

Synovial fluid lactic acid measurement in the diagnosis and management of septic arthritis

T RIORDAN, D DOYLE,* SOAD TABAQCHALI

*From the Departments of Medical Microbiology and *Rheumatology, St Bartholomew's Hospital, London EC1*

SUMMARY An improved method of lactic acid estimation by gas liquid chromatography (GLC) is described. Synovial fluid lactic acid estimation was performed on 52 patients (15 with septic arthritis and 37 with non-septic arthropathies) and compared to routine microbiological methods and white cell counts. Lactic acid was found to be a useful and rapid test for differentiating between septic and non-septic arthritis being markedly raised (> 12 mmol/l) in all the septic joints. Raised lactic acid concentrations were of particular diagnostic value in patients in whom antibiotic therapy had commenced before joint aspiration. The results of lactic acid estimation on sequential samples were helpful in assessing the response of septic arthritis to treatment.

Septic arthritis must be diagnosed rapidly so that effective treatment can be instituted and joint destruction prevented. It is often difficult to differentiate clinically between septic arthritis and a non-septic inflammatory arthropathy. The synovial fluid white cell count is raised in inflammatory arthropathies and it is not a reliable index of infection unless very high ($> 100 \times 10^9/l$).¹ A Gram stain of synovial fluid may show organisms and give rapid confirmation of a septic arthritis, but when negative a period of 24-48 h may elapse before results of cultures are available to confirm the diagnosis. Because of this delay patients may have to be treated with antibiotics on clinical assessment alone, until the bacteriological culture results are available. Furthermore, in patients in whom antibiotics have been given before joint aspiration, cultures may be negative although the infection has not been eradicated.

In the search for a method which would enable rapid diagnosis of septic arthritis, synovial fluid lactic acid has been studied. Previous studies²⁻⁴ have reported high lactic acid concentrations in septic arthritis as compared to non-infective arthropathies. There is, however, little information on the diagnostic value of lactic acid concentrations in patients who have already commenced antibiotic therapy and in monitoring response to treatment.

This study, therefore, was set up to compare the

value of lactic acid measurements to conventional methods in the diagnosis of septic arthritis in such patients.

An improved quantitative method for lactic acid measurement by gas-liquid chromatography is also reported.

Material and methods

PATIENTS

Synovial fluid was obtained under aseptic conditions from 52 patients with acutely inflamed joints and with clinically detectable effusions. These patients had septic arthritis (15), rheumatoid arthritis (18), other non-septic inflammatory arthropathies (11) and crystal arthropathy or osteoarthritis (8). Sequential samples of synovial fluid were obtained from five patients with septic arthritis, samples were obtained (a) before antibiotic therapy in patients with proven positive bacteriological culture, (b) on antibiotics but with positive culture and (c) the first samples with negative culture (see Fig. 3).

EXAMINATION OF SYNOVIAL FLUID

Samples from each fluid were submitted for synovial fluid white cell count, routine bacteriological examination and lactic acid estimation. For the latter investigation samples were either processed immediately or frozen at -20°C until assayed. For bacteriological examination, specimens were centrifuged and the deposit was examined by Gram stain, and cultured aerobically on blood agar (Oxoid)

and MacConkey agar, and anaerobically on enriched media supplemented with haemin (5 mg/l) and menadione (1 mg/l) and inoculated into thio-glycollate broth. All specimens were also cultured for mycobacteria on Löwenstein-Jensen medium.

LACTIC ACID DETERMINATION

Lactic acid estimation was performed by gas liquid chromatography (GLC). An internal standard, 2-hydroxybutyric acid sodium salt (Sigma, London Ltd) dissolved in distilled water (4 mmol/l) was used. The internal standard was assessed and shown to be stable, not detected naturally in joint fluid and gave a constant relative response factor at 0.5 over a wide range of lactic acid concentrations.

Samples were prepared for chromatography by placing 1 ml of synovial fluid in a glass bijou bottle with a screw cap. Internal standard (1 ml), 1 ml absolute ethyl alcohol (analar grade, BDH, Poole, Dorset) and 0.5 ml 50% sulphuric acid (analar grade, BDH, diluted in distilled water) were added. The mixture was heated at 100°C for 5 min and then cooled to room temperature in a water bath. Distilled water (1 ml) was then added and the sample centrifuged at 3000 rpm for 5 min to separate protein and other precipitated material. A portion (1 ml) of the supernatant was taken and mixed with 0.25 ml chloroform (chromatographic grade, BDH) in a bijou bottle and vortexed for 30 s. The emulsion was then broken by centrifuging at 1000 rpm for 30 s.

Chromatography

Samples (1 µl) were withdrawn from the chloroform layer using 1 µl syringe (SGE Ltd, Melbourne, Australia) and injected into a Pye Unicam 104 series chromatograph with a flame ionisation detector, fitted with a glass column 1.5 m long and 4 mm internal diameter, packed with 10% diethylene glycol succinate (DEGS) on celite (100-120 mesh). The gas flow rates were nitrogen carrier gas 35 ml/min, hydrogen 35 ml/min, air 350 ml/min. The amplifier current was set at 1 × 10² units and the instrument was operated isothermally at 100°C. Chromatograms were traced by a Philips 8220 potentiometric chart recorder.

Lactic acid concentration in sample

This was determined as follows:

$$\text{Concentration of lactic acid} = \frac{\text{height of lactic acid peak (HL)}}{\text{height of internal standard peak (HI)}} \times \frac{\text{concentration of internal standard}}{\text{relative response factor}}$$

$$\begin{aligned} \text{Concentration of lactic acid} &= \frac{\text{HL}}{\text{HI}} \times \frac{4}{0.5} \\ &= \frac{\text{HL}}{\text{HI}} \times 8 \end{aligned}$$

Assessment of the method

Reproducibility was assessed by processing 10 replicates of the same synovial fluid. Recovery studies were performed by adding known amounts of lactic acid standard to samples of joint fluid and measuring the total lactic acid concentration. The method was compared with the reference lactic acid method, lactic dehydrogenase (LDH) using a commercial kit (Test combination lactate, Boehringer, Mannheim), 20 samples of synovial fluid were processed in parallel by the two methods.

Results

Reproducibility and recovery studies

The 10 replicates of the same synovial fluid gave results ranging from 2.5 mmol/l to 2.9 mmol/l. The mean value was 2.74 mmol/l and the SD was ±0.13 mmol/l (±4.7%).

The recovery studies on two synovial fluids are shown in Table 1. The percentage recovery ranged from 94.6% to 106.4%. The mean recovery was 100.7% and the SD was ±3.8%.

Comparison with LDH method

The results of the comparative study between the GLC and LDH method is shown in Fig. 1. The correlation coefficient was 0.992.

CLINICAL STUDIES

The different groups of patients studied are shown in Table 2. Of the 52 patients, 11 had culture-positive septic arthritis, 4 had clinical septic arthritis with hot swollen joints and supporting evidence such as

Table 1 Lactic acid recovery studies in synovial fluid

	Lactate added (mmol/l)	Lactate determined (mmol/l)	% recovery
Specimen I	1	7.5*	
	2	8.4	98.8
	4	9.8	103.2
	6	11.3	98.3
	8	14.2	105.2
Specimen II	1	18.1	103.4
	2	2.7*	
	4	3.5	94.6
	6	5.0	106.4
	8	6.6	98.5
	6	8.8	101.1
	8	10.4	98.1

*Concentration of lactic acid in the original sample.

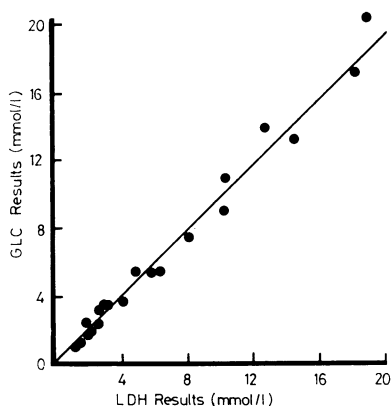


Fig. 1 Comparison of synovial fluid lactic acid estimation by gas liquid chromatography (GLC) and lactic dehydrogenase (LDH) methods.

positive blood cultures, but antibiotic therapy had been instituted and synovial fluid cultures were negative. Thirteen of the 15 cases of septic arthritis involved previously normal joints, one patient had rheumatoid arthritis and one osteoarthritis. Of the non-septic cases 18 had rheumatoid arthritis, 11 had other inflammatory arthropathies and eight had crystal arthropathy or osteoarthritis.

In seven of the 11 culture-positive cases organisms were seen in a Gram stain of synovial fluid giving rapid confirmation of the clinical diagnosis. However, the Gram stain was negative in the other four cases and the phenol-auramine fluorescence was also negative in the patient with *Mycobacteria tuberculosis* infection which was later diagnosed on culture.

The types of organisms isolated from the synovial fluid in the patients with septic arthritis were *Staphylococcus aureus* (5), *Streptococcus pyogenes* (3), *Streptococcus pneumoniae* (1), *Serratia marcescens* (1) and *Mycobacterium tuberculosis* (1).

The synovial fluid lactic acid concentrations were higher in the septic arthritis patients than in the

other two groups (Table 2, Fig. 2), and the white cell counts in the synovial fluid were also higher in the culture-positive group of patients than in the other groups. The peripheral white cell count on the other hand was variable in all groups and of little diagnostic value (Table 2).

The results of synovial fluid lactic acid measurements in the five patients in whom sequential sampling was performed are shown in Fig. 3. In the three patients with group b specimens (positive cultures while on antibiotic therapy) the levels shown represent the mean of two or more sequential samples taken over a period ranging from 2-10 days. Lactic acid concentrations remained high as long as the specimens were culture positive and fell only when bacterial cultures became negative. The time taken to render the joint fluid sterile varied from 2 to 14 days in one patient whose infection was difficult to control (Fig. 4).

Discussion

The results presented in this paper demonstrate that lactic acid measurements in the synovial fluid is a good diagnostic index of septic arthritis.

The method used is accurate, rapid and simple and compares well with the standard reference LDH method.

A GLC method was used in this study because specimens usually arrive at bacteriological departments for culture, and there is increasing acquisition and applications of GLC in these departments for taxonomic and rapid diagnostic work, particularly in anaerobic infections.⁵ Previous reports^{6,7} on lactic acid estimation by GLC showed poor resolution of the lactic acid peak from the solvent peak, making accurate measurements of peak height difficult. To overcome this problem changes in derivatisation and chromatographic conditions were applied. The introduction of an internal standard in this method obviated the need for performing a standard curve each time the assay was performed,

Table 2 Results of synovial fluid lactic acid, white cell count and peripheral white cell count

Diagnosis	No of cases	Lactic acid (mmol/l)			Synovial fluid WBC ($\times 10^6/l$)		Peripheral WBC ($\times 10^6/l$)	
		Mean	SEM	Range	Mean	Range	Mean	Range
Septic arthritis								
Culture-positive	11	24.4	3.0	13.6-44.9	147.8	113.0-193.0	12.7	3.0-48.8
Culture-negative	4	17.3	2.4	13.4-24.0	NA		10.4	7.4-14.4
Non-specific inflammatory								
Rheumatoid arthritis	18	5.9	0.9	2.8-18.0	20.4	5.8-37.7	7.6	3.5-12.1
Others	11	3.9	0.6	0.9-6.4	7.2	0.8-12.6	9.0	6.1-16.2
Degenerative								
Crystals	5	2.7	0.4	1.3-3.6	8.5	0.7-13.0	9.0	8.2-10.4
Osteoarthritis	3	1.8	0.2	1.4-2.0	0.4	0.5-1.1	6.0	4.8-6.8

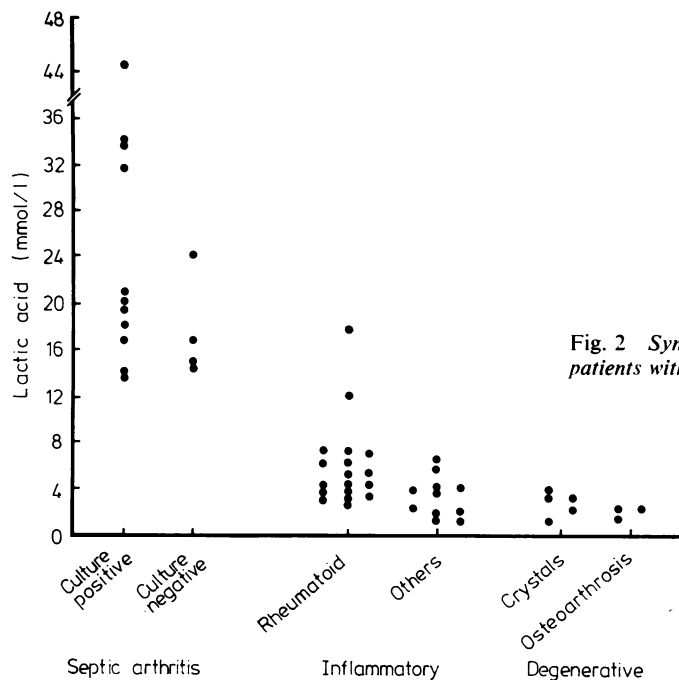


Fig. 2 Synovial fluid lactic acid concentrations in patients with septic arthritis and other arthropathies.

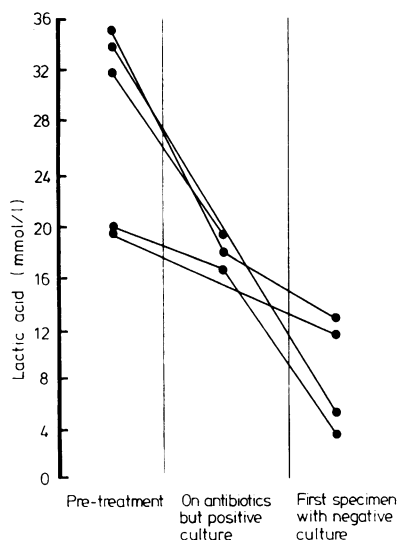


Fig. 3 Sequential synovial fluid results in five patients with culture-positive septic arthritis.

making the assay independent of small variations in the amount of sample injected and the chromatographic conditions.

All patients with septic arthritis had markedly raised synovial fluid lactic acid, even in those who had

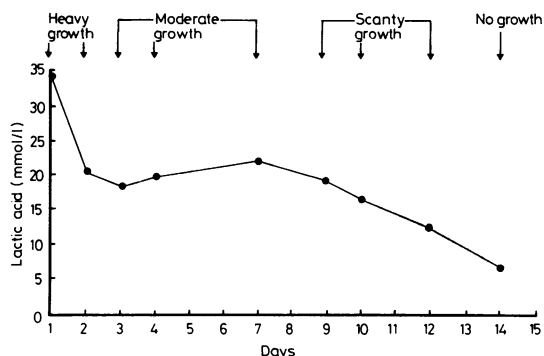


Fig. 4 Sequential synovial fluid results in a single patient with septic arthritis due to *Staphylococcus aureus*.

already commenced antibiotics and culture of their synovial fluid was negative. Whereas apart from two non-septic effusions with lactate concentrations that overlapped the septic arthritis group, the remainder were below 8 mmol/l. This indicates that lactic acid measurement is a sensitive test and is useful in differentiating between septic arthritis and non-septic arthritis. One patient of the 18 in the rheumatoid arthritis group had a lactic acid level which overlapped those in the septic group. This patient had very active rheumatoid arthritis involving the

knee joint, but there was no evidence of infection. Further work on this group of patients is necessary to assess the value of lactic acid measurements.

Synovial fluid lactic acid seemed to be more sensitive than a Gram stain. Three of the patients with acute bacterial infection had negative Gram stains but markedly raised synovial fluid lactic acid concentrations. Similarly in the tuberculous effusion the phenol-auramine fluorescence was negative but the lactic acid was clearly increased. With the long delay in myobacterial culture the level of lactic acid is particularly helpful in this situation.

Comparison with synovial fluid white cell count was made difficult because in some cases the fluid was too thick for a count to be performed. However, in those cases where it was possible to measure synovial fluid white cell count there was a good separation between septic and non-septic arthritis. The results of peripheral white cell counts show that it is very unreliable in differentiating between septic and non-septic arthritis.

The synovial fluid lactic acid results in antibiotic-treated septic arthritis are of considerable interest. The four cases of culture-negative septic arthritis (Table 2), all had raised lactic acid concentrations associated with clinically active infection. In the five septic arthritis patients with sequential samples, group b samples (on antibiotics but with clinically active infection and positive culture) still had markedly raised lactic acid concentrations, but group c samples (negative culture and clinically resolving) the lactic acid concentrations were reduced to near normal, as in Fig. 3. Thus the synovial fluid lactic acid level seems to correlate well with culture results and particularly closely with clinical activity of septic arthritis.

We conclude that synovial fluid lactic acid estimation is a useful adjunct to established methods such as Gram smear and synovial fluid white cell count in rapid differentiation between septic and non-septic arthritis. It is particularly useful in diagnosis when antibiotics have been given before joint aspiration and serial estimations are helpful in monitoring the response of septic arthritis to treatment.

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Requests for reprints to: Dr S Tabaqchali, Department of Medical Microbiology, St Bartholomew's Hospital, West Smithfield, London EC1A 7BE, England.