A comparison of serum biochemistry in ankylosing spondylitis, seronegative and seropositive rheumatoid arthritis

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SUMMARY The serum biochemistry of 31 patients with ankylosing spondylitis (AS) was compared with that of 80 patients with rheumatoid arthritis (RA) (ARA criteria), 30 of whom were negative for circulating rheumatoid factor and 50 of whom were 'seropositive'. All patients were selected because of moderate to severe disease activity. All 3 groups had distinctive biochemical profiles. Total serum sulphhydryl and haemoglobin were particularly good discriminators between AS and RA. IgG, IgA, and acute-phase reactants complemented the sheep cell agglutination test in discriminating between seropositive RA and seronegative RA. In active AS a normal erythrocyte sedimentation rate was often seen in the presence of an abnormal C-reactive protein (CRP) and plasma viscosity.

Clinical epidemiology has advanced rheumatology by allowing the recognition of an increasing number of discrete arthropathies. Thus the concept of seronegative spondylarthritides, once confused with rheumatoid arthritis (RA), is now recognised as a discrete entity on clinical, radiological, histological, and serological grounds.¹ Since specific drugs therapy for these different conditions is likely to be based on a closer understanding of the underlying pathological processes, it is relevant to compare biochemical abnormalities occurring in different variants of polyarthritis.

Biochemical changes in the blood of patients with RA have been extensively investigated.²⁻⁶ Some of these changes have been claimed to be specific for this disease, e.g., low serum histidine.⁷ Such studies invariably use the criteria of the American Rheumatism Association for the selection of their patients. These criteria include the presence of circulating rheumatoid factor, but it is not mandatory for a diagnosis of RA to be made.

Between 23%⁸ and 40%⁹ of a rheumatoid population are 'seronegative', though the recent interest in 'hidden rheumatoid factor' emphasises that even

'seronegative' patients may still have abnormal circulating immune complexes.¹⁰⁻¹² Seropositive RA (SPRA) has a poorer prognosis than seronegative RA (SNRA)¹³ and the spectrum of clinical symptoms may differ between these 2 variants.^{9, 14} However, the serum biochemistry in these variants, in relation to the nature and relevance of rheumatoid factor, has not been previously considered.

Of the seronegative spondylarthritides ankylosing spondylitis (AS) is clinically a more distant relative of RA than Reiter's syndrome, psoriatic arthropathy, or colitic arthropathy. Although several biochemical abnormalities occur in AS (raised C-reactive protein (CRP) and erythrocyte sedimentation rate (ESR)¹⁵, raised immunoglobulins,¹⁶ and high total Cu with raised caeruloplasmin¹⁷), comprehensive surveys of other factors do not appear to have been undertaken. We know of no extensive studies in which biochemical assays have been performed concurrently in the same laboratory on groups of patients with these different diseases.

We have therefore compared a broad spectrum of biochemical variables in groups of patients with RA (ARA criteria), both seropositive and seronegative for circulating rheumatoid factor, and with AS (New York criteria¹⁸). All patients were selected because of moderate to severe disease activity.

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Patients and methods

PATIENTS

Rheumatoid arthritis. Fifty patients with RA (ARA criteria) were entered into the SPRA group, all having a persistent sheep cell agglutination test (SCAT) titre of $\leq 1/64$ over the month immediately preceding the blood sampling. Thirty RA patients (ARA criteria) were included in the SNRA group, all having a persistent SCAT titre of $\geq 1/8$. Patients with SCAT titres 1/16 or 1/32 were excluded from both groups. All 80 patients showed evidence of at least moderate disease activity as previously defined.⁶

Ankylosing spondylitis. Thirty-one patients with AS, all persistently seronegative for rheumatoid factor (SCAT titre $\geq 1/8$), were included in this group. All patients conformed to the New York criteria¹⁸ and had active disease as judged by the presence of at least 2 of the following 6 criteria: (i) nocturnal pain; (ii) morning stiffness; (iii) pain and stiffness of more than 3 months' duration, not relieved by rest, in low back or dorsolumbar junction or in either or both buttocks; (iv) pain and stiffness in the thoracic region of more than 3 months' duration; (v) limitation of chest expansion; (vi) limitation in spinal anterior and lateral flexion and in spinal extension.

All patients. None of these 111 patients had previously received specific antirheumatic drug therapy (e.g., D-penicillamine or gold), and all took enteric coated aspirin alone for 2 weeks prior to the blood sampling to establish uniform conditions. A small number of the RA patients were on constant steroid therapy.

Table 1 Analytical details for biochemical variables

Variable	Method and Reference
Serum histidine	Spectrofluorometric ²⁴
Total serum sulphhydryl	Spectrophotometric ²⁵
Plasma viscosity	Capillary Viscometry ²⁶
C-reactive protein	Radial Immunodiffusion ²⁷
Haptoglobin	Radial Immunodiffusion ²⁷
Plasma gamma glutamyl transpeptidase	Spectrophotometric ²⁸

Table 2 Comparison of clinical data for SPRA and SNRA

Variable	SNRA (n = 30)		SPRA (n = 50)		F ¹
	Mean	SD	Mean	SD	
Functional grade ²	1.33	0.55	1.70	0.95	3.70
Joint size	55.7	4.67	57.1	5.90	1.21
Articular index	23.0	10.7	20.7	12.8	0.65
Pain score ²	3.20	0.76	3.04	0.93	0.60
Aspirin dose ³	8.79	3.99	9.48	3.63	0.49
Disease duration	8.66	7.86	9.95	7.97	0.48
Early morning stiffness	161	199	183	214	0.19
Age	50.6	10.7	51.0	11.0	0.02
Grip strength	90.2	56.6	90.4	50.0	<0.01

¹All nonsignificant. ²Scale 0-5. ³Number of tablets, maximum 12.

ASSESSMENTS

Six clinical assessments (pain score, Ritchie articular index, early morning stiffness, grip strength, joint size, and functional grade) were performed on all 80 RA patients at the time of blood sampling as previously described.⁶

A spectrum of 25 biochemical variables were measured in the two RA groups and 19 in the AS group—routine immunological tests being carried out on the RA groups only. Nonroutine assays are listed in Table 1 together with analytical methods.

One-way analysis of variance was used to compare the SNRA data with the SPRA data and to compare the SNRA results with the AS results.

Results

SNRA COMPARED WITH SPRA

The groups were similar in age (Table 2) and sex (F:M, SPRA 36:14, SNRA 25:5), thus allowing direct comparison of those variables known to be dependent on either of these factors. The clinical assessments (Table 2) were not significantly different between the SNRA and SPRA groups, thus suggesting approximately equivalent symptomology.

Results for the biochemical variables in the SPRA and SNRA groups are shown in Table 3. The considerable difference between the 2 groups in terms of RA latex, IgM, and IgA was to be expected and confirms the distinction based on SCAT data. Significantly higher values for plasma viscosity, CRP, and ESR were seen in the SPRA's together with significantly lower albumin and serum histidine values. All other biochemical variables failed to show a significant difference between the 2 groups.

SNRA COMPARED WITH AS

Data for the AS patients are presented in Table 4 and compared with the SNRA results. There were considerable differences in age and sex (F:M, SNRA 25:5, AS 6:25) between these 2 groups, with RA occurring predominantly in older females and AS in younger males. Thus in considering the other

Table 3 Comparison of biochemical data for SPRA and SNRA

Biochemical variable	SNRA (n=30)		SPRA (n=50)		F
	Mean	SD	Mean	SD	
RA Latex	0.46	0.69	3.52	0.73	311.73***
IgA	IU.ml ⁻¹ 211	94	318	158	10.62**
IgM	IU.ml ⁻¹ 226	133	341	157	10.41**
Plasma viscosity	cP 1.88	0.12	1.98	0.16	8.90**
Albumin	g.l ⁻¹ 41.9	3.45	40.3	2.70	5.30*
CRP	mg.dl ⁻¹ 2.67	2.54	4.60	4.23	5.11*
Serum histidine	mg.dl ⁻¹ 1.32	0.30	1.18	0.26	4.86*
ESR	mm.h ⁻¹ 47.7	26.1	60.7	25.8	4.58*
WBC	× 10 ⁹ .l ⁻¹ 7.92	2.12	8.94	2.38	3.70
Haptoglobin	g.l ⁻¹ 3.88	1.93	4.58	1.57	3.16
C4	mg.dl ⁻¹ 47	14.2	40	17.1	2.82
Urea	mmol.l ⁻¹ 5.63	1.64	5.06	1.34	2.80
GGTP	U.l ⁻¹ 22.7	14.3	29.6	19.9	2.69
Platelet count	× 10 ⁹ .l ⁻¹ 436	123	403	118	1.35
Globulin	g.l ⁻¹ 35.0	4.23	36.3	4.73	1.29
Alkaline phosphatase	KA units 9.91	3.43	11.2	5.75	1.14
Fibrinogen	g.l ⁻¹ 3.01	0.73	3.21	0.83	1.12
C3	mg.dl ⁻¹ 162	42.2	166	33.8	0.12
Bilirubin	μmol.l ⁻¹ 6.21	2.04	6.70	2.04	1.01
Protein	g.l ⁻¹ 77.6	4.33	76.7	4.20	0.87
Hb	g.dl ⁻¹ 11.7	1.63	11.9	1.38	0.50
SGOT	U.l ⁻¹ 18.4	16.6	16.6	11.4	0.32
Creatinine	μmol.l ⁻¹ 78.5	22.5	77.1	17.0	0.10
IgG	IU.ml ⁻¹ 187	67.5	190	51.9	0.04
Total sulphydryl	μmol.l ⁻¹ 380	69.8	379	70.8	<0.01

***P<0.001, **P<0.01, *P<0.05.

Table 4 Comparison of SNRA data (Table 3) with data for AS

Variable	AS (n=31)		F
	Mean	SD	
Age	35.8	9.99	30.05**
Hb	13.6	1.60	22.06**
Total sulphydryl	460	81.2	16.49**
ESR	26.7	18.6	14.28**
Creatinine	96.6	23.3	9.40**
Disease duration	13.3	7.1	5.58*
Platelet count	371	98.2	5.07*
Haptoglobin	4.72	1.30	3.92
Albumin	43.2	2.61	2.58
Bilirubin	7.26	2.87	2.53
GGTP	27.0	15.9	1.17
Globulin	33.8	4.76	0.94
CRP	2.27	1.84	0.48
Urea	5.38	1.32	0.44
WBC	8.15	1.58	0.24
Serum histidine	1.35	0.21	0.21
Alkaline phosphatase	9.59	2.89	0.16
Plasma viscosity	1.87	0.14	0.13
Protein	77.2	4.85	0.08
SGOT	17.7	5.90	0.06
Fibrinogen	3.00	0.65	<0.01

**P<0.01, *P<0.05.

variables it is necessary to allow for any dependence on age and sex. Haemoglobin, total serum sulphydryl, and creatinine were much higher (essentially normal) in the AS group, while ESR and platelet count were lower, and hence nearer normal, than in the SNRA group (Table 4). No other variable showed a significant difference between these 2 groups.

Table 5 Percentage of abnormal blood tests for SPRA, SNRA, and AS

Biochemical variable	SPRA	SNRA	AS	High (H) or low (L)*
Hb	88	87	29	L
WBC	12	7	0	H
Platelet count	6	14	6	H
ESR	100	87	55	H
Plasma viscosity	100	87	83	H
Urea	6	17	10	H
Creatinine	0	0	0	—
Bilirubin	2	4	0	L
Alkaline phosphatase	22	14	13	H
SGOT	18	14	23	H
Protein	22	29	30	H
GGTP	62	43	38	H
Total sulphydryl	88	90	40	L
Serum histidine	90	80	77	L
CRP	98	90	90	H
Albumin	4	7	0	L
Globulin	42	33	24	H
Fibrinogen	12	10	3	H
Haptoglobin	96	87	97	H
RA Latex	100	36	ND	H
IgG	46	46	ND	H
IgG	8	21	ND	L
IgM	100	79	ND	H
IgA	90	64	ND	H
IgA	2	11	ND	L
C3	71	64	ND	H
C4	50	70	ND	H
C4	11	0	ND	L

ND = no data. *Compared with reported normal range.⁶

The percentages of patients with abnormal values, as defined by comparison with reported normal ranges⁶ in all 3 groups for each of the variables, are presented in Table 5.

Discussion

SNRA COMPARED WITH SPRA

The higher levels of acute-phase reactants in the SPRA group suggest a more active disease in this group, since acute-phase proteins correlate well with disease activity.^{6 19} However, it should be noted that the 2 groups were clinically similar as shown by the clinical measurements. The high IgA and IgM levels among the SPRAs indicate greater immunological activity, possibly associated with rheumatoid factor. By contrast, no difference was seen in IgG concentration between SPRA and SNRA. It remains a possibility that IgG/IgG complexes were present in the SNRA group. The low serum histidine in the SPRA group may contribute to the formation of rheumatoid factor²⁰ or may be a more generalised reflection of enhanced disease activity.

In comparing SPRA with SNRA we found the mean values for several variables to be higher in the SPRA group even though they did not achieve a statistically significant identification. Such variables included haptoglobin and fibrinogen, in keeping with other raised acute phase proteins, alkaline phosphatase, and GGTP, indicating a greater degree of hepatic involvement. These results suggest greater systemic involvement in SPRA, though this may be localised to certain organs, since haemoglobin was depressed equally in both groups.

Total serum sulphydryl was the only other variable equally abnormal (low) in both groups (Tables 3 and 5). A rise in total serum sulphydryl levels in RA is relatively nonspecific.²¹ This rise may occur by direct interaction of drugs with serum proteins, and hence may be a temporary or false indication of disease improvement, occurring regardless of the presence or absence of rheumatoid factor. However, all the patients in the present study received aspirin alone for 2 weeks prior to bleeding, and hence the group comparisons are valid. The cause of low serum sulphydryl in RA is still not clear, though serum protein conformational abnormalities resulting in increased disulphide linkages constitute a strong possibility. Alternatively the low levels may be caused by circulating immune complexes in both SPRA and SNRA.

Although differences occurred in several biochemical variables between SPRA and SNRA, the clinical severity in the 2 groups, as defined by the 6 clinical assessments, was remarkably uniform (Table 2), possibly reflecting admission criteria to the study. However, equivalent clinical severity in the 2 groups was accompanied by comparable changes in serum sulphydryl and haemoglobin, while acute-phase reactants and liver function tests were higher

in the SPRA group. It therefore remains a possibility that acute phase reactants reflect increased systemic activity not reflected by the clinical symptoms, while the low levels of serum sulphydryl and perhaps haemoglobin are a direct consequence of the pathogenetic processes.

SNRA COMPARED WITH AS

In the case of AS the high incidence of raised acute-phase reactants was essentially the same as for the RA groups, with the exception of ESR, which was raised in only 55% of cases (Table 5). Thus a third of the AS patients had raised plasma viscosity, CRP, and haptoglobin with normal ESR. Even when allowance is made for the age and sex dependence of the ESR in a normal population²² there appears to be a clear difference between ESR and other acute-phase reactants in AS. Since the patients were selected for disease activity, it may be that CRP or plasma viscosity are both preferable to ESR as indices of disease activity.

Anaemia was far less frequent for AS than RA (Tables 4 and 5), but haemoglobin levels are sex-dependent, the normal range for males being higher than that for females.²² Low total serum sulphydryl was also less frequent in AS and showed a significant difference when compared with that of RA. Age and sex dependence has been shown in one study on patients with RA³, but this is most likely linked with disease severity and duration. These differences in haemoglobin and total serum sulphydryl between AS and RA probably reflect underlying differences in disease pathogenesis. In addition the differences in serum sulphydryl levels lend support to the idea that their low levels in RA may be related to immune complexes. Furthermore the higher haemoglobin levels in the AS group may contribute towards the low ESR, and a high correlation has been demonstrated between these 2 variables in normal subjects²² and in RA and AS patients.²³

Our biochemical findings support the concept of SNRA, SPRA, and AS as discrete disease entities with their own individual biochemical profiles provided the disease is active. At the same time there are some similarities. Where a variable becomes abnormal in a disease, the trend to abnormality is always found in the same direction, whatever the disease. While SCAT by definition remains the best discriminator between SPRA and SNRA, IgA, IgM acute-phase reactants, and serum histidine may also have a role in confirming seropositivity. Serum sulphydryl and haemoglobin were the best discriminators between AS and RA, and the behaviour of these in relation to histidine and the ESR deserves further study, particularly in

other seronegative spondarthritides. This may lead to the formulation of 'biochemical diagnostic criteria' that might complement the existing guidelines suggested for RA by the American Rheumatism Association.

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