SIGNIFICANCE OF NUCLEAR IMMUNOFLOUORESCENT PATTERNS

BY

C. A. DORSCH,* C. B. GIBBS, M. B. STEVENS, AND L. E. SHULMAN

From The Connective Tissue Division, Department of Medicine, School of Medicine, The Johns Hopkins Hospital

Immunofluorescent techniques have been used extensively to detect the presence of antinuclear factors (ANF) in sera from patients with systemic lupus erythematosus and related disorders. Furthermore, morphological differences in the character of nuclear immunofluorescence have been considered to be of clinical as well as immunological significance (Casals, Friou, and Teague, 1963; Burnham, Fine, and Neblett, 1966).

Four patterns of nuclear staining have been described, namely, homogeneous, peripheral†, speckled, and nucleolar. The homogeneous and peripheral patterns, reflecting antibodies to nucleoprotein and deoxyribonucleic acid respectively, have been correlated primarily with the clinical diagnosis of systemic lupus erythematosus (SLE) (Friou, 1958; Casals and others, 1963). Less well defined are the specific antigen-antibody systems responsible for speckled fluorescence and preferential staining of nucleoli. Moreover, these latter two immunofluorescent patterns have been correlated for the most part with disorders other than SLE, notably progressive systemic sclerosis and Sjögren's syndrome (Beck, 1963; Burnham and others, 1966).

In the present study, the clinical significance of immunofluorescent patterns and factors which influence the morphology of nuclear staining have been evaluated in sera from 76 patients with SLE, 34 patients with rheumatoid arthritis, and 39 patients with miscellaneous disorders. No pattern of nuclear immunofluorescence proved to be specific for any clinical syndrome. Furthermore, the morphology of nuclear immunofluorescence was found to vary with serum dilution. Finally, mercaptoethanol

sensitivity of antinuclear antibodies failed to correlate with either clinical syndrome or immunofluorescent pattern.

Material and Methods

Sera.—These were selected from the frozen serum bank of the Connective Tissue Division of the Johns Hopkins Hospital on the basis of previous demonstration of antinuclear factor (ANF) in undiluted serum by the immunofluorescent method, and certainty of clinical diagnosis. A single specimen of serum from each of 149 patients was studied.

Clinical Diagnosis.—76 patients had a clinical diagnosis of systemic lupus erythematosus (SLE) with L.E.-cells demonstrated at some time during the course in each individual. 34 fulfilled the A.R.A. criteria (Ropes, Bennett, Cobb, Jacox, and Jessar, 1959) for definite or classical rheumatoid arthritis. Of these, seven were patients with Felty's syndrome. The third group of 39 patients with miscellaneous disorders included eight patients with Raynaud's phenomenon, five with progressive systemic sclerosis, five with myasthenia gravis, four with Sjögren's syndrome, three with a chronic biological false positive test for syphilis, three with hepatocellular disease, and eleven with unrelated disorders.

Immunofluorescent Assay for Antinuclear Factors (ANF).

—This was performed by the method of Holborow, Weir, and Johnson (1957), using frozen 4 μ -thick sections of mouse liver as nuclear substrate and rabbit antiserum to human F-II gamma globulin, conjugated with fluorescein as indicator. Each serum containing ANF was tested undiluted and at dilutions of 1:10 and 1:100. All sera and dilutions were coded and slides were arranged in random order before fluorescent microscopy (320 $\times$ magnification).

Studies with 2-Mercaptoethanol.—Sera from 47 of the patients with SLE and 33 of those with rheumatoid arthritis were also studied after treatment with 2-mercaptoethanol. Sera were incubated at room temperature with an equal volume of 0.2 M 2-mercaptoethanol for

*Henry Strong Denison Scholar, 1967-68, for Medical Research.
This work was supported by an Arthritis Foundation Clinical Study Centre Grant, and in part by a U.S. Public Health Service Clinical Research Grant (5 MO 1 FR 35 07).
†Also termed membranous or shaggy.

16-18 hours; this mixture was then incubated with an equal volume of 0.04 *M* iodoacetic acid for 2 hours and subsequently dialysed overnight against three changes of pH 7.3 phosphate buffer. This resulted in a final serum dilution of 1:4. Untreated sera were similarly diluted 1:4 and dialysed against same equilibrating buffer. Aliquots, with and without mercaptoethanol treatment, were tested simultaneously at the dilutions 1:4, 1:10, and 1:100 by the previously stated technique.

Results

Patterns of Nuclear Immunofluorescence in Undiluted Sera

As shown in Table I, a single pattern of nuclear immunofluorescence was observed in the majority (60 per cent.) of patients when undiluted sera were tested. However, 59 (40 per cent.) of the sera showed mixed or multiple patterns of staining with more than one type of fluorescence observed in a single tissue section. Such multiple patterns occurred with sera in all clinical groups.

The frequency of each pattern or combination of patterns of nuclear staining for each clinical group is also shown in Table I. Homogeneous, peripheral, and speckled fluorescence each occurred alone and in all possible combinations. No instance of nucleolar immunofluorescence was found. The peripheral (P) pattern of immunofluorescent staining, occurring singly or in combination, was the most frequent type observed, being found in 102 (68 per cent.) of the 149 sera. Homogeneous (H) staining was found in 69 (46 per cent.) and speckled staining in 41 (28 per cent.) of the sera studied.

No immunofluorescent pattern, singly or mixed, was specific for any clinical group. Among patients with SLE, peripheral fluorescence was most frequent, while the homogeneous type was most frequent among those with rheumatoid arthritis. Least

common in these two clinical groups was the speckled pattern of immunofluorescence.

While peripheral staining was the most frequently observed pattern in patients with miscellaneous disorders, speckled fluorescence appeared in a larger proportion of these patients than was observed for the other two groups. Immunofluorescent speckling was most prominent in sera from patients with Raynaud's phenomenon (5 of 8), with Sjögren's syndrome (4 of 5), and with progressive systemic sclerosis (2 of 4).

Effect of Serum Dilution on Immunofluorescent Pattern

The number of ANF positive sera decreased with serum dilution in each of the three clinical groups (Table II). However, while 32 (42 per cent.) of the 76 sera from patients with SLE were still positive at a 1:10 dilution, only nine (26 per cent.) of those from patients with rheumatoid arthritis and six (15 per cent.) of those from patients with miscellaneous disorders remained positive at that dilution.

Table II shows that all single and mixed patterns still occurred at each dilution with the exception of the homogeneous-peripheral-speckled combination which was not found at the maximum dilution. Mixed patterns of fluorescence, which occurred in 40 per cent. of undiluted sera, were found with 57 (48 per cent.) of the 119 sera positive at 1:10 dilution and with thirteen (28 per cent.) of the 47 sera positive at 1:100 dilution.

The effect of serum dilution upon the pattern of nuclear immunofluorescence may be expressed in one of several ways:

- (1) The pattern observed in undiluted serum may persist with dilution.
- (2) The sample may become negative as serum is diluted.

TABLE I
FREQUENCY OF SINGLE, MULTIPLE, AND SPECIFIC PATTERNS OF NUCLEAR IMMUNOFLOUORESCENCE WITH UNDILUTED SERA

Clinical Diagnosis	No. of Patients	Pattern of Immunofluorescence										
		Single					Multiple					
		H	P	S	Total		H-P	H-S	P-S	H-P-S	Total	
					No.	Per cent.					No.	Per cent.
Systemic Lupus Erythematosus	76	7	30	6	43	56	18	6	6	3	33	44
Rheumatoid Arthritis	34	13	9	2	24	71	9	0	0	1	10	29
Miscellaneous	39	1	17	5	23	59	4	7	5	0	16	41
Total	149	21	56	13	90	60	31	13	11	4	59	40

H = Homogeneous P = Peripheral S = Speckled

TABLE II
PATTERNS OF NUCLEAR IMMUNOFLUORESCENT STAINING WITH SERUM DILUTION

Clinical Diagnosis	Serum Dilution	Number of Antinuclear Factor Positive Patients	Patterns of Immunofluorescence						
			Single			Multiple			
			H	P	S	H-P	H-S	P-S	H-P-S
Systemic Lupus Erythematosus	Undiluted	76	7	30	6	18	6	6	3
	1: 10	53	6	12	13	20	6	3	3
	1: 100	32	7	3	12	5	4	1	0
Rheumatoid Arthritis	Undiluted	34	13	9	2	9	0	0	1
	1: 10	32	11	7	1	10	1	1	1
	1: 100	9	4	2	1	2	0	0	0
Miscellaneous	Undiluted	39	1	17	5	4	7	5	0
	1: 10	24	3	5	4	5	6	1	0
	1: 100	6	1	0	4	0	1	0	0

H = Homogeneous P = Peripheral S = Speckled

- (3) The pattern observed in undiluted serum, whether single or multiple, may change with dilution. This change may be expressed as loss of one or more components of a mixed pattern or as the emergence of a type of immunofluorescence not observed in the undiluted sample.

The first two of these possibilities represent common phenomena in immunological assays, representing variation in the serum concentration of antibody. The emergence of new immunofluorescent staining types with serum dilution was a more surprising finding. As seen in Table III, all three types of nuclear immunofluorescence demonstrated this finding as serum was diluted. This was, however,

most common for the homogeneous and speckled types. The tendency for fluorescent types to emerge with dilution was not confined to any one of the three clinical groups of patients.

Sensitivity of Antinuclear Fluorescence to 2-mercaptoethanol

Sera from 47 patients with SLE and 33 with rheumatoid arthritis were examined before and after treatment with 2-mercaptoethanol (ME)*.

ME-sensitive ANF occurred both in patients with SLE and in those with rheumatoid arthritis (Table IV). However, 21 per cent. (7/33) of those with

*The effectiveness of ME-treatment in abolishing activity of 19S immunoglobulin is supported by the following observations:

- (1) Serum from a patient with rheumatoid arthritis whose ANF activity was completely abolished by treatment with ME was subjected to gel filtration on a Sephadex G-200 column. Before ME treatment, ANF was found only in the macroglobulin peak, further defined as IgM by Ouchterlony gel diffusion.
- (2) Rheumatoid factor was determined by the latex fixation technique of Singer and Plotz (1956) using human F-II gamma-globulin as antigen. Treatment of serum with ME resulted in loss of rheumatoid factor activity from all those sera which originally contained it.

TABLE III
EMERGENCE OF IMMUNOFLUORESCENT PATTERNS WITH DILUTION OF SERUM

Pattern of Immunofluorescence		Number of Patients		
Serum Dilution		Systemic Lupus Erythematosus	Rheumatoid Arthritis	Miscellaneous
None	1: 10			
Non-H	H	17	8	9
Non-P	P	5	8	1
Non-S	S	12	3	5

H = Homogeneous P = Peripheral S = Speckled

TABLE IV
SENSITIVITY OF ANTINUCLEAR FACTOR TO 2-MERCAPTOETHANOL (ME)

Clinical Diagnosis	Number of Patients	ME-Sensitive						ME-Insensitive	
		Complete		Partial		Total		No.	Per cent.
		No.	Per cent.	No.	Per cent.	No.	Per cent.		
Systemic lupus	47	2	4	15	32	17	36	30	64
Rheumatoid arthritis	33	7	21	11	33	18	54	15	46
Total	80	9	11	26	33	35	44	45	56

rheumatoid arthritis produced only ME sensitive ANF as compared with 4 per cent. (2/47) of those with SLE. Interestingly, four of the seven rheumatoid arthritis patients whose antinuclear fluorescence was completely abolished by ME-treatment were patients who had Felty's syndrome.

Table V presents the ME-sensitivities of individual patterns of nuclear immunofluorescence. Of the 47 sera studied from patients with SLE, forty had peripheral fluorescence, 34 had homogeneous fluorescence, and twenty had speckled fluorescence occurring at one or more dilutions, either singly or in combination with another pattern. Similarly, of the 33 sera from rheumatoid arthritis patients, 23 had peripheral, 27 homogeneous, and six speckled fluorescence occurring at some dilution as a single or mixed pattern.

The incidence of ME-sensitive homogeneous and speckled fluorescence types was essentially the same in both SLE and rheumatoid arthritis. Thus, 36 per cent. of the homogeneous fluorescence in patients with SLE was ME-sensitive compared with 29 per cent. in patients with rheumatoid arthritis. Speckled fluorescence was ME-sensitive in 35 per cent. of those SLE sera and in 33 per cent. of those rheumatoid arthritis sera which originally produced it.

However, the incidence of ME-sensitive peripheral fluorescence was significantly different in the two groups of patients. In fourteen of the 23 rheumatoid arthritis sera (61 per cent.) having peripheral fluorescence, this type was completely or partially sensitive to destruction with ME, while ME-sensitivity occurred in only seven of the forty SLE sera (18 per cent.) with peripheral fluorescence. Of interest was the fact that, in patients with Felty's syndrome, peripheral fluorescence was ME-sensitive in five of the six patients in whom this pattern occurred.

Discussion

Since the recognition of various patterns of nuclear immunofluorescent staining produced by sera from patients with connective tissue disorders, large numbers of patients have been surveyed in an attempt to establish diagnostic or prognostic significance for a given staining type. Authors have differed in the frequency with which various immunofluorescent patterns are found in different disease states (Table VI).

In our patients, homogeneous fluorescence occurred in a significant number of sera in all clinical groups. Like Beck (1961, 1963), we found homogeneous staining to be the most frequent type of

TABLE V
RELATIONSHIP OF 2-MERCAPTOETHANOL (ME) SENSITIVE ANTIBODY TO IMMUNOFLUORESCENT PATTERN

Pattern of Immunofluorescence	Clinical Diagnosis	Number of Patients	ME-Sensitive Antinuclear Factor	
			No.	Per cent.
Peripheral	Systemic lupus	40	7	18
	Rheumatoid arthritis	23	14	61
Homogeneous	Systemic lupus	34	12	36
	Rheumatoid arthritis	27	8	29
Speckled	Systemic lupus	20	7	35
	Rheumatoid arthritis	6	2	33

TABLE VI
PATTERNS OF NUCLEAR IMMUNOFLUORESCENCE IN SERIES REPORTED BY VARIOUS AUTHORS

Authors	Date	Patterns of Immunofluorescence								
		Systemic Lupus Erythematosus			Rheumatoid Arthritis			Miscellaneous		
		H	P	S	H	P	S	H	P	S
Beck	1961	3	NE	4	4	NE	1	7	NE	11
Beck	1963	33	NE	15	17	NE	4	103	NE	46
Casals and others	1963	22	5	1	NE	NE	NE	NE	NE	NE
Gonzalez and Rothfield	1966	59	39	2	32	0	11	27	0	25
Burnham and others	1966	43	17	0	27	12	0	70	23	15

NE = Not examined

immunofluorescence in patients with rheumatoid arthritis, in contrast to the observations of Friou (1958), who reported that the antinuclear factor reacting with nucleoprotein spots and responsible for the homogeneous staining of cell nuclei was almost entirely confined to SLE sera and only rarely associated with rheumatoid disease or Sjögren's syndrome.

Speckled fluorescence was most frequent in our miscellaneous group, especially in patients with systemic sclerosis, Raynaud's phenomenon, and, in agreement with Beck (1963), Sjögren's syndrome. The almost complete specificity of speckling for systemic sclerosis observed by Burnham and others, (1966) was not confirmed. However, as suggested by these authors, such a discrepancy between their observations and the reports of others might result from differences in the definition of speckled fluorescence.

Peripheral immunofluorescence has been reported to occur solely (Gonzalez and Rothfield, 1966) or predominantly (Casals and others, 1963) in patients with systemic lupus. Such a striking association was not apparent in our sera. While peripheral immunofluorescence did represent the most frequent type of staining found in our patients with SLE, it was also the most frequent type in the miscellaneous group. Burnham and others (1966) similarly observed peripheral immunofluorescence in all disease groups studied; and Barnett, Ruderman, Jeannet, and Bloch (1966) described the peripheral pattern in their patients with Felty's syndrome.

No instance of nucleolar staining was observed in our patients. Although Burnham and others (1966) found nucleolar staining to be specific for systemic sclerosis, Beck (1963) demonstrated this rare pattern in systemic and discoid lupus, Sjögren's syndrome, and pernicious anaemia also. Thus, it would appear that this immunofluorescent type of staining, like the other more common ones, cannot be considered diagnostically specific.

Among the several variables to be controlled when performing immunofluorescent assays for antinuclear factors is the dilution of serum. Patterns of immunofluorescence not apparent in undiluted sera may appear with dilution. Thus, while more than one type of nuclear immunofluorescence occurred in 59 (40 per cent.) of the 149 positive undiluted sera, when all dilutions were considered 116 (78 per cent.) of the 149 sera produced more than one type of staining. This emergence of new patterns of immunofluorescence with dilution occurred in all clinical groups and with all staining patterns. The explanation for this phenomenon remains unclear.

As noted previously by Beck (1961), when homogeneous and speckled fluorescence occur in the same undiluted serum, loss of the homogeneous pattern with dilution may allow speckled staining to become clearly visible. This visual masking of one type of fluorescence by another cannot represent the sole explanation, as evidenced by sera which, undiluted, show speckling but with dilution produce the homogeneous or peripheral patterns. While Ritchie, Bayles, and Harter (1965) have reported that 8 per cent. of rheumatoid sera which were negative undiluted became positive after a dilution of 1:8, sera from more than 1,500 patients with a wide variety of disorders (including rheumatoid arthritis) have been routinely assayed in our laboratory undiluted and at dilutions of 1:10 and 1:100 and in no instance has antinuclear factor appeared with dilution in a serum which was negative undiluted.

Finally, immunofluorescent patterns were considered in relation to the immunoglobulin class of antinuclear antibody, using 2-mercaptoethanol as a means of inactivating antibodies of high molecular weight. Mercaptoethanol has been shown completely to inactivate immunoglobulins of the IgM class (Deutsch and Morton, 1957). While IgG antibody is not affected by treatment with 2-mercaptoethanol, antibody of the IgA class may be altered to a variable degree. Barnett, Condemi, Leddy, and Vaughan (1964) found IgA antinuclear factor activity to be completely or partially destroyed in six of eight so tested. Thus, in the present study, sensitivity of antinuclear factor to inactivation by mercaptoethanol may represent IgM antibody alone or a combination of IgM and IgA antibody. Studies by other groups have failed to demonstrate in sera antinuclear antibody of only the IgA class.

While both mercaptoethanol-sensitive and mercaptoethanol-insensitive antinuclear factors were found in patients with SLE and rheumatoid arthritis, the incidence of mercaptoethanol-sensitive antibody was greater in cases of rheumatoid disease. These findings are consistent with the observations of Weir and Holborow (1962), using mercaptoethanol dissociation, and Baum and Ziff (1962), using column chromatography, who also reported an increased incidence of macroglobulin antinuclear antibody in rheumatoid arthritis. Barnett and others (1964) found antinuclear antibody of all three immunoglobulin classes in patients with SLE and rheumatoid arthritis with the mean titre of IgG antibody in rheumatoid arthritis less than its mean titre in SLE. The IgM antibody titres were comparable in the two disease states. These observations further

suggest an increased tendency in patients with rheumatoid arthritis to produce antinuclear antibody of the macroglobulin type.

Finally, in the present study, antibody sensitivity to mercaptoethanol was not specific for any pattern of nuclear immunofluorescence, either in patients with systemic or those with rheumatoid arthritis. Gonzales and Rothfield (1966) similarly found homogeneous and speckled staining to be produced equally by the three immunoglobulin classes, in contrast to the observations of Bonomo, Tursi, and Dammacco (1965) who fractionated nine sera by DEAE-cellulose chromatography and found homogeneous nuclear staining related to 7S antibody and speckling associated with antibody in the macroglobulin fraction. In our series, comparing patients with systemic lupus to those with rheumatoid arthritis, homogeneous and speckled patterns sensitive to mercaptoethanol were comparable in frequency. However, a significantly greater proportion of rheumatoid sera producing the peripheral pattern had mercaptoethanol-sensitive antibody. Sensitivity to mercaptoethanol was found in five of the six patients with Felty's syndrome and peripheral

staining, but even when these cases are excluded, the observed difference between systemic lupus and rheumatoid disease remains as further evidence supporting the trend in rheumatoid patients towards a macroglobulin antinuclear antibody response to certain, though not all, nuclear antigens.

Summary

Patterns of nuclear immunofluorescence produced by the sera from 76 patients with systemic lupus erythematosus, 34 with rheumatoid arthritis, and 39 with miscellaneous disorders have been studied.

Homogeneous, peripheral, and speckled patterns occurred singly or in all possible combinations in each of the clinical groups. No instance of nucleolar staining was found.

Serum dilution proved to be a major determinant with respect to the morphology of nuclear immunofluorescence.

Neither the pattern of immunofluorescent staining nor the patient's clinical syndrome was found to correlate specifically with sensitivity of antinuclear antibody to 2-mercaptoethanol.

REFERENCES

- Barnett, E. V., Condemni, J. J., Leddy, J. P., and Vaughan, J. H. (1964). *J. clin. Invest.*, **43**, 1104 (Gamma₂, gamma_{1A}, and gamma_{1M} antinuclear factors in human sera).
- , Ruderman, M., Jeannet, M., and Bloch, K. J. (1966). *Arthr. and Rheum.*, **9**, 846 (Felty's syndrome: Occurrence of antinuclear antibodies and leukocyte agglutinins in 14 patients).
- Baum, J., and Ziff, M. (1962). *Ibid.*, **5**, 636 (7S and macroglobulin antinuclear fluorescence factors in systemic lupus erythematosus and rheumatoid arthritis).
- Beck, J. S. (1961). *Lancet*, **1**, 1203 (Variations in the morphological patterns of "autoimmune" nuclear fluorescence).
- (1963). *Scot. med. J.*, **8**, 373 (Auto-antibodies to cell nuclei).
- Bonomo, L., Tursi, A., and Dammacco, F. (1965). *J. Lab. clin. Med.*, **66**, 42 (Characterization of the antibodies producing the homogeneous and the speckled fluorescence patterns of cell nuclei).
- Burnham, T. K., Fine, G., and Neblett, T. R. (1966). *Ann. intern. Med.*, **65**, 9 (The immunofluorescent tumor imprint technique. II. The frequency of antinuclear factors in connective tissue diseases and dermatoses).
- Casals, S. P., Friou, G. J., and Teague, P. O. (1963). *J. Lab. clin. Med.*, **62**, 625 (Specific nuclear reaction pattern of antibody to DNA in lupus erythematosus sera).
- Deutsch, H. F., and Morton, J. I. (1957). *Science*, **125**, 600 (Dissociation of human serum macroglobulins).
- Friou, G. J. (1958). *Ann. intern. Med.*, **49**, 866 (The significance of the lupus globulin-nucleoprotein reaction).
- Gonzalez, E. N., and Rothfield, N. F. (1966). *New Engl. J. Med.*, **274**, 1333 (Immunoglobulin class and pattern of nuclear fluorescence in systemic lupus erythematosus).
- Holborow, E. J., Weir, D. M., and Johnson, G. D. (1957). *Brit. med. J.*, **2**, 732 (A serum factor in lupus erythematosus with affinity for tissue nuclei).
- Ritchie, R. F., Bayles, T. B., and Harter, J. G. (1965). *Arthr. and Rheum.*, **8**, 463 (The significance of a negative and a "false negative" test for antinuclear factors in the diagnosis of rheumatic disease).

- Ropes, M. W., Bennet, G. A., Cobb, S., Jacox, R., and Jessar, R. A. (1959). *Ann. rheum. Dis.*, **18**, 49 (1958 Revision of diagnostic criteria for rheumatoid arthritis).
- Singer, J. M., and Plotz, C. M. (1956). *Amer. J. Med.*, **21**, 888 (The latex fixation test. I. Application to the serologic diagnosis of rheumatoid arthritis).
- Weir, D. M., and Holborow, E. J. (1962). *Ann. rheum. Dis.*, **21**, 40 (Serum antinuclear factor: laboratory studies).

La signification des images nucléaires immunofluorescentes

RÉSUMÉ

Les images nucléaires immunofluorescentes produites par le sérum de 76 malades atteints de lupus érythémateux disséminé, de 34 malades atteints de polyarthrite rhumatoïde et 39 malades atteints d'affection variées ont été étudiées.

Les images homogènes, périphériques et tachetées avaient apparu séparément ou dans toutes les combinaisons possibles dans chaque groupe clinique. Aucun cas de coloration nucléolaire n'avait été trouvé. La dilution du sérum a été un déterminant majeur quant à la morphologie d'immunofluorescence nucléaire.

On n'a démontré aucune corrélation spécifique entre les images de la coloration immunofluorescente ou le syndrome clinique et la sensibilité de l'anticorps anti-nucléaire au 2-mercaptoethanol.

El significado de imagenes inmunofluorescentes nucleares

SUMARIO

Se han estudiado los imagenes inmunofluorescentes nucleares producidos por el suero de 76 pacientes con lupus eritematoso sistémico, 34 pacientes con poliartritis reumatoide y 39 pacientes con desórdenes variados.

Los esquemas homogéneos, periféricos y jaspeados se presentaron separadamente o en todas las combinaciones posibles en cada uno de los grupos clínicos. No se encontró ningún caso de coloración nucleolar.

La dilución de suero resultó ser un determinante de importancia con respecto a la morfología de la inmunofluorescencia nuclear.

Se descubrió que ni el esquema de coloración inmunofluorescente ni el síndrome clínico del paciente tenían correlación específica con la sensibilidad del anticuerpo antinuclear al 2-mercaptoetanol.