Arthritis and Rheumatism

Official Journal of the American Rheumatism Association

VOL. X, NO. 2 APRIL, 1967

A Five Year Prospective Study of Rheumatoid Factor Tests in Juvenile Rheumatoid Arthritis

By JAMES T. CASSIDY AND HANS A. VALKENBURG

IT IS RECOGNIZED that tests for rheumatoid factors are less often positive in juvenile rheumatoid arthritis (JRA) than in the adult form of the disease. The explanation for this discrepancy is not completely clear. Those children who develop seropositivity may constitute a separate group of patients from the standpoint of diagnosis or prognosis. Ideally, to ascertain this, children with arthritis would be followed with serial rheumatoid factor tests well into adulthood. Data from this type of approach are not available at the present time. As a partial solution to this problem, a cross-sectional group of patients with JRA of various ages and differing durations of disease activity has been examined. It is intended that such a composite view of the disease will approximate data that might have been obtained from the more desirable, longitudinal investigation.

MATERIALS AND METHODS

The study group consisted of 110 children and adults with rheumatoid arthritis, all of whom had onset of disease at or before 14 years of age. Seventy-seven were females. Eighty-four were currently under care at The University of Michigan Medical Center. Twenty-six who had not been seen for at least five years were selected at random and recalled in order to obtain patients with longer duration of disease, and to determine whether patients currently under observation might have disease of greater severity. A complete physical examination was done on each patient to delineate current status of disease and functional capacity. Each subject was to have a minimum of two sera, separated by at least one year, drawn during the five year period of the study, 1961 through 1965. All positive results were verified by repeat examination of another serum.

For convenience in analysis, the patients were divided into three groups according to type of onset. Those categorized as having polyarticular disease had more than two joints objectively involved from the onset and otherwise met the criteria

From the Pediatric Rehabilitation Clinic, the Rackham Arthritis Research Unit, and the Department of Internal Medicine, The University of Michigan Medical School, Ann Arbor, Mich.

A preliminary report of this paper was presented at the annual meeting of the American Rheumatism Assocaition, San Francisco, Calif., June 1964.

This work was supported in part by a grant from the Michigan Chapter of The Arthritis Foundation. The Rackham Arthritis Research Unit is supported by a grant from the Horace H. Rackham School of Graduate Studies. Dr. Cassidy held a Post-doctoral Fellowship of The Arthritis Foundation, 1963–66, and Dr. Valkenburg was on a NATO Science Fellowship supported by the Netherlands Organization for the Advancement of Pure Research (Z.W.O.).

JAMES T. CASSIDY, M.D.: *Assistant Professor of Internal Medicine and Associate Physician, Rackham Arthritis Research Unit, Department of Internal Medicine, The University of Michigan Medical School.* HANS A. VALKENBURG, M.D.: *The Institute for Rheumatism Research, University Hospital, Leiden, The Netherlands.*

for definite rheumatoid arthritis established for adults,[1] as modified in this clinic for use in children. Those classified as having systemic rheumatoid arthritis had prominent manifestations of high fever, rash, lymphadenopathy, hepatosplenomegaly, and serositis, in addition to joint involvement. Those children with monarticular onset had only one joint involved during the first four months of their disease.

A general population profile for use as control sera numbered 833, of which 796 represented a random sample of the total population of 5 to 54 years of age investigated in the Tecumseh Community Health Study, Samples 1 and 2, 1962–1963. Subjects with rheumatoid arthritis were not excluded. Sera from 37 children 0 to 4 years of age were obtained from the Plymouth State Home in February of 1964. Thirty-four children with connective tissue diseases other than rheumatoid arthritis and 175 hospitalized children with diseases other than connective tissue disease, nephrotic syndrome, or liver disease were chosen from the clinic population as disease controls.

Four antiglobulin tests for rheumatoid factors were performed on each serum. Initial standardizations were carried out with sera from the laboratory of Dr. J. Ball at the University of Manchester, England, and reference sera were included with each group of determinations. The human erythrocyte agglutination test (HEAT) was performed with cells sensitized with rabbit antibody and incorporated technical modifications of the Waaler-Rose test.[2] A titer of 1:32 or more was considered positive. The latex fixation test (LFT) was a modification of the method of Singer and Plotz.[3] A positive test was defined as a titer of 1:640 or more. A further adaptation of this reaction, the inactivated latex fixation test (LFT 56), involved preincubation of the serum at 56 C. for 30 minutes. Twofold serial dilutions, starting at 1:20, were then performed in a solution of pH 8.2 containing 0.1 M glycine, 0.585 per cent NaCl, and 0.4 per cent bovine serum albumin. After incubation at 56 C. for 90 minutes, the tubes were maintained at room temperature for 20 hours, centrifuged at 1400 × g for 3 minutes, and read without agitation. A titer of 1:20 or more was considered positive. The Hyland rapid slide latex agglutination test (HST) was performed as described (Hyland RA-Test, Hyland Laboratories, Los Angeles, Calif.). In the group of patients with rheumatoid arthritis, a positive rheumatoid factor test was defined for correlations with the clinical aspects of the disease as a positive HEAT or LFT. The LFT 56 and HST were excluded from consideration in these analyses.

Total serum proteins were done by a biuret method, and paper electrophoresis was carried out on the Beckman Model R Paper Electrophoresis Apparatus. Quantitation was performed with a Spinco Analytrol. A patient with a concentration of gamma globulins of 1.5 Gm./100 ml. or greater was judged to have hypergammaglobulinemia. L.E. cell preparations were done according to the technic of Fallet and Ziff.[4]

Results

Table 1 includes data from the general population profile. In the children 0 to 4 years of age, two had minimally positive titers in the HEAT (1:32) and another had a significantly positive LFT (1:2,560). In the rest of the control group, the number of positive tests increased with age in accord with previous population surveys. The incidence of seropositivity in the LFT 56 increased markedly after 14 years of age. In Table 2 are listed 34 children with connective tissue diseases other than rheumatoid arthritis. Twelve were seropositive. In the group of 175 hospitalized children, the LFT was positive in 1.1 per cent.

Within the group of 110 patients with JRA, 102 had two or more serologic examinations separated by at least one year. Eight patients were tested on only one occasion. The distribution of positive rheumatoid factor tests is shown in Table 3. A considerable discrepancy in results was apparent among the various test systems. The HST was negative in the presence of a positive HEAT or LFT in 6.3 per cent of the patients and, conversely, was positive in 3.6 per cent in whom the HEAT and LFT were negative.

Twenty-four patients, 5 males and 19 females, had a positive HEAT or LFT or both during the course of the study. Twenty-two had strongly positive tests, and two were positive in minimally significant titer. There were two Negroes in the total group and one was seropositive. Sixteen patients were consistently positive, and four negative sera converted to positive. In this

Table 1.—*Prevalence of Positive Rheumatoid Factor Tests in a General Population Profile*

Age in Years	Number	Positive Rheumatoid Factor Tests (Per cent) HEAT	LFT	HST	LFT 56
0–4	37	5.4	2.7	5.4	5.4
5–9	82	0	0	1.2	3.7
10–14	132	0	2	0.8	9.8
15–24	144	1.4	5.6	8.3	47.3
25–34	144	0	5.6	7.6	55.6
35–44	174	4.6	12.6	19	50
45–54	120	4.2	8.3	22.5	55.8

Table 2.—*Distribution of Positive Rheumatoid Factor Tests in Children with Connective Tissue Disease Other than JRA*

Disease	Number of Patients	Number with Positive HEAT	LFT	HEAT & LFT
Probable Rheumatoid Arthritis	7	—	—	1
Rheumatoid Nodules without Arthritis	2	—	1	—
Dermatomyositis	9	—	1	3
Scleroderma	2	—	—	—
Systemic Lupus Erythematosus	3	—	2	—
Probable S.L.E.	2	—	—	2
Sjögren's Syndrome with S.L.E.	1	—	—	1
Plasma Cell Hepatitis	1	—	1	—
Palindromic Rheumatism	1	—	—	—
Rheumatic Fever	2	—	—	—
Psoriatic Arthritis	1	—	—	—
Ulcerative Colitis with Arthritis	2	—	—	—
Pigmented Villonodular Synovitis	1	—	—	—

Table 3.—*Distribution of Positive Rheumatoid Factor Tests Among 110 Patients with JRA*

Rheumatoid Factor Tests HEAT	LFT	HST	LFT 56	Number	%
+	+	+	+	12	10.9
−	+	+	+	4	3.6
+	−	+	−	1	0.9
−	+	−	+	5	4.5
−	−	+	+	4	3.6
+	−	−	−	2	1.8
−	−	−	+	38	34.6
−	−	−	−	44	40.0

latter group were two patients that had negative tests at the start of the study, then converted to positive, one strongly positive and the other weakly positive, and later reconverted to negative. Four initially positive sera converted to negative. Clinical or serologic differences were not observed in the 26 patients that were recalled relative to the larger number that were currently attending the clinic.

Correlations between positive rheumatoid factor tests and the age-dependent parameters of the patient group are shown in Table 4. The age of the patient at the time of the initial serum is presented in the first column. Half of the study group was younger than 12 years of age. Twenty-two per cent of all the patients were seropositive; however, the number of positive tests increased with advancing age. Five per cent of the children younger than 12 years of age and 38 per cent of those over 12 had positive tests. There was a significant correlation of seropositivity with age of the patient, and positive sera were not encountered in 15 children 7 years of age or younger. There was no relationship of seropositivity with sex ($\chi^2_{[1]} = 0.7334$; $p = 0.30–0.50$).

The age of onset of JRA for the study group is shown in the second column of Table 4. The peak age of onset was two to three years with a median of six years. Late onset of disease was clearly associated with seropositivity. Six patients who had onset of disease before seven years of age were, however, seropositive when tested later in life. Three were adults at the time of this study, but the others were 7, 10, and 14 years of age. This finding suggested that at least some of the children in the youngest age group would develop seropositivity if followed long enough. The relationship between duration of disease and seropositivity is detailed in the third column. Duration included the total period of continuous or

Table 4.—*Relationship of Rheumatoid Factor Tests and Age-dependent Parameters of the Patient Group with JRA*

	Age at Time of Study				Age of Onset				Duration of Disease			
Years	No.	Sero-negative %	LFT 56 positive %	HEAT/LFT positive %	No.	Sero-negative %	LFT 56 positive %	HEAT/LFT positive %	No.	Sero-negative %	LFT 56 positive %	HEAT/LFT positive %
0–4	7	43	57	0	42	48	40	12	49	49	33	18
5–9	29	55	38	7	37	43	41	16	35	29	51	20
10–14	32	41	34	25	31	26	32	42	15	40	33	27
15–19	18	33	44	22	—	—	—	—	5	80	0	20
20–24	14	36	29	36	—	—	—	—	4	0	50	50
25+	10	10	40	50	—	—	—	—	2	0	50	50
Total	110	40	38	22	110				110			
$\chi^2_{[2]}$				9.021				10.703				1.594
p				0.01–0.02				0.001–0.005				0.40–0.50

intermittent activity. Although 55 per cent of the children had active disease for five years or more, one-third of the seropositive group was within the initial five years of active disease. Seropositivity therefore did not correlate with duration of disease. At the end of the study, 17 patients were in remission. Two of these were still seropositive. Only one patient died during the five year period, a 12-year-old girl with seronegative systemic disease.

A polyarticular onset probably did not correlate with seropositivity ($\chi^2_{[2]} = 5.812$; $p = 0.05–0.10$). Thirty-one per cent of 54 patients with polyarticular onset had positive rheumatoid factor tests. The respective numbers were 14 per cent for the 14 with systemic onset and 12 per cent for the 42 with monarticular disease. The results relative to functional capacity are listed in Table 5. Although there was only one positive serum in Class I, seropositivity probably was not correlated with an increasing degree of functional impairment ($\chi^2_{[3]} = 7.045$; $p = 0.05–0.10$). Forty-three patients were found to have unequivocal erosions of bone on roentgenograms. Thirty-three per cent of this number had positive rheumatoid factor tests, and 15 per cent of those without this change were positive. Seropositivity was therefore probably not associated with erosive bone disease ($\chi^2_{[1]} = 3.796$; $p = 0.05–0.10$). Moreover, the groups with and without bone erosion were not comparable in respect to median age and duration of disease (18 and 8 years for the first group and 9 and 3 years for the second). Both seropositivity and bone erosion increased in incidence with advancing age of the patient group.

Table 5.—*Relationship of Functional Capacity to Rheumatoid Factor Tests in 110 Patients with JRA*

Class	Number	Median Age	Median Duration	Positive HEAT/LFT Number	%
I No Limitation	19	14	4	1	5
II Minimal	38	12	4	7	18
III Moderate	34	14	6	12	35
IV Severe	19	16	6	4	21

The level of gamma globulins in each serum was compared with seropositivity and with the type of onset of the disease. Thirty-five patients with polyarticular onset, 21 with monarticular, and 10 with systemic had elevated levels of gamma globulins. In no case was there a positive correlation ($\chi^2_{[2]} = 3.033$; $p = 0.20–0.30$).

Twenty-three per cent of those with and of those without elevated gamma globulins were seropositive ($\chi^2_{[1]} = 0.080$; $p = 0.70$–0.80). In addition, there was no correlation between elevated gamma globulins and impaired functional capacity ($\chi^2_{[3]} = 6.499$; $p = 0.05$–0.10). In the four classes, I–IV, the numbers of patients with hypergammaglobulinemia were 9, 19, 26, and 12. Only three patients during the course of this study had positive lupus erythematosus cell preparations.

There was no correlation of seropositivity with band keratopathy (1/9). Eighty-two per cent of 11 patients with rheumatoid nodules had positive rheumatoid factor tests. Fifteen per cent of those without nodules were positive. This is a highly significant association ($\chi^2_{[1]} = 22.034$; $p < .001$). Nodules furthermore tended to be associated with small joint disease (MCP and PIP) and with late age of onset. The 12 patients that were positive in all four tests had predominantly polyarticular disease with small joint involvement of relatively late age of onset. There was in addition a definite trend towards an increased percentage of nodules, bone destruction, elevated serum gamma globulins, and impaired functional capacity.

The therapeutic programs were evaluated and did not appear to affect the serologic reactions. The patients were treated for the most part conservatively with acetylsalicylic acid. One patient in the group with positive rheumatoid factor tests was on aurothioglucose, three were on hydroxychloroquine, and two on adrenocorticosteroids. In the patients with negative tests, four were on gold, 12 on hydroxychloroquine, and 13 on steroids.

Discussion

In comparison with the controls in Table 1 weighted for age, 5.5 times as many patients with JRA had a positive HEAT or LFT or both. This ratio is comparable to that found in population studies on adult rheumatoid arthritis, but is approximately half the expected ratio for clinic adult rheumatoid arthritis patients. The cumulative incidence of 38 per cent seropositivity in the adult patients with JRA was less than the 75 per cent that would be expected in a comparable group with postpubertal onset of the disease. No additional information was obtained by comparing the distribution of titers in the test systems to the controls. The low percentage of seropositivity in JRA may reflect one of the following suppositions: The age of the patient is the predominant factor in determining seropositivity; the juvenile group reflects a greater proportion of the total pool of these patients present in a population than do the clinic patients with adult onset of rheumatoid arthritis; or this disease category in children is different from the disease as defined for adults. The significance of the LFT 56 is uncertain at this time. It is likely that the antibody is an anti-IgG 19S macroglobulin similar or identical to rheumatoid factor.[5] In this study the LFT 56 was less specific, although more sensitive, than the HEAT and LFT. The percentage of positivity in the patients was 57 per cent as compared to 25 per cent in the population controls weighted for age.

Reports in the literature of rheumatoid factor tests in patients with juvenile rheumatoid arthritis are in general agreement with this study. Selected aspects of some of the larger series are summarized in Table 6. In order to facilitate a more direct comparison of results with the present study, the data provided by these reports have been analyzed by comparable statistical methods.

During 4 to 8 years, the differential sheep cell agglutination test was performed on 142 patients seen at the Rheumatism Research Unit at Taplow.[6] Sixty-four patients had positive tests at some time during the

Table 6.—*A Summary of Statistical Analysis of Previous and Current Studies of Positive Rheumatoid Factor Tests in JRA*

Correlation of Seropositivity with:	Investigation, Rheumatoid Factor Tests, and Number of Patients				
	Bywaters et al. DAT 142	Toumbis et al. SCAT 45	Sievers et al. SCAT LFT 200	Laaksonen SCAT LFT 439	Present HEAT LFT 110
Sex	(−) (M)			+ (F)	−
Age of Patient		++	+++		+
Late Age of Onset	+	−	+	+++	++
Duration of Disease	−	(−)	−		−
Type of Onset		−		+	(−)
Functional Class				+++	(−)
Stage		−	−	+++	
Disease Activity	−	−			
Bone Erosion					(−)
Nodules	+++	−			+++
Band Keratopathy					−
Therapy					−
Positive L.E. Cell Test					−
Hypergammaglobulinemia					−
Prognosis	−			+	−

Symbols used: −, Not significant; (−), p = 0.05–0.10; +, p = 0.01–0.02; ++, p = 0.001–0.01; +++, p < 0.001.

study. Of this number, 35 had negative results in the first year and became positive later. In a comparison of the same group of children with adult patients,[7] 50 per cent of adults were seropositive on first testing compared with 13.4 per cent of children. In a report from New York City,[8] the sensitized sheep cell agglutination test was positive in 40 per cent of 45 patients and the latex fixation test in 13 per cent of 31 patients with JRA. The incidence of positive tests was greater in patients with arthritis for more than five years, and there was a positive association with the age of the patient. There were four positive sensitized cell tests in six patients with subcutaneous nodules. The titers were not significantly higher in patients with systemic disease.

In a series of 100 children and 100 adults with JRA treated between 1960 and 1962 at the Rheumatism Foundation Hospital, Heinola, Finland,[9] the Waaler-Rose and latex fixation tests were markedly more positive in the adult group. The tests were in most instances negative if onset had occurred before six years of age irrespective of the patient's present age. There was no increase in percentage of positivity with advancing age of onset in the latter group, as was the case in the present study; therefore, the relation between late age of onset and seropositivity was primarily due to the results observed in the adult group. Laaksonen found the latex slide test positive in 29.5 per cent of 363 patients and the Waaler-Rose reaction in 11.2 per cent of a group of 384 with JRA studied at the same Rheumatism Foundation Hospital from 1951–61.[10] There was 7.6 per cent positivity in children with onset at less than six years of age and 54 per cent in those with onset after the age of six. Seropositivity correlated with a poor prognostic outlook. Only one patient in the oligoarticular category had a positive latex fixation test, and those with persistently monarticular course were seronegative.

Except for the ability to prevent or cure rheumatoid arthritis, accurate prognostica-

tion has been one of the most elusive features of JRA, although it would more than anything else aid in deciding on the type of treatment. The observation that only 21 per cent of the present series was relatively severely incapacitated from the standpoint of articular function was in agreement with the impression that onset of rheumatoid arthritis during childhood heralded a better outcome than the adult form of the disease.[10-11] Erosive disease, although a typical rheumatoid development, was often late in appearance.[12] A significantly and persistently positive rheumatoid factor test has been considered one indication of a more guarded prognosis. A lack of strong correlation in the present study with impaired functional capacity, type of onset, and bone erosion suggested that seropositivity could not be interpreted as an unequivocal indication of a poor functional outcome. There was likewise an impressive incidence of seropositivity in children with connective tissue diseases other than rheumatoid arthritis, negating in part the value that the tests might have as discriminating diagnostic measures.

ACKNOWLEDGMENTS

The authors wish to thank Drs. T. Francis, Jr., F. H. Epstein, W. M. Mikkelsen, and H. J. Dodge for sera from the Tecumseh Community Health Study; Drs. David Dickinson and Donita Sullivan of the Pediatric Rehabilitation Clinic, and the following persons for technical assistance during certain periods of the study: Paula v. Bortel, Ann Burt, R. Ann Dalbey, Joan Himes, Scott Himes, and Bauwien v.d. Oord.

Summary

Four rheumatoid factor tests were performed during a five year period in 110 patients with rheumatoid arthritis in whom onset of the disease was at or before age 14 years. Twenty-two per cent were seropositive. There was a significant correlation of seropositivity with age of the patient, late age of onset, and rheumatoid nodules. A less impressive association was found with type of onset of the disease, impaired functional capacity, and bone erosion. There was no direct correlation with duration of disease, sex, band keratopathy, type of therapy, positive L.E. cell test, or hypergammaglobulinemia. Although 5.5 times as many patients with JRA had a positive rheumatoid factor test in comparison with population controls, there was a substantial incidence of seropositivity in a group of children with connective tissue diseases other than rheumatoid arthritis.

Summario in Interlingua

Quatro tests pro factor rheumatoide esseva effectuate durante un periodo quinquenne in 110 patientes con arthritis rheumatoide in qui le declaration del morbo habeva occurrite al etate de 14 annos o minus. Vinti-duo pro cento esseva seropositive. Esseva constatate un correlation significative inter le presentia de seropositivitate e (1) le etate del patiente, (2) le etate plus alte del patiente al tempore del declaration del morbo, e (3) l prsntia d nodulos rheumatoide. Esseva trovate un association minus frappante inter le presentia de seropositivitate e le typo del declaration del morbo, defectos del capacitate functional, e erosion ossee.

REFERENCES

1. Ropes, M., Bennett, G. A., Cobb, S., Jacox, R., and Jessar, R. A.: 1958 revision of diagnostic criteria for rheumatoid arthritis. Bull. Rheum. Dis. 9:175, 1958.
2. Valkenburg, H. A.: Human erythrocyte agglutination test (HEAT). *In:* The Epidemiology of Chronic Rheumatism. Kellgren, J. H., Jeffrey, M. R., and Ball, J., Eds. Philadelphia, Pa., F. A. Davis, Co., 1963, Vol. 1, p. 330.
3. Valkenburg, H. A.: Latex fixation test (LFT). *In:* The Epidemiology of Chronic Rheuma-

tism. Kellgren, J. H., Jeffrey, M. R., and Ball, J., Eds. Philadelphia, Pa., F. A. Davis, Co., 1963, Vol. 1, p. 337.
4. Fallet, G. H., and Ziff, M.: Leucocyte disc method for L.E. cell test in serum and serum fractions. Arthritis Rheum. 1:70, 1958.
5. Klein, F., Valkenburg, H. A., van Zwet, T. L., and Lafeber, G. J. M.: Characterization of two different agglutinators in the latex fixation test, occurring in normal human sera. Immunology 10:87, 1966.
6. Bywaters, E. G. L., Carter, M. E., and Scott, F. E. T.: Differential agglutination titre (D.A.T.) in juvenile rheumatoid arthritis. Ann. Rheum. Dis. 18:225, 1959.
7. Bywaters, E. G. L., Carter, M. E., and Scott, F. E. T.: Comparison of differential agglutination titre (D.A.T.) in juvenile and adult rheumatoid arthritis. Ann. Rheum. Dis. 18: 233, 1959.
8. Toumbis, A., Franklin, E. C., McEwen, C., and Kuttner, A. G.: Clinical and serologic observations in patients with juvenile rheumatoid arthritis and their relatives. J. Pediat. 62: 463, 1963.
9. Sievers, K., Ahvonen, P., Aho, K., and Wager, O.: Serological patterns in juvenile rheumatoid arthritis. Rheumatism (London) 19:88, 1963.
10. Laaksonen, A.: A prognostic study of juvenile rheumatoid arthritis. Acta Paediat. Scand. (Suppl. 166), 1966.
11. Ansell, B. M., and Bywaters, E. G. L.: Prognosis in Still's disease. Bull. Rheum. Dis. 9:189, 1959.
12. Martel, W., Holt, J. F., and Cassidy, J. T.: Roentgenologic manifestations of juvenile rheumatoid arthritis. Amer. J. Roentgen. 88: 400, 1962.