); hospital interns, 14 (8.91%); general medicine, 6 (3.82%); orthopedics and neurology, 3 (1.91%) each one, and finally, hematology, infectology and research unit, 1 (1.38%) each for each department (Figure 2). The studies requested were: ANA, 131 requests; anti-DNA, 50; ACL, 35; anti-ENA antibodies, 4, and C3 and C4 complement, 9 (Figure 3). The analysis of previous diagnoses related to the results by specialty and subspecialty showed that diagnoses of rheumatic pathology were made in 91 cases, hematologic in 14 cases, neurological in 13 cases, gastroenterological in 9 cases, nephrology in 11 cases, obstetrical and for the study of fever of unknown origin in 2 cases each one, and previous suspect diagnosis was not mentioned in 15 cases. Being the area of rheumatology the one with a greater frequency of requests in this study, a subanalysis of the most frequent diagnoses was made, and a total of 62 diagnoses of SLE, 10 diagnoses of APS, 5 of spondyloarthropathies, 7 of rheumatoid arthritis, 3 of idiopathic inflammatory myopathy, 2 of autoimmune hepatitis, and 2 of primary biliary cirrhosis were made. All diagnoses preceded the results. The evaluation of the profile of the doctor who made the request showed us that of the 157 received requests,

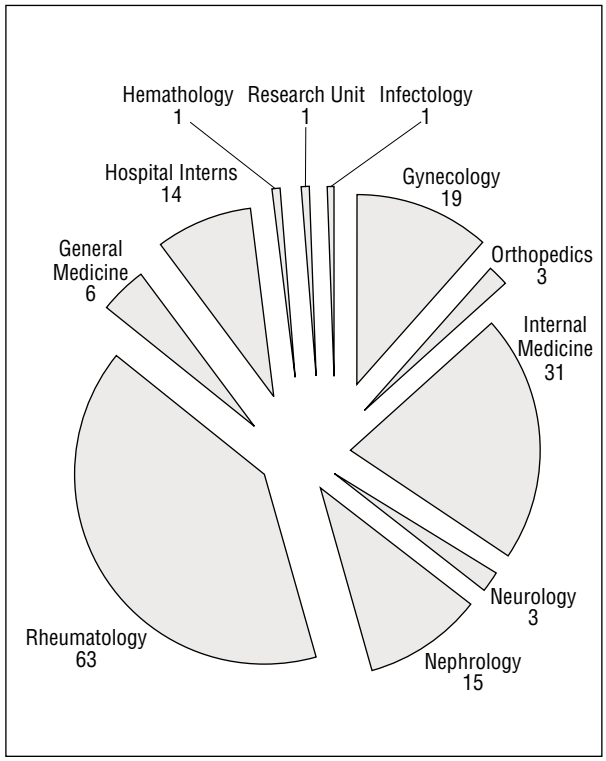

Figure 2. *Number of requests per service.*

86 were done by specialists; of these, 62 were done by rheumatologists, 15 nephrologists, 5 by neurologists, 2 by infectologists, and 2 by hematologists; 51 requests were done by residents; 33 of these corresponded to internal medicine, 14 to gynecology and obstetrics, and 4 to orthopedics; 14 requests were made by interns and 6 by general physicians. With respect to the ANA in the diagnosis of SLE, a sensitivity of 70% was calculated

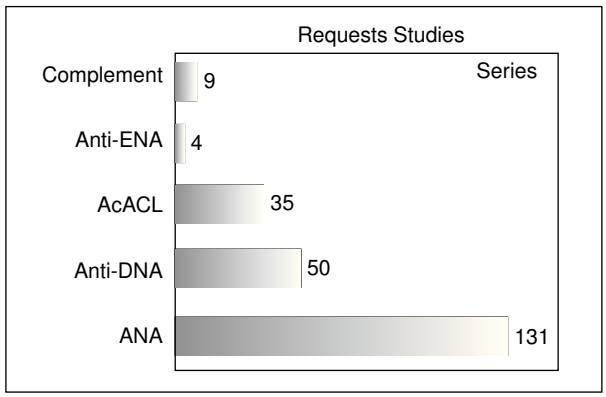

Figure 3. *Number of requests for each type of study. AcACL indicates IgG and IgM anticardiolipin antibodies; ANA, antinuclear antibodies; anti-DNA, anti-DNA antibodies; anti-ENA, antibodies against nuclear extractable antigen; complement, C3-C4 complement.*

TABLE 1. Diagnostic Properties of Antinuclear Antibodies in Systemic Lupus Erythematosus (SLE)*

	SLE (+)	SLE (–)	95% CI
Anti-DNA (+)	30		7
Anti-DNA (–)	13		81
Total		131	
Sensitivity	0.7	70%	0.55-0.81
Specificity	0.92	92%	0.85-0.97
VR (+)	8.73	8.73	4.2-18
VR (–)	0.33	0.33	0.20-0.51
PV (+)	0.81	81%	0.66-0.91
PV (–)	0.86	86%	0.78-0.92
Prevalence	0.33	33%	
Exactitude	0.85	85%	

*CI indicates confidence interval; RV (+), positive verisimilitude ratio; RV (–), negative verisimilitude ratio; PV (+), positive predictive value; VP (–), negative predictive value.

(95% confidence interval [CI], 0.55-0.81) and a specificity of 92% (95% CI, 0.85-0.97), with a positive probability of 8.73 (95% CI, 4.2-18) and negative probability of 0.33 (95% CI, 0.20-0.51), as well as a positive predictive value of 81% (95% CI, 0.66-0.91), and a negative predictive value of 86% (95% CI, 0.78-0.92) (Table 1); with respect to anti-DNA antibodies in the same disease, a sensitivity of 78% was demonstrated (95% CI, 0.62-0.88), a specificity of 50% (95% CI, 0.27-0.73), with a positive probability of 1.56 (95% CI, 0.90-2.7), and a negative probability of 0.44 (95% CI, 0.20-0.99), with a positive predictive value of 80% (95% CI, 0.64-0.90), and a negative predictive value of 46% (95% CI, 0.50-0.70) (Table 2). As far as ACL in the diagnosis of APS, a sensitivity of 78% was calculated, with a specificity of 92%, a positive probability of 10 and a negative probability of 0.24, a positive predictive value of 78% and a negative predictive value of 92% (Table 3). After the result, the change in therapeutic strategies was analyzed; in 18 cases the treatment was modified after the laboratory result, in 129 cases the same treatment was continued and in 10 cases treatment is mentioned neither before nor after the result.

Discussion

The request of immunology studies has become a common practice in numerous medical attention centers. Most of the times there is little information on the correct use of these, either by ignorance of the published guideline for their use or the more frequently to the nonexistence of such guidelines, which would certainly be of great utility in benefitting the patients who day to day seek medical attention. In the present study we analyzed the clinical utility of different rheumatology tests in a second level of attention hospital, since numerous studies published worldwide exist about the utility of the above mentioned tests in third level of attention hospitals or in centers of reference of patients with rheumatic disease; nevertheless, we did not find publications about the utility of these in

TABLE 2. Diagnostic Properties of Anti-DNA Antibodies in Systemic Lupus Erythematosus (SLE)*

	SLE (+)	SLE (–)	95% CI
Anti-DNA (+)	28	7	
Anti-DNA (–)	8	7	
Total		50	
Sensibility	0.78	78%	0.62-0.88
Specificity	0.5	50%	0.27-0.73
RV (+)	1.56	1.56	0.90-2.7
RV (–)	0.44	0.44	0.20-0.99
PV (+)	0.8	80%	0.64-0.90
PV (–)	0.46	46%	0.25-0.70
Prevalence	0.72	72%	
Exactitude	0.7	70%	

*CI indicates confidence interval; RV (+), positive verisimilitude ratio; RV (–), negative verisimilitude ratio; PV (+), positive predictive value; VP (–), negative predictive value.

TABLE 3. Diagnostic Properties of Anticardiolipin Antibodies (ACL) in Antiphospholipid Syndrome (APS)*

	APS (+)	APS (–)	95% CI
ACL (+)	7	2	
ACL (–)	2	24	
Total		35	
Sensitivity	0.78	78%	0.45-0.93
Specificity	0.92	92%	0.75-0.97
RV (+)	10	10	2.55-40.05
RV (–)	0.24	0.24	0.07-0.82
PV (+)	0.78	78%	0.45-0.93
PV (–)	0.92	92%	0.75-0.97
Prevalence	0.26	26%	
Exactitude	0.89	89%	

*CI indicates confidence interval; RV (+), positive verisimilitude ratio; RV (–), negative verisimilitude ratio; PV (+), positive predictive value; VP (–), negative predictive value.

second level of attention centers. Contrary to what we expected, the frequency of use of the mentioned studies is scarce, since in average approximately 1 study per day was asked for. We excluded from the analysis files of patients in which any of the studies had been previously asked for in a different laboratory, with the intention of not overestimating their utility to complement the diagnosis of any suspected diseases. The sera of the patients without a clinical file were of patients seen sometimes by general medicine, department which only has daily registries of consultation and not complete clinical files; this last one is obtained after being sent to the specialty consultation. The high frequency of request of studies in women can be explained by the frequency of immune diseases in rheumatology in that gender; there was also agreement with age, since generally this type of affection appears in young adults.[9,10] It differs from the total number of requests for ANA in comparison with the total of requests for anti-DNA antibodies, because in some patients ANA are the only ones measured. It was of no surprise that most of the requests came from the rheumatology department or by residents of internal medicine, because those are the departments that most frequently are in direct contact with patients with this type of disease and, consequently, are also more informed on the real utility of such studies; nevertheless, it is noticeable we also received a considerable number of requests from gynecology and obstetrics for the study protocol of patients with recurrent abortions, since, as they mention Gomez-Puerta et al[11] in a long term follow-up study of 128 patients with primary APS, found a considerable prevalence, nearly 50% of the patients, with recurrent abortion as a part of an ample range of clinical manifestations in this disease. Similarly, the number of requests by nephrologists is remarkable, generally in search of an immune cause of renal disease, which is not infrequent either; as Lange et al[12] analyzed, the presence of glomerulonephritis due to immune causes is frequent, specifically in SLE, forcing the meticulous study of patients in which renal disease has developed because, as mentioned Lai et al[13] in his review on lupus nephritis, this is one of the main causes of morbidity and mortality in patients with SLE. We found that the ANA study was requested, present in 83% of total requests and when 2 or more studies were asked for simultaneously, the association of these with anti-DNA antibodies was the most frequent; concordant with the suspected disease before the result was obtained, which was SLE, in a widely known relationship. This was also related to the frequency of requests by areas, being specialists in rheumatology those responsible of 40% of the total requests for specialized studies. There is abundant information in the literature about the association of certain antibodies against nuclear structures and that they are most frequently related to a very characteristic type of diseases, as mentioned by Solomon et al,[6] Wicck,[3] and Kavanaugh et al,[2] to mention only some of the published guidelines for the use of these studies in which such a relation is clearly described. The results obtained in the present study demonstrate that there is a certain discrepancy between the type of study asked for and the suspected affection and, consequently, the real clinical utility of specialized rheumatologic studies is decreased by the lack of information among those who asks for them. In our study we found a sensitivity of 70% and a specificity of 92% in the use of ANA to complement the diagnosis of SLE, whereas in studies published in other centers a sensitivity of 93% was communicated and specificity of 57%,[6] and in some the sensitivity came close to 95% and 98%.[3,15] The same happens with anti-DNA antibodies, for which, though we found a sensitivity of 78% (similar to that published elsewhere, 65%-80% sensitivity), a 50% specificity is discordant, since some studies publish a specificity near 99%,[3,16] the same tendency observed with respect to the antiphospholipid antibodies. We must comment the following likely possible explanations for the low diagnostic yield: that the 95% CI in our study is within what is published in universal literature and perhaps the punctual result had to do with a small sample of studies analyzed. We knows that the concentrations of antibodies in SLE can vary with clinical activity and it could influence the low sensitivity found and that its presence is not diagnostic of the disease; other autoimmune diseases can present them and that reason can explain the low specificity.[20] It is also important to comment the cross-sectional nature of our study and that the displayed data involve only one isolated determination of the laboratory tests. In conclusion, the use of specialized rheumatology studies is scarcely frequent in the Hospital General Dr. Miguel Silva after 2 years since their introduction. The services that more frequently use this type of specialized tests are rheumatology and internal medicine. In our hospital, the sensitivity and the specificity of ANA and anti-DNA antibodies seem to not correspond with that in literature with respect to the diagnosis of SLE; something similar happens with ACL antibodies and the diagnosis of APS, and its utility is low when influencing therapeutic decisions after the results. For that reason, the elaboration of guidelines is necessary to regulate the request of specialized rheumatology studies and improve their diagnostic yield.

References

1. Hepburn AL. LE cell. Rheumatology. 2001;40:826-7.
2. Kavanaugh A, Tomar R, Reveille J, Solomon DH, Homburger HA. Guidelines for clinical use of the antinuclear antibody test and test for especific autoantibodies to nuclear antigens. Arch Pathol Lab Med. 2000;1:71-81.
3. Wikk AS. Anti-nuclear autoantibodies: clinical utility for diagnosis, prognosis, monitoring, and planning of treatment strategy in systemic immunoinflammatory diseases. Scand J Rheumatol. 2005;34:260-8.
4. Anaya JM. Autoinmunidad y enfermedad autoinmune. Medellín: Corporación para Investigaciones Biológicas; 2005.
5. Shojania K. Rheumatology: 2. What laboratory tests are needed? CMAJ. 2000;162:1157-63.
6. Solomon DH, Kavanaugh AJ, Schur PH. Evidence Based Guidelines for the use of immunologic tests: Antinuclear antibody testing. Arthritis Rheum.

2002;47:434-44.
7. Benito-Garcia E, Schur PH, Lahita R. Guidelines for immunologic laboratory testing in rheumatic diseases: Anti-Sm and anti-RNP antibodies test. Arthritis Rheum. 2004;51:1030-44.
8. Viveros ME, Gómez VH, Campos ID, Cornejo RH, Cardiel MH. Utilidad clínica de la determinación de anticuerpos antinucleares, anti DNA por ELISA, anticuerpos anticardiolipina, y anti-ENA en un hospital de segundo nivel de atención. Rev Mex Reumatol. 2005;20:75-6.
9. Harrison KD. Principios de Medicina Interna. Madrid: McGrawHill; 2005.
10. Malleson PN, Sailer M, Mackinnon MJ. Usefulness of antinuclear antibody testing to screen for rheumatic diseases. Arch Dis Child. 1997; 77:299-304.
11. Gómez-Puerta JA, Martín H, Carmen Amigo MC, Aguirre MA, Camps MT, Cuadrado MJ, et al. Long-term follow-up in 128 patients with primary antiphospholipid syndrome. Medicine (Baltimore). 2005;84:225-30.
12. Lange K, Wasserman E, Slobody LB. The significance of serum levels for the diagnosis and prognosis of acute and subacute glomerulonephritis and lupus erythematosus disseminatus. Ann Intern Med. 1960;53:636-46.
13. Lai KN, Tang SC, Mok CC. Treatment for lupus nephritis: A revisit. Nephrology (Carlton). 2005;10:180-8.
14. Lyons R, Narain S, Nichols C, Satoh M, Reeves WH. Effective use of autoantibody tests in the diagnosis of systemic autoimmune disease. Ann N Y Acad Sci. 2005;1050:217-28.
15. Ward MM. Laboratory testing for systemic rheumatic diseases. Postgrad Med. 1998;103:93-100.
16. Egner W. The use of laboratory tests in the diagnosis of SLE. J Clin Pathol. 2000;53:424-32.
17. Slater CA, Davis RB, Shmerling RH. Antinuclear antibody testing. A study of clinical utility. Arch Intern Med. 1996;156:1421-5.
18. Hahn BH. Mechanism of disease: Antibodies to DNA. N Engl J Med. 1998;338:1359-68.
19. Tan EM. Autoantibodies to nuclear antigens (ANA): their immunobiology and medicine. Adv Immunol. 1982;33:167-240.
20. Kurien BT, Scofield RH. Autoantibody determination in the diagnosis of systemic lupus erythematosus. Scand J Immunol. 2006,64:227-35.
21. Hochberg M. Updating the American college of rheumatology revised criteria for the classification of systemic lupus erythematosus. Arthritis Rheum. 1997;40:1725.
22. Ravelli A, Martín A. Antiphospholipid syndrome. Pediatr Clin North Am. 2005,52:469-91.