December 1986:1307–11

Antinuclear Antibodies in Primary Pulmonary Hypertension

STUART RICH MD, FACC,* KAREN KIERAS, RN,* KIM HART, RN,*
BERTRON M. GROVES, MD, FACC,† JOHN D. STOBO, MD,‡ BRUCE H. BRUNDAGE, MD, FACC*

Chicago, Illinois, San Francisco, California, Denver, Colorado

The association of positive antinuclear antibodies with the clinical and hemodynamic features of 43 patients with primary pulmonary hypertension and 16 patients with secondary pulmonary hypertension was investigated. Each patient had determinations of antinuclear antibodies using a KB cell substrate immunofluorescent test. Of the patients with primary pulmonary hypertension, 40% had positive antinuclear antibodies at titers of 1:80 dilutions or greater. There were no differences between patients with primary pulmonary hypertension and positive antinuclear antibodies compared with those with negative antinuclear antibodies in relation to clinical or hemodynamic status. A 6% incidence rate of antinuclear antibodies was found in patients with secondary pulmonary hypertension, similar to that in the normal population.

The clinical, hemodynamic, serologic and histologic similarity between patients with primary pulmonary hypertension and those with unexplained pulmonary hypertension associated with collagen vascular disorders suggests that primary pulmonary hypertension in some patients may represent a collagen vascular disease confined to the lungs. The frequency of positive antinuclear antibody tests would place primary pulmonary hypertension between rheumatoid arthritis and scleroderma in the spectrum of collagen vascular diseases. Further studies are necessary, however, before one might expect that immunosuppressive therapy would be beneficial to these patients.

(J Am Coll Cardiol 1986;8:1307–11)

Primary pulmonary hypertension has been associated with autoimmune phenomena in previously reported studies (1). The meaning of this association is unclear, but because of the occurrence of pulmonary hypertension in the collagen vascular diseases it has been speculated that there may be a link between primary pulmonary hypertension and collagen vascular or autoimmune disorders (2,3). We investigated this relation by evaluating the frequency and titer of antinuclear antibodies in patients with both primary and secondary pulmonary hypertension.

Methods

Study patients. The study group consists of 43 patients referred to the University of Illinois at Chicago, University of California in San Francisco and University of Colorado with primary pulmonary hypertension, as well as 16 patients from the former two universities with secondary pulmonary hypertension. All patients sought medical attention because of clinical symptoms related to their disease. Each patient underwent an extensive evaluation to search for any secondary cause of pulmonary hypertension (4). Patients were also screened for recent therapy with drugs known to cause positive antinuclear antibody (ANA) tests, and underwent thorough clinical examinations for eye, skin, joint or other organ system findings that might suggest the presence of any type of collagen vascular disorder.

Patient characterization. Patients were classified from their history according to functional capacity by the New York Heart Association classification system, and the duration of their disease was estimated as the period between the time that the patient first noted symptoms that could be attributed to pulmonary hypertension until the current evaluation. Patients who had no identifiable cause for pulmonary hypertension were classified as having primary pulmonary hypertension. Those with congenital or acquired heart disease or lung disease were classified as having secondary pulmonary hypertension, even if the degree of the pulmonary hypertension seemed out of proportion to their underlying disease state.

From the *Department of Medicine, University of Illinois, Chicago, Illinois, the †Department of Medicine, University of Colorado, Denver, Colorado and the ‡Department of Medicine, University of California, San Francisco, California.

Manuscript received February 28, 1986; revised manuscript received June 25, 1986, accepted July 7, 1986.

Address for reprints: Stuart Rich, MD, Section of Cardiology, University of Illinois Hospital, P.O. Box 6998, Chicago, Illinois 60680.

0735-1097/86/$3.50

Hemodynamic evaluation. Baseline hemodynamic values were determined with the patients at rest, supine, after an overnight fast while receiving no cardiac medications other than digoxin and diuretic agents. A thermodilution flow-directed catheter was introduced into the pulmonary artery, and a short Teflon cannula was placed into a radial or femoral artery. Pressures were recorded on a multichannel recorder with mean pressures determined by electronic integration. Cardiac output was taken as the average of three serial determinations using a thermodilution computer. The calculation of systemic and pulmonary vascular resistance (in Wood units) was according to conventional formulas.

Antinuclear antibody determinations. Two sets of antinuclear antibody determinations were performed on each patient, with their informed consent. One was through the conventional hospital laboratory for the respective hospital, and employed indirect fluorescent antibody testing using the Hep2 cell line at the University of Illinois, Swiss-Webster mouse kidney cell at the University of Colorado and KB cell line at the University of California. In addition, each sample was tested again in the University of Illinois research laboratory for antinuclear antibody with an indirect fluorescent antibody test utilizing the human KB cell line (Electro-Nucleonics, Inc.). Peripheral blood serum samples were either analyzed immediately, or frozen at −20°C and analyzed at a later date. Each serum was diluted 1:10, followed by doubling dilutions up to 1:1,280. Goat antihuman immunoglobulinG conjugated with fluorescein isothiocyanate (Electro-Nucleonics, Inc.) was used as the second antibody. The slides were provided with a cover glass using phosphate-buffered glycerol (pH 8.0) and read on a Nikon epifluorescent microscope with standard fluorescein filters and a fluorite 40× objective. A positive test was determined by the presence of nuclear fluorescence at a dilution of 1:20 or greater. The end point for the titer was the highest dilution showing a 1+ intensity. Samples that showed a positive reaction at 1:20 were then titered for the end point of reactivity. The staining pattern was also noted, and subcategorized as homogeneous, peripheral, speckled or nucleolar. Internal controls from sera with known positive and negative antinuclear antibodies were established before the testing of each group of samples.

Control population. To reestablish the frequency of elevated titers in a presumed normal population, serum samples were obtained from 20 university employees who were healthy and taking no medications. These specimens were tested on two separate occasions for antinuclear antibody using the KB cell substrate. Our normal control subjects had the following results: negative, 11 subjects; positive (1:20), 5 subjects; positive (1:40), 4 subjects and positive (>1:40), no subjects.

Statistical methods. The mean and standard deviation for variables in patients with and without a positive antinuclear antibody test were computed, and differences be-

Table 1. Patients With Primary Pulmonary Hypertension and a Positive Antinuclear Antibody Test

Case	Age (yr) & Sex	NYHA Class	Duration (yr)	PAP (mm Hg)	PVR (units)	ANA Titer	Type
1	28F	II	2.0	41	7	1280	S
2	22F	II	0.5	35	5.7	1280	P
3	20F	II	1.5	77	29	1280	P
4	45M	IV	7.0	67	22.9	1280	P
5	27F	III	4.0	65	47.7	1280	S
6	11F	III	0.6	83	38.7	640	S
7	59F	III	4.0	71	7.9	640	S
8	53F	IV	2.0	63	44.0	640	S
9	35F	III	2.6	78	23.9	640	S
10	52F	III	4.0	62	13.6	320	S
11	60F	III	5.0	58	11.6	320	S
12	40F	IV	1.5	50	19.2	320	S
13	55M	II	0.2	65	12.6	160	S
14	55F	III	8.0	55	14.4	80	H
15	20F	IV	1.0	49	10.3	80	H
16	22F	III	0.6	42	12.3	80	P
17	42M	II	5.0	48	13.8	80	S
Mean (n = 8)	38	2.9	2.9	59.3	19.7		
±SD	16	0.7	2.3	13.9	13		

ANA = antinuclear antibody; F = female; H = homogeneous; M = male; NYHA class = New York Heart Association functional class; P = peripheral; PAP = mean pulmonary artery pressure; PVR = pulmonary vascular resistance; S = speckled.

tween them were evaluated by use of the Student's *t* test for unpaired data. Significance was taken to be at a probability of less than 5% ($p < 0.05$).

Results

It has been shown that the titer that one uses to categorize patients with a positive antinuclear antibody test will in part determine the sensitivity and specificity of the test (5). Based on the results of our normal control subjects we classified patients with primary pulmonary hypertension as having a positive antinuclear antibody test if they had a titer of 1:80 or greater with the KB cell substrate.

Antinuclear antibodies in primary pulmonary hypertension with titers of 1:80 or greater. The demographic, hemodynamic and antinuclear antibody results of testing with the KB cell line in patients with primary pulmonary hypertension is listed in Table 1. Of the 43 patients with primary pulmonary hypertension tested, 17 (40%) had an antinuclear antibody titer of 1:80 or greater. Of the 17 with positive antinuclear antibodies, 14 were women and 3 were men versus 18 women and 8 men with negative antinuclear antibodies (Table 2). The mean age for all patients was 39 ± 15 years with similar ages for those with positive and negative antinuclear antibodies. Functional class based on the New York Heart Association classification averaged 3.0 ± 0.7 with no difference between those with a positive and those with a negative antinuclear antibody test. In addition, the duration of the disease as determined by the onset of symptoms attributable to pulmonary hypertension was 3.6 ± 3 years, and was similar for both subgroups. Two of 17 patients with a positive antinuclear antibody test and 3 of 25 with a negative test had a history of Raynaud's phenomenon. At the time of the rest hemodynamic measurements, all of the patients had severe pulmonary hypertension as manifested by extremely high mean pulmonary artery pressure (mean 59 ± 18 mm Hg) and pulmonary vascular resistance (mean 19 ± 7 Wood units). There was no significant difference in the pulmonary artery pressure, cardiac output or pulmonary vascular resistance of the patients with a positive as opposed to those with negative antinuclear antibody test.

Staining patterns of antinuclear antibodies. The staining pattern of the patients with a positive antinuclear antibody test with the KB cell substrate was studied. In the patients with primary pulmonary hypertension the staining pattern was speckled in 11, peripheral in 4 and homogeneous in 2. There were no significant clinical or hemodynamic differences among patients based on the staining pattern of the antinuclear antibody.

Relative sensitivity of different antinuclear antibody tests. The sensitivity of the KB antinuclear antibody test was compared with the Hep2 test utilized at the University of Illinois, and the mouse kidney test utilized at the University of Colorado in those patients who were tested with both (Table 3). Six of the 11 patients with a positive KB test had a positive titer at 1:80 or greater dilutions on the

Table 2. Patients With Primary Pulmonary Hypertension and Negative Antinuclear Antibody Test

Case	Age (yr) & Sex	NYHA Class	Duration (yr)	PAP (mm Hg)	PVR
18	41F	4	7.0	47	19.2
19	55F	4	0.3	74	19.7
20	26F	3	9.0	61	17.8
21	51F	3	5.0	65	13.1
22	39M	2	2.0	57	14.5
23	17F	4	1.5	79	15.9
24	21M	4	0.5	127	34.8
25	34F	3	7.0	54	19.1
26	61F	4	2.8	73	25.4
27	33M	3	5.4	50	14.0
28	22F	2	9.0	56	11.2
29	38F	3	7.0	41	13.7
30	31F	2	10.0	62	20.6
31	40M	2	1.5	49	3.5
32	33F	2	2.5	42	8.8
33	50M	2	8.0	55	9.0
34	25F	4	0.2	43	17.5
35	30F	4	2.3	68	33.5
36	57M	3	2.0	40	11.7
37	25F	4	0.4	68	17.4
38	19F	2	6.0	73	21.0
39	13F	3	0.8	46	13.3
40	11M	2	2.0	115	38.6
41	44F	3	0.7	60	23.0
42	33M	3	2.0	70	23.0
43	38F	2	1.8	77	16.7
Mean	32.8	3.0	3.7	63.5	18.3
±SD	13.0	0.8	3.2	20.7	8.1

Abbreviations as in Table 1.

Table 3. Results Using Different Antinuclear Antibody Tests in Patients With Primary Pulmonary Hypertension and a Positive Antinuclear Antibody Test

Titer	KB (n = 20)	Hep2
1:20	3	0
1:40	6	2
1:80	1	0
1:160	0	1
1:160	10	5
	KB (n = 4)	**Mouse Kidney**
1:20	0	1
1:40	2	0
1:80	2	0
1:160	0	0

Table 4. Patients With Secondary Pulmonary Hypertension

Case	Age (yr) & Sex	Diagnosis	PAP (mm Hg)	ANA Titer	Type
44	28F	ASD	60	Neg	
45	32F	ASD	95	Neg	
46	30M	VSD/TGA	73	Neg	
47	19M	AS/AI	52	Neg	
48	29F	TGA	45	Neg	
49	36F	ASD	85	Neg	
50	66F	PE	38	Neg	
51	62F	ILD	32	Neg	
52	34M	PDA	69	160	S
53	46F	PE	59	Neg	
54	59F	MS	73	40	S
55	23M	VSD	80	Neg	
56	37F	VSD	54	40	S
57	40M	MS	59	Neg	
58	61M	MS	59	40	S
59	49M	PE	60	40	S

ASD = atrial septal defect; AS/AI = aortic stenosis/aortic insufficiency; ILD = interstitial lung disease; MS = mitral stenosis; Neg = negative; PDA = patent ductus arteriosus; PE = pulmonary emboli; TGA = transposition of the great arteries; VSD = ventricular septal defect; other abbreviations as in Table 1.

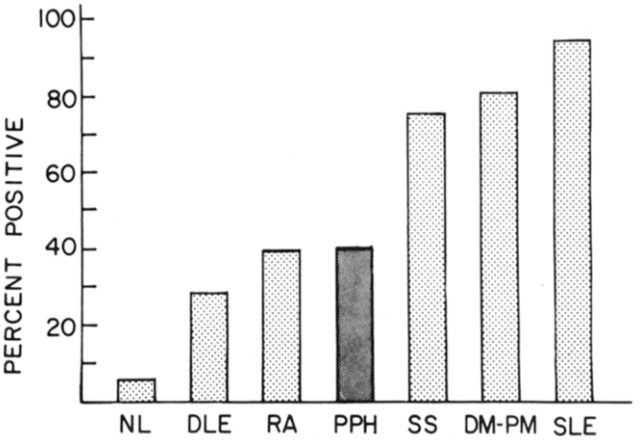
Hep2 line. None of the two patients with a positive KB test from the University of Colorado had a positive antinuclear antibody test using the mouse kidney cell substrate. Specimens from the University of California were tested twice with a KB test. The percent of positive tests was identical, and the titer never differed by more than one dilution between the two tests.

Secondary pulmonary hypertension. To examine whether a positive antinuclear antibody test in a patient with primary pulmonary hypertension is a manifestation of the disease as opposed to a phenomenon related to the presence of an elevated pulmonary artery pressure, (for example, endothelial damage with exposure of "new antigens"), we obtained sera from 16 patients with pulmonary hypertension of similar magnitude secondary to other causes and evaluated the presence of antinuclear antibodies using the KB cell test. Table 4 describes these 16 patients, 8 of whom had pulmonary hypertension secondary to congenital heart disease, and 8 of whom had pulmonary hypertension secondary to adult acquired heart or lung disease. Only one patient had a titer of greater than 1:40 (Patient 52). Although we have classified this patient as having pulmonary hypertension secondary to congenital heart disease, he had a patent ductus arteriosus closed during infancy and developed the pulmonary hypertension in adult life, which raises a question as to whether the development of the pulmonary hypertension was perhaps primary in nature.

Discussion

Prevalence and significance of positive test for antinuclear antibodies. Although an association between antinuclear antibodies and primary pulmonary hypertension has been previously made, no prior study has determined the frequency of positive antinuclear antibody tests with this disease. We found a positive antinuclear antibody test in 40% of cases using a KB cell substrate and using a titer of 1:80 or greater to determine positivity. Had the prevalence in our group been based on standard antinuclear antibody tests performed at the respective hospitals, patients tested at the University of Illinois would have had only 23% pos-

Figure 1. Relative frequency of positive (1:80) antinuclear antibody titers in various collagen vascular diseases using the KB cell substrate. Data from patients with collagen vascular disease come from sera of 100 patients at the University of Texas (6). Data for primary pulmonary hypertension (PPH) come from the 43 patients in this study. Primary pulmonary hypertension appears to fall between rheumatoid arthritis (RA) and scleroderma (systemic sclerosis [SS]) with respect to the frequency of positive tests. DLE = discoid lupus erythematosus; DM-PM = dermatomyositis-polymyositis; NL = normal subjects; SLE = systemic lupus erythematosus.



itive tests (versus our result of 37%), and those at the University of Colorado would have had no positive tests (versus our result of 40%). This probably accounts for the varying frequencies in the association between positive antinuclear antibodies and primary pulmonary hypertension in the past. The KB cell substrate is perhaps the most sensitive test for antinuclear antibodies. One study (5) showed that a large number of patients who were originally categorized as having "seronegative" lupus were "seropositive" when retested on the KB cell line. The specificity of the KB antinuclear antibody test obviously depends on the titer that one uses to define a positive test. At titers of 1:160 or greater, less than 5% of the normal population will have a positive test (6). At titers of 1:20 or greater this rises to 23%. Consequently, low titer positive antinuclear antibody results are often considered nonspecific.

We defined a positive antinuclear antibody test as a titer that was 1:80 or greater, based on our own control group. There was no way to distinguish on clinical grounds patients with primary pulmonary hypertension who had a positive antinuclear antibody test from those who did not. We saw no difference between them with respect to sex, age, functional class, duration of symptoms or hemodynamic status, nor did there appear to be any difference in their clinical course or survival. Even with exclusion of the four patients whose positivity was borderline (1:80), the prevalence rate is 30%, still much higher than that expected from a normal population. When these four patients are classified as having a "negative" antinuclear antibody test, there is no significant difference between the groups with a positive and a negative antinuclear antibody test.

Primary pulmonary hypertension as a collagen vascular disease. The increased prevalence of antinuclear antibodies in patients with primary pulmonary hypertension raises the possibility that the etiologic basis of primary pulmonary hypertension in some patients may be an autoimmune mechanism. Pathologic specimens of patients with primary pulmonary hypertension and pulmonary hypertension secondary to collagen vascular disease show very similar patterns. A review of the published data (2,3,7,8) on pulmonary vascular hypertension in patients with collagen vascular disease examined for lung disease consistently documents plexogenic arteriopathy indistinguishable from primary pulmonary hypertension with the absence of active vasculitis. The point has been made repeatedly from these studies that primary pulmonary hypertension and pulmonary hypertension in patients with collagen vascular disease without interstitial lung disease appear identical histologically.

If primary pulmonary hypertension is a collagen vascular disorder in some patients, it might be placed between rheumatoid arthritis and scleroderma with regard to the frequency of positive antinuclear antibody tests found (Fig. 1). The prevalence of the speckled pattern in both the primary and secondary group is of interest, but it is difficult to speculate on the clinical significance, because speckled patterns appear to be the most common patterns seen in all collagen vascular diseases.

The 6% incidence rate of antinuclear antibodies in patients with secondary pulmonary hypertension may be a nonspecific finding, because it is similar to that of the normal population. This would support the view that a positive antinuclear antibody test of 1:80 dilutions or greater in patients with primary pulmonary hypertension is neither nonspecific nor secondary to elevated pulmonary artery pressures, but rather a feature of the disease state itself.

Therapeutic implications. The implications of the presence of antinuclear antibodies in patients with primary pulmonary hypertension with regard to therapy must be addressed. Unfortunately, it is often difficult to show significant benefits of immunosuppressive therapy in patients with overt collagen vascular disorders. However, case reports (9,10) have appeared describing patients with pulmonary hypertension secondary to collagen vascular disease whose hypertensive state appears to be ameliorated somewhat with treatment by steroids and immunosuppressives. At present it is premature to recommend immunosuppressive therapy for primary pulmonary hypertension but clearly all feasible avenues of treating these patients need to be explored.

We are grateful to Electro-Nucleonics, Inc., for supplying the KB antinuclear antibody testing kits, and to Mary F. Lipscomb, MD and David Hellmann, MD for helpful comments regarding this study.

References

1. Rawson AJ, Woske HM. A study of etiologic factors in so-called primary pulmonary hypertension. Arch Intern Med 1960;105:233–43.
2. Kobayashi H, Sano T, Fi K, Hizawa K, Yamanoi A, Otsuka T. Mixed connective tissue disease with fatal pulmonary hypertension. Acta Pathol Jpn 1982;32:1121–9.
3. Schwartzberg M, Lieberman DH, Getzoff B, Erlich GE. Systemic lupus erythematosus and pulmonary vascular hypertension. Arch Intern Med 1984;144:605–7.
4. Rich S, Brundage BH. Primary pulmonary hypertension: current update. JAMA 1984;251:2252–4.
5. Maddison PJ, Provost TT, Reichlin M. Serological findings in patients with "ANA-negative" systemic lupus erythematosus. Medicine 1981;60:87–94.
6. Lipscomb HF, Cope LD, Stephens GL, Deng JS, Gilliam JN. Comparison of substrates for the detection of antinuclear antibodies in normals and in patients with connective tissue and other diseases. Diagn Immunol 1984;2:181–7.
7. Bunch TW, Tancredi RG, Lie JT. Pulmonary hypertension in polymyositis. Chest 1981;79:105–7.
8. Salerni R, Rodnan GP, Leon DF, Shaver JA. Pulmonary hypertension in the CREST syndrome variant of progressive systemic sclerosis (scleroderma). Ann Intern Med 1977;86:394–9.
9. Pines A, Kaplinsky N, Goldhammer E, Olchovsky D, Frank LO. Corticosteroid responsive pulmonary hypertension in systemic lupus erythematosus. Clin Rheumatol 1982;1:301–4.
10. Rosenberg AM, Petty RE, Cumming GR, Koehler BE. Pulmonary hypertension in a child with mixed connective tissue disease. J Rheumatol 1979;6:700–4.