Alkaline phosphatase isoenzyme activities in rheumatoid arthritis: hepatobiliary enzyme dissociation and relation to disease activity

Sumihisa Aida

Abstract

***Objectives*—Hyperphosphatasaemia has been observed occasionally in patients with rheumatoid arthritis (RA), and it has been suggested that the serum alkaline phosphatase (ALP) level is related to the activity of the disease. Therefore, the relationship between serum ALP and RA was studied.**

***Methods*—The serum activities of hepatobiliary enzymes (ALP isoenzymes, γ-glutamyltranspeptidase (GTP), leucine aminopeptidase (LAP), aspartate aminotransferase (AST), and alanine aminotransferase (ALT)), immunoglobulins, RA haemagglutinin test (RAHA), C reactive protein (CRP), and erythrocyte sedimentation rate (ESR) were observed in 288 patients with rheumatoid arthritis.**

***Results*—Serum biliary ALP (ALP_1) activity was detected in 31·6% of the patients. In patients positive for ALP_1 the respective values of total ALP (ALPt) ($p<0·001$), liver ALP (ALP_2) ($p<0·001$), bone ALP (ALP_3) ($p<0·05$), γ-GTP ($p<0·001$), LAP ($p<0·001$), immunoglobulins IgG ($p<0·01$), IgA ($p<0·01$), and IgM ($p<0·01$), RAHA ($p<0·001$), CRP ($p<0·001$), ESR ($p<0·001$), and articular index ($p<0·001$) were significantly higher than in patients who did not have ALP_1. Significant Spearman's rank correlations (r_S) were demonstrated between serum ALP_2 level and the respective values of ALPt ($r_S=0·9128$, $p<0·001$), ALP_1 ($r_S=0·4443$, $p<0·001$), ALP_3 ($r_S=0·5898$, $p<0·001$), γ-GTP ($r_S=0·2903$, $p<0·001$), LAP ($r_S=0·3093$, $p<0·001$), IgA ($r_S=0·2299$, $p<0·01$), IgM ($r_S=0·1773$, $p<0·05$), RAHA ($r_S=0·2420$, $p<0·01$), CRP ($r_S=0·3532$, $p<0·001$), ESR ($r_S=0·4108$, $p<0·001$), and the articular index ($r_S=0·4006$, $p<0·001$). However, no significant difference or correlation was noted for either AST or ALT. In many patients who showed abnormal hyperphosphatasaemia, hepatobiliary enzyme dissociation was observed: levels of ALPt (in 12·8%), ALP_1 (in 31·6%), ALP_2 (18·8%), γ-GTP (in 4·3%), and LAP (in 19·3%) were abnormally high, but both AST and ALT were within normal limits.**

***Conclusion*—These findings are considered to be characteristic of RA, and suggest the existence of latent or subclinical hepatobiliary involvement and an association between the expansion of hepatobiliary involvement and the mechanism of disease activation. Thus measurement of the serum levels of ALP and its isoenzymes in RA is considered to be important.**

(*Ann Rheum Dis* 1993; **52**: 511–516)

Department of Anesthesiology (Pain Clinic), Niigata University School of Medicine, Niigata, Japan
S Aida

Correspondence to:
Dr S Aida,
Department of Anesthesiology, Niigata University School of Medicine, 1-757 Asahimachi, Niigata 951, Japan.

Accepted for publication
4 March 1993

The level of serum alkaline phosphatase (ALP) in patients with rheumatoid arthritis (RA) is thought to be within normal limits. Hyperphosphatasaemia has been observed occasionally in patients with RA, however, despite the absence of any clinical hepatobiliary disorder.[1–12] Comparison between patients with RA and osteoarthritis has shown a higher serum ALP level in RA.[3 10 12] These reports suggest an increase of the baseline serum ALP level in RA, although the underlying mechanism remains uncertain. Most reports have described that the raised serum ALP activity originates in the liver or bone, or both, from observations of ALP isoenzymes.[4–11] Rosalki, Foo, and Tanner[11] reported detection of serum biliary ALP isoenzyme (ALP_1) activity, which is macromolecular and is thought to be detectable only in cholestasis.[13–15]

Recently, observation of serum samples from patients with RA suggested that ALP_1 activity was detectable at a high prevalence of about 30%.[12] Furthermore, this report suggested that serum levels of liver ALP (ALP_2) and bone ALP (ALP_3) isoenzymes, as well as total ALP (ALPt) in patients with RA, were higher than in those with osteoarthritis, and that these levels in patients with RA who were ALP_1 positive were higher than those who were negative. No ALP_1 activity was detectable in serum samples from patients with osteoarthritis. Based on these findings, serum hepatobiliary enzyme activities in RA were observed in the present study. It is suggested that the serum ALP level is related to RA disease activity.[1–3 5–12] The relation between serum ALP and RA disease activity was also studied, therefore, based on observations of immunoglobulins, C reactive protein (CRP), erythrocyte sedimentation rate (ESR), and articular index.[16]

Materials and methods

Two hundred and eighty eight patients with RA (226 women and 62 men; mean age, 54

Table 1 Cases demonstrating abnormal hyperphosphatasaemia (more than 300 IU/l)

Patient No	Age	Sex	ALPt* (IU/l)	ALP_1* (IU/l)	ALP_2* (IU/l)	ALP_3* (IU/l)	γ-GTP* (U)	LAP* (U)	AST* (U)	ALT* (U)
1	55	F	311	0	183	128	16	135	23	13
2	72	F	312	0	121	157	20	156	26	25
3	72	F	316	0	198	118	18	190	22	18
4	62	F	338	0	184	154	29	155	15	13
5	62	F	300	28	160	80	57	122	22	17
6	55	F	300	13	188	94	92	395	19	8
7	61	F	301	53	172	76	107	NR	14	13
8	61	F	303	47	151	105	46	251	21	18
9	50	F	348	18	240	90	46	221	16	11
10	71	M	366	27	178	131	31	220	17	12
11	57	F	391	38	251	102	38	NR	12	15
12	53	F	404	37	286	80	42	211	22	20
13	50	F	428	57	252	119	13	141	14	10
14	45	F	454	31	277	128	47	271	14	10
15	57	F	465	54	244	133	28	161	23	17
16	72	M	476	138	209	102	117	NR	26	32
17	72	F	504	80	321	102	25	181	18	10
18	59	F	703	191	419	93	232	703	35	43
19	57	F	720	82	435	204	58	257	27	20
20	38	F	1756	497	787	402	578	871	40	43

ALPt=total alkaline phosphatase; ALP_1=biliary ALP; ALP_2=liver ALP; ALP_3=bone ALP; γ-GTP=γ-glutamyltranspeptidase; LAP=leucine aminopeptidase; AST=aspartate aminotransferase; ALT=alanine aminotransferase.
*Normal range: ALPt=70–260 IU/l; ALP_1=0 IU/l; $ALP_2 \leq 140$ IU/l; $ALP_3 \leq 110$ IU/l; γ-GTP=0–60 U; LAP=103–190 U; AST=5–40 U; ALT=5–35 U.
Although abnormal values are seen in ALPt, ALP_1, ALP_2, γ-GTP, and LAP, both AST and ALT are approximately within normal limits. ALP was measured by Bessey, Lowry and Brock's method.[18]
The sum of ALP_1, ALP_2, and ALP_3 is not equal to the value of ALPt in some cases because intestinal ALP is not shown.

Table 2 Comparison between patients with rheumatoid arthritis with and without biliary alkaline phosphatase activity

	ALP_1-positive		ALP_1-negative	
ALPt (IU/l)***	255(20)	(n=91)	162(3)	(n=197)
ALP_2 (IU/l)***	149(10)	(n=91)	90(2)	(n=197)
ALP_3 (IU/l)*	73(5)	(n=91)	62(2)	(n=197)
AST (U)	17·1(0·8)	(n=88)	16·4(0·5)	(n=170)
ALT (U)	14·8(1·0)	(n=88)	13·2(0·6)	(n=170)
γ-GTP (U)***	44(8)	(n=86)	14(1)	(n=167)
LAP (U)***	207(2)	(n=74)	140(2)	(n=149)
IgG (g/l)***	21·1(0·6)	(n=91)	19·1(0·4)	(n=197)
IgA (g/l)***	5·3(0·3)	(n=91)	3·8(0·1)	(n=197)
IgM (g/l)***	2·8(0·1)	(n=91)	2·4(0·1)	(n=197)
RAHA (/l)***	1096(173)	(n=91)	272(41)	(n=197)
CRP (mg/l)***	51(4)	(n=91)	17(1)	(n=197)
ESR (mm/h)***	83(5)	(n=91)	42(2)	(n=197)
Articular index***	16·2(1·2)	(n=91)	6·5(0·4)	(n=197)

ALP_1=biliary alkaline phosphatase; ALPt=total ALP; ALP_2=liver ALP; ALP_3=bone ALP; AST=aspartate aminotransferase; ALT=alanine aminotransferase; γ-GTP=γ-glutamyl transpeptidase; LAP=leucine aminopeptidase; RAHA=rheumatoid arthritis haemagglutinin test; CRP=C reactive protein; ESR=erythrocyte sedimentation rate.
ALP was measured by Bessey, Lowry, and Brock's method.[18]
*p<0·05, ***p<0·001 between patients positive or negative for ALP_1 by the Mann-Whitney U test.
Values are given as mean (SE).

(SE 1) years), diagnosed according to the revised criteria of the American Rheumatism Association,[17] were entered into the present study. They had been receiving treatment for RA at our pain clinic from 1985 to 1991. The articular index was scored according to the method of Ritchie *et al.*[16] No other diagnosed disorders were clinically evident in these patients, such as biliary, hepatic, skeletal, intestinal, or uterine diseases, and there was no overlap with other autoimmune or connective tissue diseases, such as systemic lupus erythematosus, or primary biliary cirrhosis. Non-steroidal anti-inflammatory drugs, corticosteroids, or disease modifying anti-rheumatic drugs had been given to all patients.

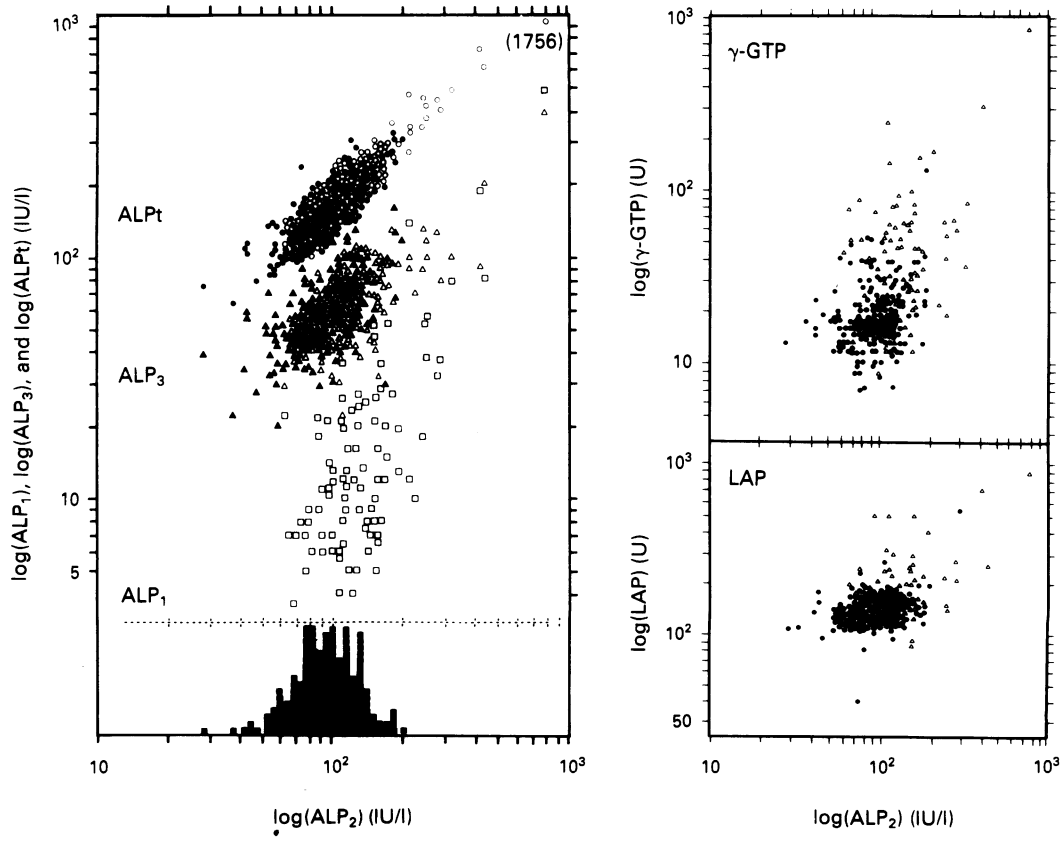

Figure 1 Left: relation between liver alkaline phosphatase (ALP_2) and total ALP (ALPt), biliary ALP (ALP_1), or bone ALP (ALP_3). Significant Spearman's rank correlation (r_S) was demonstrated between ALP_2 and each value of ALPt (●,○; r_S=0·9128; p<0·001), ALP_1 (■,□; r_S=0·4443; p<0·001), or ALP_3 (▲,△; r_S=0·5898; p<0·001). Right: relation between ALP_2 and γ-glutamyl transpeptidase (γ-GTP) (r_S=0·2903; p<0·001), or leucine aminopeptidase (LAP) (r_S=0·3093; p<0·001). Open symbols represent patients positive for ALP_1 and filled symbols, patients who had no ALP_1 activity. The bar graph below the broken line (left) shows ALP_2 in these patients negative for ALP_1. The values for patients positive for ALP_1 are distributed to the upper right.

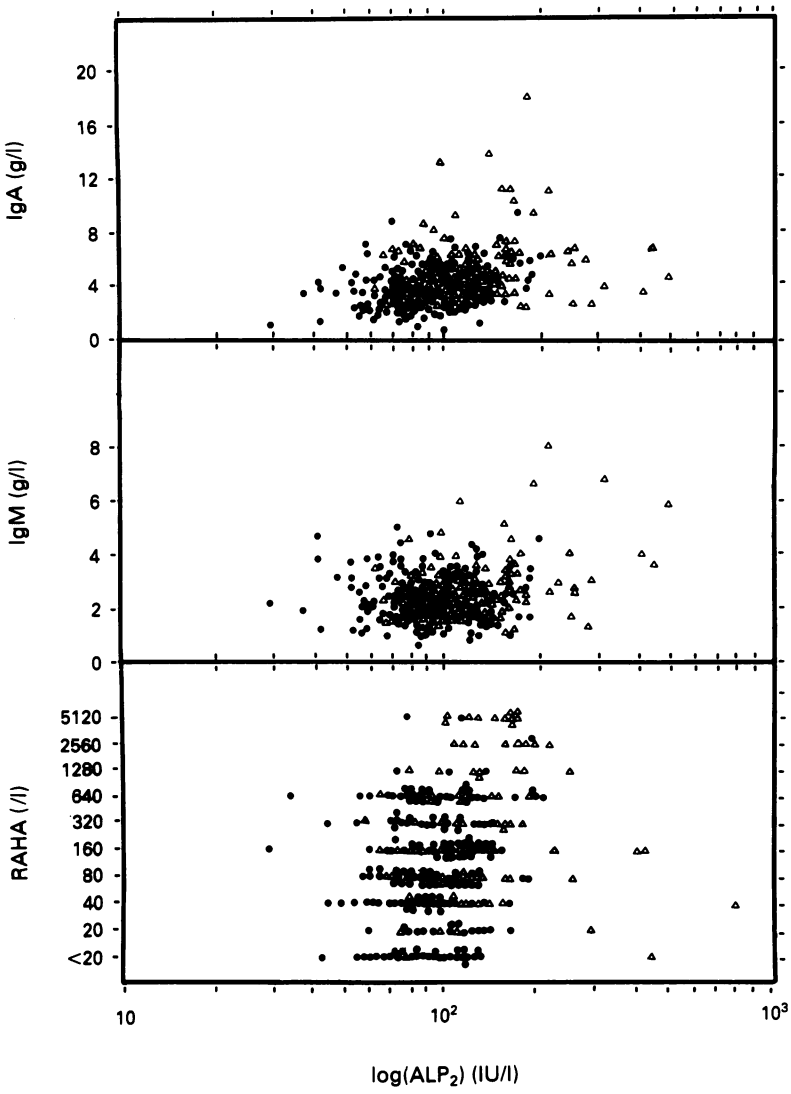

Figure 2 Relation between liver alkaline phosphatase (ALP_2) and IgA, IgM, or rheumatoid arthritis haemagglutinin test (RAHA). Significant Spearman's rank correlations (r_S) were demonstrated between ALP_2 and each value of IgA (r_S=0·2299; p<0·01), IgM (r_S=0·1773; p<0·05), or RAHA (r_S=0·2420; p<0·01). Open triangles represent patients positive for biliary ALP (ALP_1) and filled circles, patients with no ALP_1 activity. The values of patients with ALP_1 activity are distributed to the upper right.

All clinical assessments of articular index and laboratory data were performed as a routine clinical examination on the earliest consultation day after the start of this investigation in 1985, when informed consent was obtained. ALPt activity was measured by the method of Bessey, Lowry, and Brock,[18] and ALP isoenzymes were separated by electrophoresis on cellulose acetate membranes (Helena Laboratories, Urawa, Japan). Immunoglobulins (IgG, IgA, and IgM) were determined by single radial immunodiffusion (Hoechist Japan, Tokyo, Japan). Rheumatoid arthritis haemagglutination test (RAHA) was performed by the microtitre method (Fuji Reibo, Tokyo, Japan). An autoanalyser (Hitachi 736-60, Hitachi Medico, Tokyo, Japan) and Clinimate kits (Daiichi Pure Chemicals, Tokyo, Japan) were used for measurements of ALPt, γ-glutamyl transpeptidase (γ-GTP) (γ-glutamyl CPA substance method), leucine aminopeptidase (LAP) (Tuppy, Wiesbauer and Wintersberger's method[19]), aspartate aminotransferase (AST) (IFCC method), alanine aminotransferase (ALT) (IFCC method), and CRP (immunonephelometry). ESR was obtained by Westergren's method.

The resuslts were expressed as mean and standard error of the mean (mean (SE)). Data were analysed statistically using the Mann-Whitney U test, or Spearman's rank correlation coefficient (r_S). Differences at p<0·05 were considered significant.

Results

Serum ALP_1 activity was detected in 91 out of 288 (32%) patients with RA and was undetectable in the remaining 198 patients. Values of ALP_1 varied from 2 to 497 IU/l, but 279 (97%) were less than 50 IU/l (table 1). In the patients positive for ALP_1 the respective values of ALPt (p<0·001), ALP_2 (p<0·001), ALP_3 (p<0·05), γ-GTP (p<0·001), LAP (p<0·001), IgG (p<0·01), IgA (p<0·01), IgM (p<0·01), RAHA (p<0·001), CRP (p<0·001), ESR (p<0·001), and the articular index (p<0·001) were significantly higher, by the Mann-Whitney U test, than in those without ALP_1 activity. No significant differences were noted in either AST or ALT (table 2).

On the other hand, significant Spearman's rank correlations were demonstrated between the serum ALP_2 level and the respective values of ALPt (r_S=0·9128, p<0·001), ALP_1 (r_S=0·4443, p<0·001), ALP_3 (r_S=0·5898, p<0·001), γ-GTP (r_S=0·2903, p<0·001), LAP (r_S=0·3093, p<0·001), IgA (r_S=0·2299, p<0·01), IgM (r=0·1773, p<0·05), RAHA (r_S=0·2420, p<0·01), CRP (r_S=0·3532, p<0·001), ESR (r_S=0·4108, p<0·001), and the articular index (r_S=0·4006, p<0·001). The values of these parameters in patients positive for ALP_1 were distributed to the upper right, as shown in figs 1–3.

Many patients demonstrating hepatobiliary enzyme dissociation were noted: high values of ALPt, ALP_1, ALP_2, γ-GTP, and LAP were seen in many patients, whereas the values of both AST and ALT were approximately within normal limits in all the patients tested (tables 1 and 3). No jaundice was observed in any of the patients. Table 1 shows the clinical data for 20 patients who demonstrated abnormally high serum ALPt activity (more than 300 IU/l). Tables 1 and 3 suggest that hepatobiliary enzyme dissociation was demonstrated frequently for ALP_1 (31·6%), often for ALPt (12·8%), ALP_2 (18·8%), and LAP (19·3%), and occasionally for γ-GTP (4·3%). However, four patients showing abnormal hyperphosphatasaemia but ALP_1 negativity were also noted. In these cases, the increase of ALPt was not so severe, and ALP_3 elevation was seen dominantly (table 1).

In a few cases a pattern considered to indicate binding to immunoglobulin was found. These cases showed electrophoretic patterns quite different from those of patients positive for ALP_1 (fig 4), and were in agreement with the results obtained by Maekawa, Sudo, and Kano.[20] Significant relations between the immunoglobulin binding and hepatobiliary enzymes were not noted.

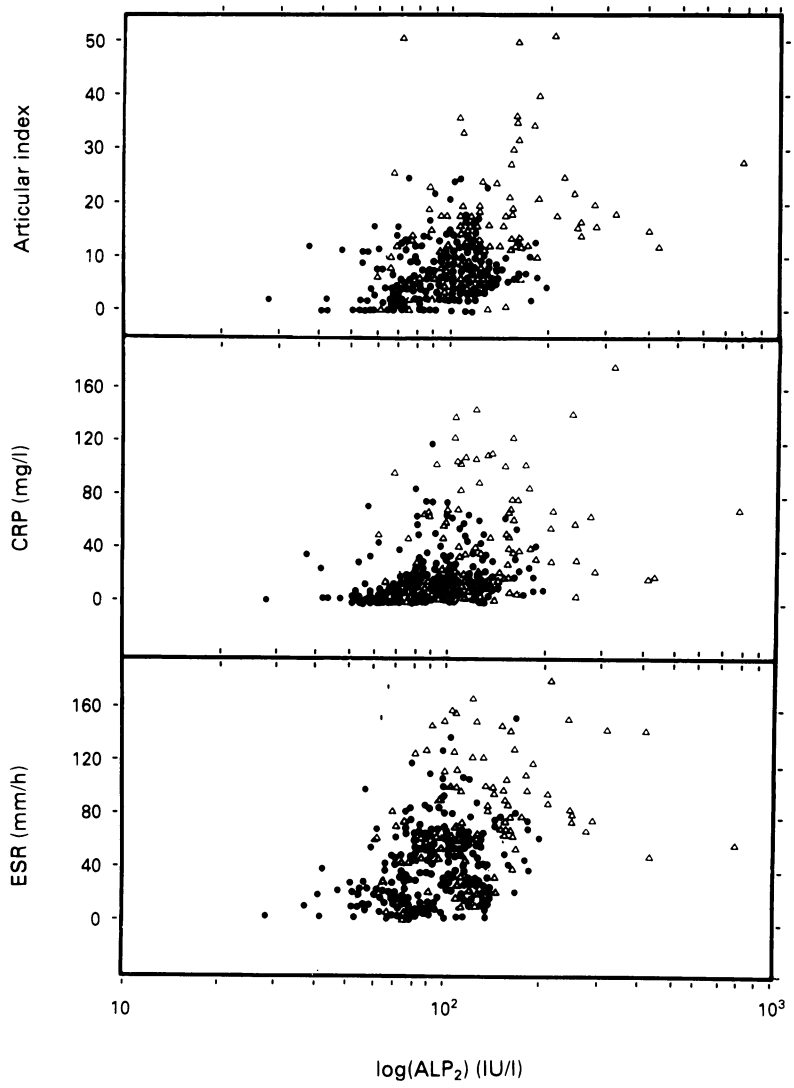

Figure 3 Relation between liver alkaline phosphatase (ALP_2) and the articular index, C reactive protein (CRP) or erythrocyte sedimentation rate (ESR). Significant Spearman's rank correlations (r_S) were demonstrated between ALP_2 and each value of the articular index (r_S=0·4006; p<0·001), CRP (r_S=0·3532; p<0·001) or ESR (r_S=0·4108; p<0·001). Open triangles represent patients positive for biliary ALP (ALP_1) and filled circles, patients with no ALP_1 activity. The values of patients with ALP_1 activity are distributed to the upper right.

Intestinal ALP isoenzyme activity was seen in the serum of type B or O blood, but this activity, 20 (SE 2) IU/l, was considered to be physiologically normal.[21] No other ALP isoenzyme activity was seen.

Discussion

Hyperphosphatasaemia in patients with RA has been observed on occasion.[1-12] Several authors have recorded a rise of ALP_2 activity[2 4-8 11 12] accompanied by concurrent increases in γ-GTP[4 6 8 12] or 5'-nucleotidase,[2 4] or both, but normal AST and ALT levels.[2 5 6 12] On the other hand, it has been reported that serum ALP_1 activity is detectable in patients with RA.[11 12] In the present study serum ALP_1 activity was detectable in 31·6% of patients examined. This prevalence rate is thought to be very high, because serum ALP_1 activity is usually detectable only in biliary disorders, as the internal pressure of the bile capillaries increases.[13-15]

In the present study high serum values of hepatobiliary enzymes were evident in many patients (tables 1 and 3). Significant correlations between ALP_2 and the respective values of ALPt, ALP_1, ALP_2, ALP_3, γ-GTP, and LAP were demonstrated (fig 1), and these values were higher in those patients positive for ALP_1 than in those who had no ALP_1 activity. These findings indicate the probable existence of subclinical hepatobiliary involvement in RA. This involvement is supported by the histopathological observations by Kendall, Cockel, and Hawkins,[2] Webb *et al*,[5] Lefkovits and Farrow,[26] and Dietrichson *et al*,[27] indicating that the histology of the liver in RA is non-specific, and includes findings of Kupffer cell hyperplasia, fatty infiltration, and infiltration of peripheral areas with mononuclear cells.[28] Neither AST nor ALT was high, however, even in patients who showed abnormally high levels of ALPt, ALP_1, ALP_2, γ-GTP, and LAP in the present study (tables 1–3). These phenomena are considered to indicate hepatobiliary enzyme dissociation.

In the previous study,[12] the baseline serum ALP_2 level was significantly raised, even in patients without ALP_1, in comparison with patients with osteoarthritis. In the patients positive for ALP_1, the respective values of ALPt, ALP_2, γ-GTP, and LAP were significantly higher than in patients without ALP_1 activity (table 2). These results suggest more frequent underlying subclinical hepatobiliary involvement in RA than is generally assumed. Because the serum levels of these enzymes are known to increase more in biliary tract disorders than in hepatocellular injury,[21-25] this involvement seems to start from the biliary capillaries at the latent stage. As involvement of the bile capillaries proceeds further, ALP_1 leaks into serum and the serum ALP_1 activity may be detectable. Concomitantly, the ALP_2 level still rises. The leakage seems not to be attributable to an increase in internal pressure of the bile capillaries but to an increase in the permeability of the bile capillaries in RA. If the pressure had increased, jaundice would accompany the presence of ALP_1. Even in the case showing a very high level of ALPt—that is, 1756 IU/l (ALP_1, 497; ALP_2, 787 IU/l), jaundice was not observed. Thus ALP_2, γ-GTP, and LAP increase considerably, as shown in fig 1, but remain at subclinical levels in most cases (table 2). Considering the normal levels of both AST and ALT at this stage, therefore, hepatocellular injury is thought to be slight. When the involvement expands to hepatocytes, obvious hepatitis, such as that

Table 3 Hepatobiliary enzyme dissociation

	ALPt (n=288)	*ALP_1 (n=288)*	*ALP_2 (n=288)*	*γ-GTP (n=253)*	*LAP (n=223)*	*AST (n=258)*	*ALT (n=258)*
Cases showed high value (n)	37	91	54	11	43	0	2
Cases showed high value (%)	12·8	31·6	18·8	4·3	19·3	0*	0·8
Range	261–1756 (IU/l)	2–497 (IU/l)	141–787 (IU/l)	61–578 (U)	191–871 (U)	— (U)	43 (U)

ALPt=total alkaline phosphatase; ALP_1=biliary ALP; ALP_2=liver ALP; γ-GTP=γ-glutamyl-transpeptidase; LAP=leucine aminopeptidase; AST=aspartate aminotransferase; ALT=alanine aminotransferase.
Although high values are demonstrated in ALPt, ALP_1, ALP_2, γ-GTP, and LAP of many patients with RA, both AST and ALT are approximately within normal limits.
*Values of AST were less than 40 U in all patients.

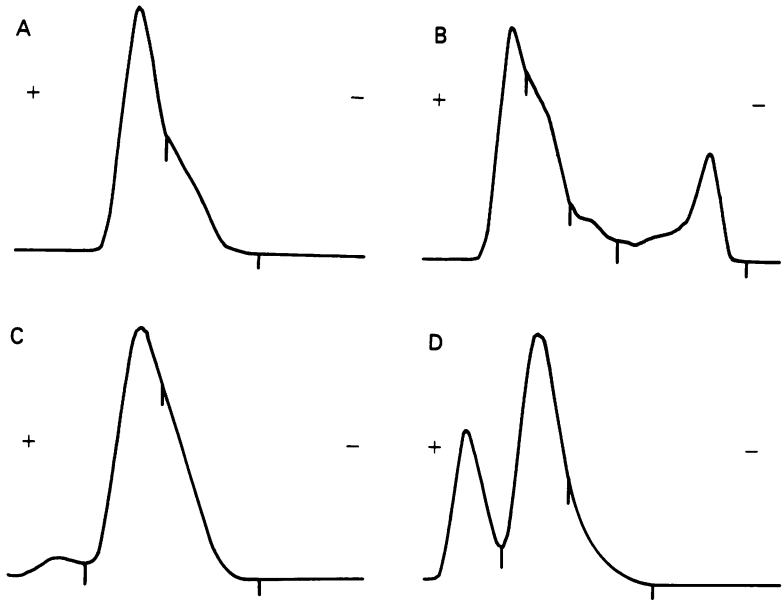

Figure 4 Electrophoretic patterns of serum alkaline phosphatase in patients with rheumatoid arthritis. A: normal pattern. B: a pattern considered to indicate binding to immunoglobulin. C and D: biliary ALP (ALP_1) activity is evident. ALP_1 has moved towards the anode side.

reported by Job-Deslande *et al*,[29] may become clinically evident. Of course, the levels of AST and ALT would then increase. However, Job-Deslande *et al*[29] demonstrated that increases in serum bilirubin (10–63 μmol/l), AST (54–92 U), and ALT (54–152 U) were mild in hepatitis during RA, and jaundice was present in two of six cases. These facts are thought to support the results of the present study. Therefore, it is thought that we observed the latent or subclinical involvement of RA as hepatobiliary enzyme dissociation. Such dissociation, which is considered to be characteristic of RA, was observed frequently for ALP_1, sometimes for ALPt, ALP_2, and LAP, and occasionally for γ-GTP, and became more frequent in the patients positive for ALP_1 (tables 1 and 3). The number and level of parameters showing abnormal values are thought to indicate the grade and intensity of hepatobiliary involvement.

A connection between serum ALP and RA disease activity has already been shown by several authors.[1-3 5-12] In the present study significant correlation between ALP_2 and the respective values of IgA, IgM, RAHA, CRP, ESR, and the articular index was demonstrated (figs 2 and 3), and IgG, IgA, IgM, RAHA, CRP, ESR, and the articular index in patients positive for ALP_1 were all significantly raised compared with patients without ALP_1 activity (table 2). These facts suggest a relation between the two phenomena in hepatobiliary involvement and disease activation.

It is known that promotion of disease activity induces more active bone resorption.[31 32] Activated bone resorption is accompanied by concomitant bone formation and a rise in serum ALP_3.[21] Therefore, several authors[1-3 6 7 10] have considered that disease activity in RA is related to the level of ALP_3. A relation between RA disease activity and ALP_3 might not be wholly denied. The higher ALP_3 level in patients with RA, however, is considered to be produced by the activated bone formation: this is to say, a rise of ALP_3 is not a cause but a result of disease activation. Therefore, a significant correlation between ALP_2 and ALP_3 was considered to be present (fig 1). In four patients negative for ALP_1 showing abnormal hyperphosphatasaemia, however, the ALP_3 level was considerably raised (patients 1–4 in table 1). From these facts, the existence of other disease activation mechanisms, which are not associated with hepatobiliary enzyme dissociation, is also suggested. In actual cases, both mechanisms would work concurrently. Whichever mechanism operates, serum ALPt activity may have to increase in parallel with disease activity in RA. In fact, three of these four patients also had a high ALP_2 level (table 1). Therefore, ALP_1, ALP_2, and ALP_3 are considered important for the evaluation of disease activation. To determine whether the disease activity is associated with ALP activities, however, other markers that directly indicate the disease activity should be monitored in the long term.

There is also a possibility that rises in ALP, γ-GTP, and LAP are due to liver toxicity resulting from treatment with non-steroidal anti-inflammatory drugs, disease modifying antirheumatic drugs, or corticosteroids. In the present study, however, no pathological increase of AST and ALT was observed in any subject even though treatment with these drugs was continued. The previous study[12] also showed that this was unlikely, as ALP isoenzyme activities did not differ among patients treated with these drugs. Doube *et al*[30] also reported that non-steroidal anti-inflammatory drugs had no influence on ALP activity in patients with RA. Therefore, rises in these enzymes are considered to be attributable to RA itself.

Webb *et al*,[5] Spooner *et al*,[8] and Kantharia and Woolf[33] demonstrated cases of RA and primary biliary cirrhosis overlapping. In the present study, however, patients with apparent hepatobiliary disorders, such as cirrhosis or hepatitis, were excluded and both AST and ALT were at normal levels. Thus the present findings indicate that serum biliary enzymes, expecially ALPt, ALP_1, and ALP_2, are related closely to the activity of RA and subclinical hepatobiliary involvement by RA, which is observed in the latent and subclinical stages as hepatobiliary enzme dissociation.

1 Frank Von O, Klemmayer K. Die alkalische Serumphosphatase bei Erkrankungen des rheumatischen Formenkreises unt ihre Beeinflussung druch Kortikosteroide. *Z Rheumaforsch* 1968; **27:** 142–9.
2 Kendall M J, Cockel R, Hawkins C F. Raised alkaline phosphatase in rheumatoid arthritis. An index of liver dysfunction? *Ann Rheum Dis* 1970; **29:** 537–40.
3 Perlík F, Kůtová M. The evaluation of bone changes in patients with rheumatoid arthritis. *Z Rheumatol* 1983; **42:** 261–4.
4 Thompson P W, Houghton B J, Clifford C, Jones D D, Whitacker K B, Moss D W. The source and significance of raised serum enzymes in rheumatoid arthritis. *Q J Med* 1990; **76:** 869–79.
5 Webb J, Whaley K, MacSween R N M, Nuki G, Dick W C, Buchanan W W. Liver disease in rheumatoid arthritis and Sjögren's syndrome. *Ann Rheum Dis* 1975; **34:** 70–81.

6 Fernandes L, Sullivan S, McFarlane I G, *et al.* Studies on the frequency and pathogenesis of liver involvement in rheumatoid arthritis. *Ann Rheum Dis* 1979; **38:** 501–6.
7 Siede W H, Seiffert U B, Merle S, Goll H G, Oremek G. Alkaline phosphatase isoenzymes in rheumatic diseases. *Clin Biochem* 1989; **22:** 121–4.
8 Spooner R J, Smith D H, Bedford D, Beck P R. Serum gamma-glutamyltransferase and alkaline phosphatase in rheumatoid arthritis. *J Clin Pathol* 1982; **35:** 638–41.
9 Cimmino M A, Accardo S. Changes in the isoenzyme pattern of alkaline phosphatase in patients with rheumatoid arthritis. *Clin Chem* 1990; **36:** 1376–7.
10 Cimmino M A, Buffrini L, Bsrisone G, Bruzzone M, Accardo S. Alkaline phosphatase activity in the serum of patients with rheumatoid arthritis. *Z Rheumatol* 1990; **49:** 143–6.
11 Rosalki S B, Foo A Y, Tanner P A S. Serum gamma-glutamyltransferase and alkaline phosphatase in rheumatoid arthritis. *J Clin Pathol* 1982; **35:** 1395.
12 Aida S. Relation between alkaline phosphatase isoenzymes and disease activity in rheumatoid arthritis. *Acta Med Biol* 1992; **40:** 79–83.
13 Price C P, Sammons H G. The nature of serum alkaline phosphatases in liver diseases. *J Clin Pathol* 1974; **27:** 392–8.
14 Brocklehurst D, Lathe G H, Aparicio S R. Serum alkaline phosphatase, nucleotide pyrophosphatase, 5'-nucleotidase and lipoprotein-X in cholestasis. *Clin Chim Acta* 1976; **67:** 269–79.
15 Crofton P M, Smith A F. High-molecular-mass alkaline phosphatase in serum and bile: Physical properties and relationship with other high-molecular-mass enzymes. *Clin Chem* 1981; **27:** 860–6.
16 Ritchie D M, Boyle J A, McInnes J M, *et al.* Clinical studies with an articular index for the assessment of joint tenderness in patients with rheumatoid arthritis. *Q J Med* 1968; **147:** 393–406.
17 Arnett F C, Edworthy S M, Bloch D A, *et al.* The American Rheumatism Association 1987. Revised criteria for the classification of rheumatoid arthritis. *Arthritis Rheum* 1988; **31:** 315–24.
18 Bessy O A, Lowry O H, Brock M J. A method for the rapid determination of alkaline phosphatase with five cubic millimeters of serum. *J Biol Chem* 1946; **164:** 321–9.
19 Tuppy H, Wiesbauer U, Wintersberger E. Aminozäure-p-nitroanilid als Substrate für Aminopeptidasen und andere proteolytische Fermente. *Phys Chem* 1962; **329:** 278–88.
20 Maekawa M, Sudo K, Kanno T. A case of rheumatoid arthritis with various enzyme-immunoglobulin complexes. *Clin Chim Acta* 1986; **157:** 45–54.
21 Posen S. Alkaline phosphatase. *Ann Intern Med* 1967; **67:** 183–203.
22 Arthur Y, Wellman-Bednawska M, Jacquier A, Siest G. Associations between serum gamma-glutamyltransferase and apoliproteins: Relationships with hepatobiliary diseases. *Clin Chem* 1984; **30:** 1318–21.
23 Rutenberg A M, Goldgarg J A, Pineda E P. Serum γ-glutamyl transpeptidase activity in hepatobiliary pancreatic disease. *Gastroenterology* 1963; **45:** 43–8.
24 Wachstein M. Enzyme histochemistry of the liver. *Gastroenterology* 1959; **37:** 525–37.
25 Pineda E P, Goldbarg J A, Banks B M, Rutenberg A M. Serum leucine aminopeptidase in pancreatic and hepatobiliary diseases. *Gastroenterology* 1960; **38:** 698–712.
26 Lefkovits A M, Farrow I J. Liver in rheumatoid arthritis. *Ann Rheum Dis* 1955; **14:** 162–8.
27 Dietrichson O, From A, Christoffersen P, Juhl E. Morphological changes in liver biopsies from patients with rheumatoid arthritis. *Scand J Rheumatol* 1976; **5:** 65–9.
28 Weinblatt M E, Tesser J R P, Gillian III J H. The liver in rheumatic diseases. *Semin Arthritis Rheum* 1982; **11:** 399–405.
29 Job-Deslandre C, Feldmann J L, Djian Y, Menkes C J. Chronic hepatitis during rheumatoid arthritis. *Clin Exp Rheumatol* 1991; **9:** 507–10.
30 Doube A, Davies J, Davis M, Maddison P J. Influence of non-steroidal anti-inflammatory drugs and disease activity on serum alkaline phosphatase concentrations in rheumatoid arthritis, osteoarthritis, and polymyalgia rheumatica. *Ann Rheum Dis* 1989; **48:** 368–71.
31 Sambrook P N, Ansell B M, Foster S, *et al.* Bone turnover in early rheumatoid arthritis. 1. Biochemical and kinetic indexes. *Ann Rheum Dis* 1985; **44:** 575–9.
32 Rico H, Hernandez E R, Gomez-Castresana F, Yague M, Cabranes J A, Valor R. Osteopenia in rheumatoid arthritis: a biochemical, hormonal and histomorphometric study. *Clin Rheumatol* 1990; **9:** 63–8.
33 Kantharia B K, Woolf A D. Raised alkaline phosphatase in rheumatoid arthritis: Primary biliary cirrhosis? *Br J Rheumatol* 1987; **26:** 313–4.