A Possible Defective Estimation of Antineutrophil Cytoplasmic Antibodies in Systemic Lupus Erythematosus Due to the Coexistence of Periodontitis: Preliminary Observations

ERNESTO NOVO, DDS, PhD*; ERNESTO GARCÍA-MAC GREGOR, MD†; SORAYA NAVA, DDS*
LENIS PERINI, MD†.

ABSTRACT. The objectives of this study is to determine if periodontitis-related ANCA hinder the accurate estimation of this kind of autoantibodies in systemic lupus erythematosus (SLE), due to the frequent coexistence of SLE and periodontitis, and the high incidence of antineutrophil cytoplasmic antibodies (ANCA) in this periodontal condition. Thirty SLE, thirty periodontitis lacking systemic involvement patients, and twenty healthy controls were utilized in this study. The periodontal condition and the presence of ANCA in sera of all individuals was carefully evaluated. For ANCA determination an EIA assay was utilized, directed to a neutrophil granular extract and six neutrphil granule proteins. Sixty percent of SLE patients had eeriodontitis, and sixtyfive percent were ANCA positive. Eighty three percent of all ANCA cases were coexisting with periodontitis. A significant association ($p > 0.005$) between periodontitis and ANCA was found (Chi Square Test). Fifty percent of the patients with periodontitis lacking systemic involvement were ANCA positive. The results obtained in this study suggest that the figures of ANCA previously reported for SLE, might be overestimated due to the inadvertent presence of periodontitis.

Antineutrophil cytoplasmic antibodies are a type of autoantibodies directed against the enzymes located in the primary granules of polimorphonuclear leucocytes, and lysosomes of monocytes. They were first described in 1982 by Davies (1) in patients with necrotizing glomerulonephritis. Since then, ANCA have been detected in a wide range of inflammatory, infectious and neoplastic conditions (2,3,4) and are currently utilized as a sensitive, specific marker for Wegener's granulomatosis (5).

*Ernesto Novo, DDS, PhD. Chief, Oral Biology Section, Dental Research Institute, Universidad del Zulia, Maracaibo, Venezuela.
†Ernesto Garcia-Mac Gregor, MD. Chief, Rheumatology Unit, Hospital Central, Maracaibo, Venezuela.
*Soraya Nava, DDS. Epidemiology and Dental Practice Section, Dental Reasearch Institute, Universidad del Zulia, Maracaibo, Venezuela.
†Lenis Perini, MD. Rheumatology Unit, Hospital Central, Maracaibo, Venezuela.

This research was supported by The Council for Development of Science (CONDES), Universidad del Zulia, Maracaibo, Venezuela, grant No. 2172-95

Address for correspondence: Ernesto Novo, PhD. Apartado Postal 15111 Maracaibo, Venezuela. Phone and Fax Number: (58)(16) 625579
E-mail: enovo@europa.ica.luz.ve

Acording to a number of studies they might be involved in the pathophysiology of some disorders such as microscopic polyarteritis and the idiopathic form of pauci-immune necrotizing crescentic gromerulonephritis (6).

ANCA have fairly been described in SLE (7-10),although no correlation has been found so far, between the presence of these autoantibodies and SLE, with respect to disease activity or presence of vasculitis (7-10). In a previous paper, we reported a high frequency of ANCA in patients affected with periodontitis (11), a condition that has become the major cause of tooth loss in adults (12-14).

Pocket formation, bleeding, bone loss, and tooth mobility are usual clinical features of periodontitis, an affection which is frequently associated to systemic conditions (15,16) where neutrophil impairement is ussually present e.g., scleroderma, hyperthyroidism, hypoadrenocorticism and diabetes mellitus (16,17).

The elevated number of SLE patients with alleged signs of periodontal disease, and the high occurrence of ANCA observed in periodontitis patients, prompted this study,

which is directed to investigate the distribution of these autoantibodies in SLE patients with and without periodontitis in order to ascertain, if the bulk of ANCA observed in SLE is attributable to periodontitis rather than SLE.

We have reported previously high numbers of ANCA in patients with periodontitis (11). If, as we assume, there is a considerable number of periodontitis in SLE patients, and we can find a tight association between ANCA and periodontitis in SLE, it would be clear, that a high number of ANCA positive cases accredited to SLE, rather should be ascribed to periodontitis.

In the present study, two patient groups were investigated: The first one, which will be herefrom, referred to as the SLE group, was composed by 30 individuals with a SLE diagnosis. The second group, referred to, as the periodontitis group, was composed by 30 patients with periodontitis and no systemic involvement whatsoever. Both groups, were thoroughly examined to assess their periodontal condition, and tested for the presence of ANCA through an indirect EIA assay directed to a neutrophil granular extract, and six granule constituents: myeloperoxidase (MPO), elastase (HLE), cathepsin G (CAT), lysozyme (LYZ), lactoferrin (LF), and proteinase 3 (PR3). Data generated from the SLE Group, will provide information about the number of SLE patients with and without periodontitis, whose sera display reactivity for ANCA, and should allow to detect the existence of any association between periodontitis and ANCA on this type of patients. The data from the periodontitis group, will provide information about the number of patients with periodontitis, whose sera display reactivity for ANCA, when this periodontal condition does not coexist with any other local or systemic disease.

This investigation is conceived as a pilot study designed to explore a possible relationship between a defective estimation of ANCA in SLE and periodontitis.

The data to be collected in our study, will pave the way for the designing of additional, more controlled investigations that would allow us to attain a sound, more comprehensive view of this interesting relationship.

Materials and Methods

Patients. The SLE Group consisted in thirty patients from the Rheumatology Unit, Central Hospital, Maracaibo, Venezuela. They were diagnosed as SLE according to the American College of Rheumatology criteria for the classification of this systemic illness (18). Patients with drug-induced lupus were excluded. The patients in this group were on different stages of the disease, and it was composed by one 45 year old hispanic male, and 29 female, including three indian, four caucasian and twenty two hispanic (mean age + SD = 31.63 + 9.28; range 19 to 45). As inclusion criteria for the SLE and the Periodontitis Group, these patients should have at least 16 teeth and, age between 18 and 45 years.

The Periodontitis Group consisted in thirty patients from the Periodontics Postgraduate Clinic of the School of Dentistry, Universidad del Zulia, Maracaibo, Venezuela; they should present the following clinical conditions in order to be considered as periodontitis patients: 1) three or more periodontal pockets deeper than 4mm, which bled on gentle probing on two separate sites of the mouth. 2) Evidence of alveolar bone loss, estimated from the cemento-enamel junction at affected sites.

Sixteen patients were male, including one caucasian and fifteen hispanic (37.30 + 8.07; r 22 to 45); Fourteen were female, including three caucasian, one black, and ten hispanic (28.78 + 8.14; r 18-43).The general inclusion criteria for the Periodontitis Group were: 1) age between 18 and 45 years. 2) presence of at least 16 teeth. 3) good general health with no history of systemic disease, no blood dyscrasias or anomalies of the immune system. 4) no use of medication in the past three months that may influence the immune system or the inflammatory response. 5) no history of surgical or periodontal therapy in the previous six months.

The control group consisted in 20 periodontally and systemically healthy individuals, age between 18 and 45 years old, recruited from laboratory staff, students, and employees of the School of Dentistry. This group included six male, one caucasian and five hispanic (36.66 + 6.37; r 27 to 45), and fourteen female, one black, two caucasian and eleven hispanic (30.78 + 5.45; r 25-40).

Periodontal Evaluation of the Study Groups. This evaluation was accomplished applying the following standard procedures (19,20): a) assessment of probing depth in mm (PD). Probeable depths were measured to the nearest mm using a Michigan type "O" probe at six locations on each tooth (midbuccal, midlingual and the proximal aspects) while keeping the probe in line with the long axis of the tooh. Pocket depth in mm was measured from the base of the periodontal pocket to the free gingival margin. All examinations were performed by one examiner. b) Bleeding on gentle probing. This parameter was registered as present or absent. c) Bone loss, which was estimated by periapical radiographs. Any areas suggestive of bone loss were also examined on bite-wing radiographs, measured and registered as localized or generalized, according to the location and extent of the affected area, and considered as mild, moderate or severe, when it extended over one, two, or more than two thirds of the dental roots.

EIA Asssay. An indirect EIA assay was utilized to test sera for the presence of antibodies directed to a granular extract and to the purified enzymes MPO, HLE, LF, CAT, LYZ and PR3. Purified proteins used in this study were purchased from Sigma Chemical Co., St. Louis, Missouri, USA. For anti-PR3 antibodies detection, a kit from Scimedx Corp., Denville, New Jersey, USA, was used. A 1:50 dilution of patients and control sera in PBS-Tween (PBST), was utilized for the EIA assays.

The granular extract consists of a mixture of different granule proteins which cause positive reactions in ANCA positive sera. This extract was obtained by the method described by Riches et al. (21) Briefly, neutrophils were isolated from peripheral blood healthy donors by Ficoll-Hypaque density (1.077 g/cm^3) and subsequent lysis of eythrocytes with ammonium choride. The cells were placed in cold PBS plus the proteolysis inhibitors PMSF and TPCK (6 mM) and disrupted by sonication in an Artek sonic dismembrator model 300 (Artek Systems Co., Farmingdale, NY, USA), in ice cold water at full power for 4min with 30s intervals. Differential centrifugation was based on the method described by Klempner et al. (22)

The granule containing supernatant was sonicated as described above and stored at -70°C until used. An 1 ml aliquot of the granular extract was utilized for protein determination according to the method described by Lowry (23). The EIA plates were sensitized with a concentration of 5-10mg/ml of each protein, and the granular extract. An alkaline phosphatase labeled, anti-human IgG, g-chain specific $F(ab')_2$ obtained from Sigma Chemical Co.,St. Louis, MO, USA, was used as conjugate.

Data Analysis. For ANCA determination, sera were considered as positive when its optical density values were more than 2 SD above the mean of the healthy control group. Presence of periodontitis in the SLE group, and ANCA reactivity in both experimental groups was registered as percentage of positive cases.

In order to determine a possible association between periodontitis and ANCA in SLE patients, a Chi Square contingency table and the correction method for small numbers of Pirie and Hamden was utilized. A value of $p < 0.005$ was considered significant.

Results

The periodontal examination and, the ANCA testing on sera of both SLE and the Periodontitis Group allowed to draw the following data:

SLE Group. Eighteen out of thirty patients (60%) had periodontitis, hence, twelve (40%) had an healthy periodontal condition (Fig 1). Also, nineteen out of thirty (65%) sera were ANCA positive. From the eighteen patients with periodontitis, fifteen (83.3%) were ANCA positive and only three (16.6%) were not (Table I and Fig 1). When sera from the twelve patients with a healthy periodontal condition was tested for ANCA reactivity, eight (66.6%) were negative and only four (33.4%) showed a positive response for ANCA (Table I). A significant correlation ($p < 0.005$) between periodontitis and ANCA in SLE patients was found (Chi square test).

Table 1. Distribution of Periodontitis and ANCA in SLE Group

Category	Frequency	
	Number	Percentage
Healthy Periodontium, ANCA Positive	4	13.3
Healthy Periodontium, ANCA Negative	8	26.6
Periodontitis, ANCA Positive	15	50
Periodontitis, ANCA Negative	3	10

Periodontitis group . When sera from patients from this group were tested for ANCA reactivity, fifteen out thirty (50%) were positive (Fig 1). In addition, the results obtained show that proteinase 3 is the granule protein with the highest reactivity from both SLE and Periodontitis Group sera. Nine out nineteen in the SLE Group and, five out fifteen in the Periodontitis Group showed ANCA reactivity for this protein. Likewise, seven out nineteen sera on the SLE Group and, five out fifteen on the Periodontitis Group showed reactivity for lactoferrin (Table 2). For other granule proteins cathepsin G, elastase, myeloperoxidase, and lysozyme, the reactivity in both groups was slightly lower.

Table 2. Antibody Reactivity of Sera for Proteinase 3 and Lactoferrin

	SLE Group		Perio Group*	
	Number	Percentage	Number	Percentage
Proteinase 3	9/19	47.36	5/15	33.3
Lactoferrin	7/19	36.8	5/15	33.3

* Periodontitis Group

Dicussion

The relationship between SLE and the oral cavity has been known since long time ago, due to the oral lesions occurring in aproximately 45% of SLE patients, mainly as erythematous areas often accompanied by edema and petechiae (24,25).

This investigation found a high occurrence (60%) of

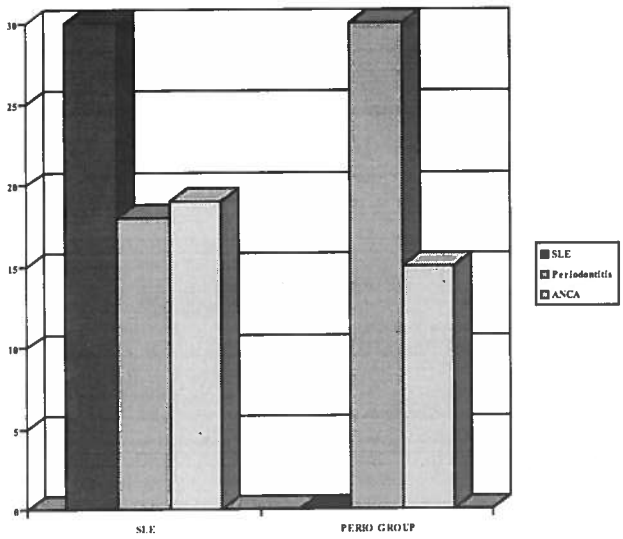

Figure 1. Number of patients with Periodontitis in SLE group, and number of patients whose sera showed ANCA reactivity in both SLE, and Periodontitis group.
Abbreviations: PERIO GROUP Periodontitis Group.

periodontitis in SLE, which seems to indicate that the coexistence of SLE and periodontitis is a common feature (Fig. 1). Surprisingly, most of the ANCA positive sera in SLE (83.3%) were concurrent with periodontitis (Table I, Fig 1), which lead us to investigate a possible association between periodontitis and ANCA in SLE.

On the other hand, testing for ANCA on sera from periodontitis patients with no systemic involvement, revealed that almost a half of them (45%) were ANCA positive (Fig. 1). This finding sustains results of a previous report (11), which reveals a high incidence of ANCA in adult periodontitis, the most common form of periodontitis. According to data provided by this investigation, it is clear that patients with SLE and a coexisting periodontitis, have an additional source of ANCA that is not being accounted for, and hence, may mislead clinicians and/or investigators in the data analysis of ANCA in SLE patients, because some sera which might display ANCA reactivity, could exclusively have this type of autoantibodies due to the presence of periodontitis, not being related to SLE.

The significant correlation found between periodontitis and ANCA, has a great influence in the analysis of ANCA values in SLE. Former ANCA figures reported for SLE patients in the past, may be not accurate, because they do not take in account the presence of periodontitis and therefore, they do not subtract the number of ANCA positive sera which belong exclusively to the presence of periodontitis. This fact may have profound implications; for some reports show that no correlation has been found so far between the presence of ANCA and SLE with respect to disease activity or presence of vasculitis. Undoubtedly, these reports may have included some ANCA positive sera which are originated from a coexisting periodontal condition and not from the SLE status of the patient. This fact, creates a bias that turns appropriate inferences extremely difficult to make.

The results obtained in the present study, seem to indicate that the concomitant occurrence of periodontitis and SLE modifies the net figures of ANCA in this systemic condition which, makes mandatory the periodontal evaluation of SLE patients when the presence of ANCA is going to be determined on this type of individuals.

We think that periodontitis could be considered as part of the clinical pathology of SLE. The high number of SLE patients having this periodontal condition gives to this hypothesis a reasonable support. In the classification of periodontitis there is a vast number of systemic diseases which go along, and are somehow associated with periodontitis (16). This periodontal condition is not detected in routine clinical examination of SLE patients. Periodontal evaluation requires specialized personnel, and equipment which is not easily available for most of the Rheumatology Clinics. We think that additional more controlled investigations evaluating other periodontal and and systemic parameters such as : clinical attachment levels, SLE drug therapy, etc., have to be done, in order to confirm the preliminary observations obtained in this investigation.

On the other hand, the results of this investigation show a high ANCA reactivity for proteinase 3 in sera of SLE and periodontitis patients. We do not recall previous reports of this specific reactivity in SLE, a fact which also deserves further attention.

Resumen

El propósito de esta investigación es determinar si la presencia de ANCA relacionados a una condición periodontal, impide efectuar una precisa evaluación de estos autoanticuerpos en lupus eritematoso sistémico (LES), debido a la alta incidencia de ANCA en periodontitis.

En este estudio se investigaron treinta pacientes con LES, treinta con periodontitis sin compromiso sistémico y veinte controles sanos. La evaluación periodontal de todos los pacientes se realizó cuidadosamente. Para la determinación de ANCA, se utilizó un ensayo de ELISA dirigido contra un extracto granular de neutrófilos y seis proteínas granulares. Sesenta por ciento de los pacientes con LES tenían periodontitis y el sesenta y cinco por ciento

fueron ANCA positivos. El ochenta y tres por ciento de todos los casos positivos de ANCA en LES coexistían con periodontitis. Se encontró una asociación entre ANCA y periodontitis estadísticamente significativa ($p < 0.005$), a través de una prueba de Chi cuadrado. El cincuenta por ciento de los pacientes con periodontitis sin compromiso sistémico fueron ANCA positivos.

Los resultados de este estudio sugieren que la presencia de periodontitis no detectada en pacientes con LES es la responsable de la mayoría de los casos de ANCA reportados para esta enfermedad sistémica.

Acknowledments

The authors like to thank Dr. Ramón Soto, Chief of the Dental Service, Central Hospital, Maracaibo, Venezuela, for the invaluable assistance provided by his department staff, and Dr. Yolanda Crozzoli, Dr. Neira Chaparro and MS Ninoska Viera for their help in the aspects related to periodontal managing and ANCA testing of the study groups. Also, we want to express our gratitude to Dr. Jesus Mosquera for his assistance related to the graphics work.

References

1. Davies DJ, Moran JE, Niall JF, Ryan GB. Segmental necrotizing glomerulonephritis with antineutrophil antibody: possible arbovirus aetiology. Br Med J 1982;2:606.
2. Hauschild S, Schmitt W, Csernok E, Flesh B, Rautmann A, Gross W. ANCA in systemic vasculitides, collagen vascular diseases, rheumatic disorders and inflammatory bowel diseases. In: Gross W. (ed). ANCA-Associated Vasculitides. Plenum Press, London, 1993.
3. Savige J, Davies D, Gatenby P. Anti-neutrophil cytoplasmic antibodies (ANCA): their detection and significance: report from workshops. Pathology 1994;26:186-193.
4. Ardiles LG, Valderrama G, Moya P, Mezzano A. Incidence and studies on antigenic specificities of antineutrophil-cytoplasmic antibodies (ANCA) in poststreptococcal glomerulonephritis. 1997; 47:1-15.
5. Van der Woude FJ, Rasmussen N, Lobato S, Wiik A, Permin H, Van Es L, Van der Giessen M, Van der Hem G. The T autoantibodies to neutrophils and monocytes: a new tool for diagnosis and a marker of disease activity in Wegener's granulomatosis. Lancet 1985;ii:425-429.
6. Kallenberg C, Brouwer E, Weening J, Cohen Tervaert J. Antineutrophil cytoplasmic antibodies: current diagnostic and pathophysiological potential. Kidney Int 1994;46:1-15.
7. Kuster S, Apenberg S, Andrassy K, Ritz E. Antineutrophil cytoplasmic antibodies in systemic lupus erythematosus. Contrib Nephrol 1992;99:94-98.
8. Schnabel A, Csernok E, Isenberg DA, Mrowka C, Gross WL. Antineutrophil cytoplasmic antibodies in systemic lupus erythematosus. Prevalence, specificities, and clinical significance. Arthritis Rheum 1995;38: 633-637.
9. Pauzner R, Urowitz M, Gladman D, Gough J. Antineutrophil cytoplasmic antibodies in sistemic lupus erythematosus. J Rheumatol 1994;21:1670-1673.
10. Wong SN, Shah V, Dillon MJ. Antineutrophil cytoplasmic antibodies in childhood systemic lupus erythematosus. Eur J Pediatr 1995;154:43-45.
11. Novo E, Viera N. Antineutrophil cytoplasmic antibodies: a missing link in the pathogenesis of periodontal disease? J Periodont Res 1996;31:365-368.
12. Socransky SS, Haffajee AD, Goodson JM, Lindhe J. New concepts of destructive periodontal disease. J Clin Periodontol 1984;11:21-32.
13. Schluger S, Yuodelis RA, Page RC. Periodontal Disease. 2nd ed. Philadelphia, Lea and Febinger, 1990.
14. Ismail AJ, Morrison EC, Burt BA, Caffessee RG, Karanah MR. Natural history of periodontal disease in adults: findings from the Tecumseh periodontal study, 1959-1987. J Dent Res 1990; 69:430-435.
15. Haffajee AD, Socransky SS, Dzink JL, Taubman MA, Ebersole JL, Smith DJ. Clinical, microbiological, and immunological features of subjects with destructive periodontal disease. J Clin Periodontol 1988;15:240-246.
16. Genco RJ, Goldman HM, Cohen DW. Contemporary Periodontics. St. Louis, Mosby, 1990.
17. Safkkan-Seppala A. Periodontal conditions in insulin-dependent diabetes mellitus. J Clin Periodontol 1992;19:24-29.
18. Tan EM, Cohen AS, Fries JF, Masi AT, McShane DJ, Rothfield NF, Schaller JG, Talal N, Winchester RJ. The 1982 revised criteria for the classification of systemic lupus erythematosus. Arthritis Rheum 1982;25:1271-1277.
19. Polson AM, Goodson JM. Periodontal diagnosis. Current status and future needs. J Periodontol 1985;56:25-34.
20. Goodson JC. Clinical measurements of periodontitis. J Clin Periodontol 1986;13:446-450.
21. Riches DW, Young SK, Seccombe JF, Clay KL, Henson PM. The subcellular distribution of platelet-activating factor in stimulated human neutrophils. J Immunol 1990;145:3062-3069.
22. Klempner MS, Mikkelsen RB, Corfman DH, Schwartz JA. Neutrophil plasma membranes. I. High-yield purification of human neutrophils plasma membrane vesicles by nitrogen cavitation and differential centrifugation. J Cell Biol 1980; 86: 21-25.
23. Lowry OH, Rosebrough NJ, Farr AL, Randall RJ. Protein measurement with the Folin phenol reagent. J Biol Chem 1951; 193:265-275.
24. Jonsson R, Heyden G, Westberg G, Nyberg G. Oral mucosal lesions in systemic lupus erythematosus. A clinical, histopathological and immunopathological study. J Rheumatol 1984;11:38-42.
25. Rose LF, Kaye DK. editors: Internal medicine for dentistry. St. Louis, Mosby, 1983.