

## Plasma lactate dehydrogenase: a marker of disease activity in cryptogenic fibrosing alveolitis and extrinsic allergic alveolitis?

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**ABSTRACT:** Total plasma lactate dehydrogenase (LDH) activity may be elevated in cryptogenic fibrosing alveolitis (CFA) and extrinsic allergic alveolitis (EAA), and may be a useful monitor of disease progress.

In a retrospective, primary referral centre study, we compared LDH at presentation, prior to bronchoalveolar lavage BAL, and after treatment and follow-up with changes in pulmonary function, in patients with CFA, EAA and pulmonary sarcoidosis.

Plasma levels of LDH at presentation in CFA (n=47) and EAA (n=10) were significantly higher than in patients with sarcoidosis (n=36). LDH activity decreased in patients with improving lung function (EAA, p=0.008; CFA, p=0.02), whereas it increased in CFA patients with deteriorating lung function (p=0.015).

Total plasma LDH is a simple, though nonspecific test, which appears to reflect changes of disease activity in patients with CFA and EAA.

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There is no reliable, simple method to assess disease activity in patients with cryptogenic fibrosing alveolitis (CFA). This makes management decisions difficult, since the disease responds unpredictably and often poorly to treatments, which may have significant adverse effects [1, 2].

CFA can be thought of as having two components: a potentially reversible inflammatory component associated with, to a varying degree, an interstitial fibrotic element, which is regarded as irreversible [3]. Disease activity is usually assessed from tissue obtained by open or trans-bronchial lung biopsy. However, histological changes are not uniform throughout the lung and, therefore, small samples may be unrepresentative [4]. Serial open lung biopsies are not practicable. The prognostic value of bronchoalveolar lavage (BAL) differential cell counts and gallium-67 scanning are substantially less than initially suggested [4, 5]. Erythrocyte sedimentation rate, immunoglobulins, autoantibodies and immune complexes may be elevated in CFA patients, but show no consistent relationship with disease activity [4, 6].

Physiologically, CFA is characterized by decreased lung volumes, reduced diffusing capacity, and hypoxaemia on exertion or at rest. Hence, pulmonary function testing remains the most useful monitor of disease progress. The need for a quantitative and reproducible assessment of clinical activity led to the development of a clinical, radiographic and physiological (CRP) score. The CRP score described by WATTERS *et al.* [7] used seven variables (dyspnoea, chest radiograph, spirometry, lung volume, diffusing capacity, resting

alveolar/arterial oxygen tension (Po<sub>2</sub>) and exercise O<sub>2</sub> saturation), with an arbitrary scoring system weighted towards physiological measurements. Whilst this provides an overall quantitative assessment of disease progress and response to treatment, it is cumbersome and time consuming.

We have observed that some patients with CFA have elevated lactate dehydrogenase LDH values in contrast with sarcoidosis patients. Previously, DE REMEE [8] reported elevation of serum LDH in five cases of "Interstitial pneumonitis". LDH is an ubiquitous intracellular enzyme used primarily, in clinical practice, as a marker of cardiac and liver injury. However, it has been suggested that increased serum LDH may reflect disease activity in renal systemic lupus erythematosus [9], joint disease in rheumatoid arthritis (RHA) [10], and severe dermatitis [11]. Elevated LDH has recently been reported in alveolar proteinosis [12], and pneumocystis pneumonia associated with acquired immune deficiency syndrome (AIDS) [13]. In CFA, LDH could be released from damaged inflammatory or parenchymal cells. There is *in vitro* evidence of LDH leakage from Type II pneumocytes, pulmonary endothelium and alveolar macrophages, following a number of insults, including hypoxia [14-17]. In animal studies, BAL LDH provides a useful indicator of lung injury [17].

We have reviewed plasma LDH in patients with CFA, extrinsic allergic alveolitis (EAA) and sarcoidosis attending a primary referral centre, and have compared changes in blood levels with lung function: 1) acutely in response to therapy; and 2) over longer term follow-up.

## Methods

A disease-based card index system identified patients with CFA (n=81), EAA (n=10) and sarcoidosis (n=49) attending the Northern General Hospital, Edinburgh, between 1984 and 1991. The diagnoses were based on clinical and radiographic features, history of environmental exposure, serum precipitins, transbronchial biopsy, BAL differential cell counts and, if indicated, a Kveim test. Ten percent of patients with CFA or EAA had open lung biopsies. All CFA and EAA patients presented with chronic symptoms, of more than three months duration. The majority of patients with radiological Grade I sarcoidosis presented acutely with erythema nodosum and/or respiratory symptoms. Most patients with Grade II and III disease presented with chronic respiratory symptoms. However, there were a small number in all grades with few or no symptoms. Patients with documented cardiac failure, oedema, liver disease or other abnormal "liver enzymes", carcinoma or connective tissue disease (including RhA) were excluded. LDH levels obtained within the three months prior to death or those with other "liver enzyme" abnormalities were not included in the analyses, in order to try to exclude possible nonpulmonary sources of LDH. All initial blood samples at presentation were taken prior to any therapy. Subsequent samples were taken at clinic reviews (1-6 monthly). The decision to use immunosuppressive therapy was taken by the individual clinician.

BAL was performed in the middle lobe or lingular segment of the left upper lobe with 240 ml (8x30 ml) warmed physiological saline. BAL differential counts were assessed by light microscopy counting 500 haematoxylin/eosin stained cells from cytocentrifuge preparations. Macrophages, lymphocytes, neutrophils and eosinophils were expressed as a percentage (%) of BAL cells.

## Assay

Total plasma LDH activity was determined at 37°C, according to the recommendations of the Deutsche Gesellschaft für Klinische Chemie [18, 19] using a kit (Test-Combination LDH opt., (Cat No. 124907), Boehringer Mannheim) on a Monarch centrifugal fast analyser (IL Ltd, Warrington, UK). Briefly, the conversion of pyruvate to L-lactate was measured spectrophotometrically by the decrease in absorbance at 340 nm due to the removal of reduced nicotinamide adenosine dinucleotide (NADH) from the reaction solution. The initial concentrations of reagents in the reaction cuvette were; phosphate buffer 50 mmol·l<sup>-1</sup>, pH 7.5; pyruvate 0.6 mmol·l<sup>-1</sup>; and NADH 0.18 mmol·l<sup>-1</sup>.

The between batch coefficient of variation was 1.5% at a level of 300 U·l<sup>-1</sup> and 1.9% at a level of 700 U·l<sup>-1</sup>. The reference range was 72-395 U·l<sup>-1</sup>.

## Pulmonary function

Pulmonary function was assessed by vital capacity (VC), total lung capacity (TLC), transfer factor of the lungs for carbon monoxide (Tlco) and transfer coefficient for carbon monoxide (Kco), using helium dilution and single-breath carbon monoxide methods. A significant change in pul-

monary function was defined as a 10% or greater change in one or more parameters, provided the remaining parameters were unchanged.

## Statistical analyses

Nonparametric tests were used. These were the Mann-Whitney U-test, Kruskal-Wallis (analysis of variance (ANOVA)) and Spearman Correlation. Results were considered significant at p<0.05. Analyses refer to Mann-Whitney tests unless otherwise stated.

## Results

Case records of 47 of 81 patients with CFA, all 10 with EAA and 36 of 49 with sarcoidosis were analysed. Thirty four CFA patients were excluded, because records were not available or LDH was not measured (21), or due to the presence of cardiac failure (4), liver disease with abnormal transaminases (2), concomitant connective tissue disease (5), or invalid diagnosis (2). Thirteen out of 49 sarcoidosis patients were excluded, because records were not available or LDH was not documented. Twenty out of 47 CFA patients received immunosuppressive therapy (corticosteroids, cyclophosphamide, azathioprine or cyclosporin).

## LDH at presentation

Patients with CFA (n=47, median LDH 444 U·l<sup>-1</sup>, range 280-746 U·l<sup>-1</sup>) and EAA (n=10, median LDH 537 U·l<sup>-1</sup>, range 369-702 U·l<sup>-1</sup>) had plasma LDH activity above the laboratory normal range (72-395 U·l<sup>-1</sup>) at presentation and significantly higher than the sarcoidosis group (n=36, median LDH 337 U·l<sup>-1</sup>, range 253-454 U·l<sup>-1</sup>) both p<0.0001 (ANOVA p<0.0001) (table 1, fig. 1). LDH levels within the radiological grades of sarcoidosis did not differ (mean (median); Stage I n=17, 346(346); Stage II n=6, 330(336); Stage III n=13, 342(337)).

## LDH and changes in pulmonary function in CFA (long-term follow-up)

Long-term data on 35 out of 47 CFA patients were available. "Long-term follow-up" was defined (with a pre-set minimum of three months) as the last available LDH measurement before December 1991 or three months prior to death. Mean follow-up was 29 months (median follow-up 24 months) with a range of 7-84 months. Six CFA patients (mean follow-up 45 months) showed a sustained improvement in pulmonary function; in 18 (mean follow-up

Table 1. - Plasma LDH at presentation

Diagnosis	n	Male n	Age† yrs	LDH at presentation* U·l <sup>-1</sup>
CFA	47	26	64 (46-84)	471±104
S	36	16	40 (24-67)	342±52
EAA	10	1	45 (14-89)	522±90

†: data presented as mean and range in parenthesis; \*: data presented as mean±SD. LDH: lactate dehydrogenase; CFA: cryptogenic fibrosing alveolitis; S: sarcoidosis; EAA: extrinsic allergic alveolitis.

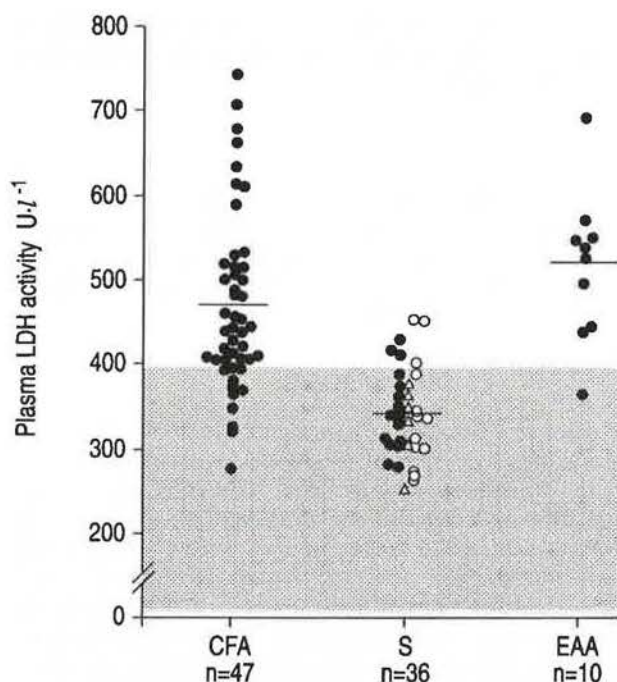


Fig. 1. - Plasma lactate dehydrogenase (LDH) levels at presentation for patients with cryptogenic fibrosing alveolitis (CFA), sarcoidosis (S) and extrinsic allergic alveolitis (EAA). The median values are indicated by the bar and the normal range by the shaded area. For Sarcoidosis percentage, chest X-ray Stage I (●); Stage II (Δ); Stage III (○).

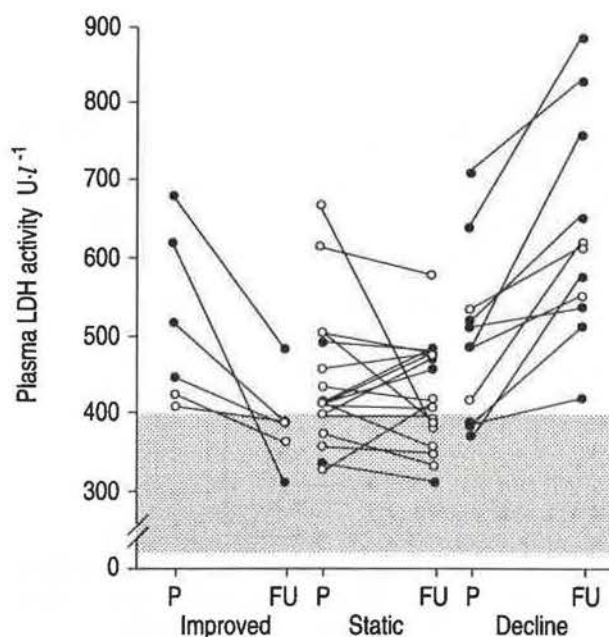


Fig. 2. - Lactate dehydrogenase (LDH) levels at presentation (P) and follow-up (FU) in patients with cryptogenic fibrosing alveolitis (CFA), related to changes in pulmonary function (see text). ●: patients given immunosuppressive therapy after presentation; ○: patients given no treatment.

Table 2. - Pulmonary function and LDH: progress over time

Diagnosis	Pulmonary function change >10%	n	Duration of follow-up† months	Mean LDH		p
				Presentation U.l <sup>-1</sup>	Follow-up U.l <sup>-1</sup>	
CFA	Improved (↑)	6	45 (24-84)	515	404	0.02*
CFA	No change	18	32 (7-72)	437	422	0.75
CFA	Decline (↓)	11	18 (7-43)	495	630	0.018*
EAA	Improved (↑)	6	12 (5-29)	563	361	0.015*
EAA	No change	3	20 (6-45)	492	499	0.82

†: data presented as mean and range in parenthesis. \* statistically significant by Mann Whitney test. For abbreviations see legend to table 1.

32 months) there was no change, and in 11 (mean follow-up 18 months) there was deterioration. Deteriorating lung function was associated with a rise in LDH (mean rise 140 U.l<sup>-1</sup>,  $p=0.0018$ ), and improving lung function with a fall in LDH (mean fall 130 U.l<sup>-1</sup>,  $p=0.02$ ) (table 2, fig. 2).

#### LDH before and after six months immunosuppressive therapy for CFA

Only 10 CFA patients had pulmonary function and LDH measured immediately before and six months after starting immunosuppression (nine prednisolone, one cyclosporin and prednisolone). On treatment, three patients had improved lung function and their mean LDH fell from 617 to 349 U.l<sup>-1</sup>. Four patients with unchanged lung function showed no change in LDH (mean LDH from 432 to 430 U.l<sup>-1</sup>). In three patients in whom lung function deteriorated LDH levels rose (mean 566 to 823 U.l<sup>-1</sup>). (fig. 3).

#### LDH and changes in pulmonary function in EAA

On removal of a causative agent, with or without supplementary corticosteroid therapy, lung function improved in six EAA patients (mean follow-up 12 (3-29) months). In three there was no change (mean follow-up 20 (6-45) months). For one patient, follow-up LDH data were not available. In the six patients in whom lung function improved there was a reduction in LDH from 563 to 361 U.l<sup>-1</sup> ( $p=0.0018$ ). Three patients with unchanged lung function had no change in LDH (mean LDH from 492 to 499 U.l<sup>-1</sup>) (fig. 4).

#### LDH and BAL differential cell count in CFA and sarcoidosis

BAL differential cell counts were available for 27 out of 47 CFA and 14 out of 35 sarcoid patients with LDH measured

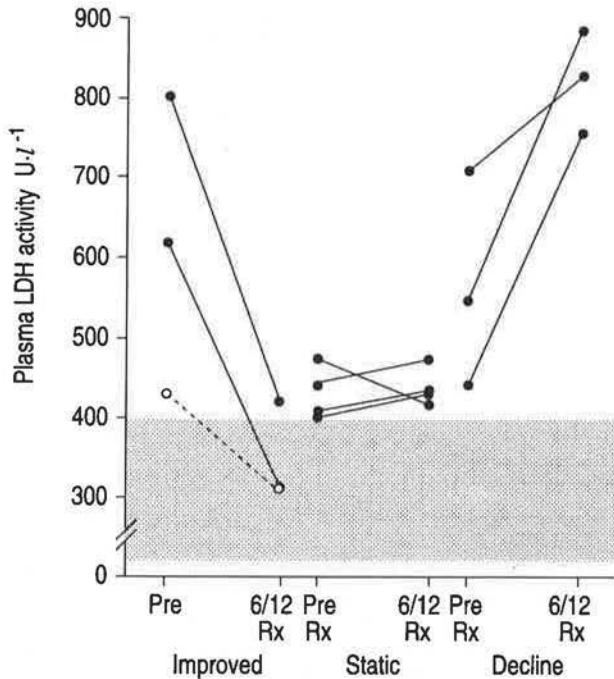


Fig. 3. - Lactate dehydrogenase (LDH) levels in patients with cryptogenic fibrosing alveolitis pretreatment (Pre-Rx) and after six months (6/12-Rx) immunosuppressive therapy (prednisolone (●); cyclosporin (○) related to changes in pulmonary function. Rx: treatment.

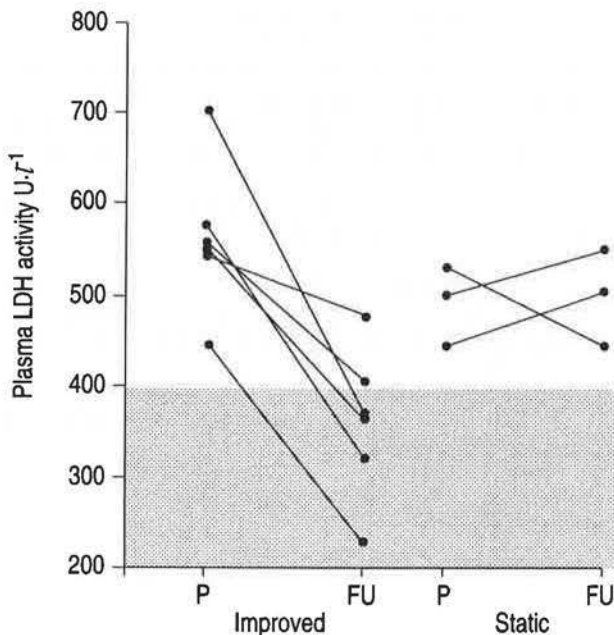


Fig. 4. - Lactate dehydrogenase (LDH) levels at presentation (P) and follow-up (FU) in patients with extrinsic allergic alveolitis (EAA), related to changes in pulmonary function.

prebronchoscopy and before treatment. BAL differential cell counts for CFA (mean % (range)) were: macrophages 80 (98-24)%, lymphocytes 9 (47-0)%, neutrophils 6 (46-1)%, eosinophils 5 (21-0)%; and for sarcoidosis were: macrophages 58 (87-34)%, lymphocytes 40 (64-10)% neutrophils 1 (3-0)%, eosinophils 1 (4-0)%.

In CFA, increasing % BAL neutrophil ( $r_s=0.48$ ,  $p=0.011$ ) and eosinophil ( $r_s=0.44$ ,  $p=0.02$ ) counts correlated with

plasma LDH, and % BAL macrophages were inversely related to LDH activity ( $r_s=-0.46$ ,  $p=0.016$ ). In sarcoidosis, % BAL lymphocytes ( $r_s=0.63$ ,  $p=0.017$ ) correlated with LDH and % macrophage counts were inversely related ( $r_s=-0.63$ ,  $p=0.016$ ).

**Discussion**

At presentation, plasma LDH levels were elevated in CFA and EAA but not in pulmonary sarcoidosis. The wide overlap of values, however, would preclude using LDH to discriminate between CFA, EAA and sarcoidosis. CFA and EAA patients whose lung function improved showed a fall in LDH. Conversely, LDH increased in CFA patients whose lung function deteriorated. We observed that changes in LDH in some patients predated changes in pulmonary function. However, due to the lack of data points in this retrospective study, this aspect could not be assessed for many patients.

In the UK, open lung biopsy is not a standard diagnostic procedure for patients with interstitial lung diseases. Our relatively low proportion of open lung biopsies was therefore not unusual. However, we believe the diagnoses to be secure in the majority of patients by application of stringent criteria in establishing a diagnosis as CFA rather than EAA; namely the absence of exposure to environmental antigens, negative serum precipitins, absence of granulomata in multiple transbronchial biopsies, and the cell profile of the bronchoalveolar lavage. In the event, LDH appears to be elevated in both disorders. The diagnosis of sarcoid was adequately established in the absence of open lung biopsy by the presence of noncaseating well-formed granulomata on transbronchial lung biopsies or positive Kveim test, and supported by elevated angiotensin converting enzyme levels. We were unable to determine the LDH isoenzyme profile retrospectively in our patients, and the origin of the LDH is uncertain. Lung parenchymal cells or local inflammatory cells, including macrophages [20], may be a source. *In vitro* studies of Type II pneumocytes and alveolar macrophages demonstrate release of LDH from either cell type, when subjected to a noxious stimulus [14, 16]. Elevated serum LDH and BAL LDH activity is reported in alveolar proteinosis [12]. It is reasonable to suggest that the moderate elevation of LDH activity seen in CFA and EAA could be of pulmonary origin, since other inflammatory diseases have been associated with elevated LDH [9-11]. Case reports have suggested the presence of macromolecular LDH (immunoglobulin-LDH complexes) in patients with autoimmune diseases [21]. This complex increases the half-life of LDH in the blood and thus increases "plasma level". At least one case of macromolecular LDH has been reported in association with CFA [22]. Increased LDH in CFA patients with deteriorating lung function may be due to nonpulmonary sources. In particular, the liver could release LDH, in response to hypoxia or "right" heart failure. However, patients with overt heart failure were excluded from this study.

We were surprised to find correlations between LDH and % BAL neutrophils and eosinophils, in CFA, and % BAL lymphocytes in sarcoidosis. Whilst this raises the

possibility that the inflammatory cell component of the alveolitis may contribute to the elevated LDH, we would not wish to misinterpret what are relatively weak correlations.

The reasons why LDH levels should be elevated in CFA and EAA but not sarcoidosis, are unclear. Could the degree of pulmonary involvement affect the LDH levels? For the sarcoidosis patients as a group, the radiographic changes were less marked than in those with CFA. However, within the sarcoidosis patients there were no differences in LDH levels between the different radiological stages. We have also observed that in two patients (data not shown) with open lung biopsy confirmation of bronchiolitis obliterans-organising pneumonia (BOOP), with marked lymphocytic exudate and extensive X-ray changes, LDH levels (327, 365 U·l<sup>-1</sup>) were normal.

Elevated LDH activity as a feature of EAA has not been reported. It is uncertain why the association between LDH and CFA has not been more widely recognized. Our laboratory has measured total plasma LDH as a "liver function test". Many laboratories do not measure LDH routinely or measure specific or urea stable isoenzymes in serum. This study suggests plasma total LDH may provide a helpful and simple method of monitoring patients with CFA and EAA.

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