REVIEW

Usefulness of lactate dehydrogenase and its isoenzymes as indicators of lung damage or inflammation

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Usefulness of lactate dehydrogenase and its isoenzymes as indicators of lung damage or inflammation. M. Drent, N.A.M. Cobben, R.F. Henderson, E.F.M. Wouters, M. van Dieijen-Visser. ©ERS Journals Ltd 1996.
ABSTRACT: This review describes the usefulness of monitoring the activity level of lactate dehydrogenase (LDH) and its isoenzyme pattern as indicators of pathological conditions in the lungs, such as cell damage or inflammation.

Cytoplasmatic cellular enzymes, like LDH, in the extracellular space, although of no further metabolic function in this space, are still of benefit because they serve as indicators suggestive of disturbances of the cellular integrity induced by pathological conditions. Since LDH is an enzyme present in essentially all major organ systems, serum LDH activity is abnormal in a large number of disorders.

Although the increase in total serum LDH activity is rather nonspecific, it is proposed that measurement of LDH activity levels and its isoenzyme pattern in pleural effusion and, more recently, in bronchoalveolar lavage fluid may provide additional information about lung and pulmonary endothelial cell injury.
Eur Respir J., 1996, 9, 1736–1742.

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Cellular enzymes in the extracelluar space, although of no further metabolic function in this space, are still of benefit because they serve as indicators suggestive of disturbances of the cellular integrity induced by pathological conditions. Lactate dehydrogenase (LDH) is a cytoplasmatic enzyme present in essentially all major organ systems. The extracellular appearance of LDH is used to detect cell damage or cell death [1–3]. Due to its extraordinarily widespread distribution in the body, serum LDH is abnormal in a host of disorders [3–9]. It is released into the peripheral blood after cell death caused by, *e.g.* ischaemia, excess heat or cold, starvation, dehydration, injury, exposure to bacterial toxins, after ingestion of certain drugs, and from chemical poisonings [1, 2]. Therefore, the total serum LDH is a highly sensitive, but nonspecific test.

In order to optimize the diagnostic value, LDH isoenzymes can be measured [3]. Although the serum LDH isoenzyme activity pattern has a slightly better specificity, the clinical value is still rather low. In contrast, measurement of LDH and LDH isoenzymes in pleural fluid, and more importantly in bronchoalveolar lavage (BAL) fluid, is useful to determine lung tissue damage and pulmonary endothelial cell injury [10–14].

Cellular changes in BAL fluid (BALF) during acute inflammation include an influx of polymorphonuclear neutrophils (PMNs) and activation of alveolar macrophages (AMs) [15–18]. The neutrophil has been implicated as contributing to the lung injury incurred during an inflammatory response [15, 18]. It has been suggested that the neutrophil influx plays a major role in increasing the permeability of the alveolar/capillary barrier and in producing cellular toxicity during the inflammatory response [18–20]. In line with this, it was indicated that the cytotoxicity of neutrophils was associated with, *e.g.* reperfusion injury and adult respiratory distress syndrome (ARDS) [5].

If cell lysis occurs, or cell membranes are damaged, cytoplasmic enzymes, such as LDH and glutathione reductase (GR), are released into the extracellular space [4]. The enzymatic activities found in the BALF provide important information concerning the degree of cytotoxicity present, measured by extracellular activities of cytoplasmatic enzymes, such as LDH, and the release of lysosomal enzymes [4]. Some increase in LDH is caused by transudation of serum, but in previous studies this has been a minor component of the increased LDH activity in BALF [21]. The LDH in BALF appeared to originate from lung cells, probably AMs or PMNs [22–24]. However, the exact cellular source of these enzymes is still unknown.

The usefulness of measurement of LDH activity and its isoenzymes in serum, but especially in BALF and pleural effusions as indicators of lung damage or inflammation is reviewed.

Biochemistry and physiology of LDH

Lactate dehydrogenase (LDH, EC 1.1.1.27) is a hydrogen transfer enzyme that catalyses the oxidation of L-lactate to pyruvate with nicotinamide-adenine dinucleotide (NAD)+ as hydrogen acceptor, the final step in the metabolic chain of anaerobic glycolysis. The reaction is reversible and the reaction equilibrium strongly favours the reverse reaction, namely the reduction of pyruvate (P) to lactate (L) [1, 2, 25]:

LDH, pH 8.8–9.8

$$L\text{-lactate} + NAD^+ \quad \Leftrightarrow \quad Pyruvate + NADH + H^+$$

pH 7.4–7.8
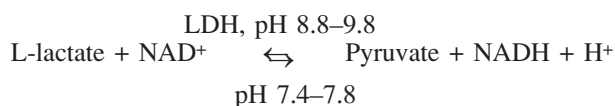
A multiplicity of procedures for measurement of LDH activity have been developed over the last 25 yrs; the procedures use the forward (L→P) and the reverse (P→L) in almost equal numbers. The L→P assay has the following advantages: substrate inhibition by lactate is less than that produced by pyruvate; and the reaction linearity is more prolonged than that of the P→L assay. Advantages of the P→L assay are: a less expensive assay formulation (because of the much lower concentration of the reactants); the greater absorbency with time (thus allowing more precise measurements); and greater stability of the working reagents once they are prepared as assay solutions.

The enzyme is composed of four peptide chains of two types: M and H, each under separate genetic control. Heart (H) subunit or muscle (M) subunit are so named because of their predominance in the respective tissues. In this way, five different isoenzymes, having different chemical and physical properties can be found. The isoenzymes all catalyse the same biochemical reaction but differ in their molecular structure, and are more or less organ specific [1, 2]. Therefore, isoenzyme patterns can be used to localize the source of LDH release. The isoenzymes differ in reactivity to substrates, sensitivity to inhibitors, resistance to heat inactivation, cold lability, and electrophoretic mobility in tertiary structure and charge. Therefore, isoenzymes are separable electrophoretically. The subunit compositions of the five isoenzymes in order of decreasing anodal mobility in an alkaline medium are: LDH-1 (HHHH; H_4); LDH-2 (HHHM; H_3M); LDH-3 (HHMM; H_2M_2); LDH-4 (HMMM; HM_3); and LDH-5 (MMMM; M_4) [1, 2].

Using the assay at 37°C by an enzymatic rate method, with pyruvate as a substrate, the reference ranges for LDH activity are 200–450 $U \cdot L^{-1}$ in serum [25], and 10–50 $U \cdot L^{-1}$ in BALF. The reference ranges in serum for LDH isoenzymes are: LDH-1 19–30%; LDH-2 32–48%; LDH-3 12–22%; LDH-4 5–11%; and LDH-5 5–13%, [25]. Moreover, in BALF, reliable determination of LDH isoenzymes is only possible when the total LDH level in BALF is higher than 50 $U \cdot L^{-1}$.

Tissue activities of LDH and its isoenzymes

Activity of LDH is present in almost all cells of the body and is found only in the cytoplasm of the cell [1, 2]. The activities of isoenzymes are present in brain, kidney, liver, lung, lymph nodes, myocardium, skeletal muscle, spleen, erythrocytes, leucocytes, and also platelets [1, 2]. Enzyme levels in various tissues (in $U \cdot g^{-1}$ wet weight) are very high compared to those in serum: liver 9,000; heart 25,000; kidney 15,000; skeletal muscle 9,000; lung 9,500 [2]. Thus, tissue levels are about 500 fold higher than those normally found in serum, and leakage of the enzyme from even a small mass of damaged tissue can increase the observed serum level of LDH to a significant extent. In addition to their higher enzyme concentration, many of these tissues show a different isoenzyme composition. In cardiac muscle, erythrocytes and kidney, the electrophoretically faster moving isoenzymes LDH-1 and LDH-2 predominate. In liver and skeletal muscle, the more cathodal LDH-4 and LDH-5 isoenzymes predominate. Isoenzymes of intermediate mobility account for the LDH of many tissues. Specific percentages of isoenzymes for the lung are estimated: LDH-1, 10%; LDH-2, 20%; LDH-3, 30%; LDH-4, 25%; and LDH-5, 15% [1].

Elevation of total LDH activity in lung tissue was found several days after exposure of rodents to high or low levels of O_2, to cadmium aerosols, or to NO_2 [26–29]. However, these studies did not distinguish between LDH of lung tissue and of infiltrating cells. In a study using histochemical staining techniques, a viral pulmonary infection in mice resulted in a decreased LDH activity in lung cells after 6 days. In unexposed mice, LDH activity was highest in the bronchial and type II alveolar epithelial cells lining the airways [29]. Thus, there seems to be a time-related effect; and, therefore, knowing the moment that the LDH activity was measured is of vital importance in interpreting the results of the analysis. Similarly, in extrinsic allergic alveolitis, the BALF profile was also recently found to be highly dependent on the time-point at which the material was obtained in relation to the last exposure to the causative antigen [30].

HENDERSON and co-workers [31] found a predominantly interstitial reaction rather than an exudative one, with mononuclear phagocytes and lymphocytes rather than PMNs being the predominant inflammatory cell types after inhalation of toxicants inducing pulmonary injury in hamsters. The elevated LDH tissue levels remained for more than 3 weeks after exposure, suggesting some residual subacute inflammation. Airway enzymatic and cytological responses were shown to be potentially useful as indicators of lung damage in toxicological screening, as well as screening of lung injury of unknown origin [18, 22, 31, 32].

Serum LDH in pulmonary disease

Lung-related disorders as possible sources of serum LDH abnormalities have been underreported, and isoenzyme patterns are seldom measured. A summary of several pulmonary disorders associated with elevated serum LDH levels in human is given in table 1. These disorders all have in common that cell damage or inflammation or both are involved in the pathogenesis [9–11, 32–39]. In contrast, mostly in lung cancer - in the absence of liver metastases - no increased serum or BALF LDH was found (unpublished data). This suggests that no derangement of the alveolar/capillar barrier is involved in lung cancer.

An increase in airway LDH activity might arise from diverse sources, including: 1) rupture (necrosis) of airway and/or alveolar epithelial cells, AMs, or other pulmonary cell types; 2) increased flux of plasma derived LDH through an air/blood barrier rendered more permeable by pulmonary injury (e.g. oedema, haemorrhage); and 3) elevated plasma LDH concentration resulting in an increased plasma/alveolus concentration gradient, with consequent increased rate of passage of LDH across the air/blood barrier of a normal lung [13]. Studies have

Table 1. – Pulmonary diseases associated with increased serum LDH activity

Pulmonary disorder	Main pathogenetic process
Obstructive and other diseases	Cell damage or cell death
Emphysema	
Chronic obstructive pulmonary disease (COPD)	
Pneumothorax	
Pulmonary embolism	
Microbial pulmonary disease	Inflammation
Bronchopneumonia	
Tuberculosis	
Pneumocystis carinii pneumonia (PCP)	
Acquired immune deficiency syndrome (AIDS)	
Interstitial lung diseases	Inflammation and/or cell damage, remodelling/ fibrosis
Desquamative intersitial pneumonitis (DIP)	
Idiopathic pulmonary fibrosis (IPF)	
Hypersensitivity pneumonitis (HP)	
or extrinsic allergic alveolitis (EAA)	
Drug-induced pneumonitis	
Pulmonary alveolar proteinosis (PAP)	
Pneumoconiosis, silicosis pulmonum	
Acute respiratory distress syndrome (ARDS)	
Acute respiratory insufficiency	

suggested that the pulmonary interstitium can be seen as a significant part of the blood-lymph barrier when looking at protein transport. Lung parenchymal cells, or local inflammatory cells including AMs and PMNs, may be a potential source of elevation of serum LDH associated with pulmonary diseases. *In vitro* studies have demonstrated LDH leakage from type II pneumocytes, pulmonary endothelium and AMs following cellular damage caused by various stimuli. Injury to the basolateral aspect of endothelial cells would be expected to release LDH into the alveolar interstitium, and a compromised alveolar epithelial barrier would allow access of the enzyme into alveolar spaces. Mechanisms for increased levels of enzymes in the lavage fluid are usually considered to reflect cell damage, increased production and release from cells, and impaired catabolism [1]. Thus, LDH might be released from injured cells of the lung into the pulmonary interstitium and alveoli, or from damaged inflammatory cells that infiltrate the lung after treatment [14].

Only a few human studies have been carried out. DeRemee [37] reported elevated serum LDH activity in five cases of interstitial pneumonitis, suggesting that LDH would be helpful in differentiating interstitial pneumonitis from sarcoidosis. More recently, Matusiewicz *et al.* [10] reported serum LDH to be a simple though nonspecific test, which appears to reflect changes of disease activity in patients with cryptogenic fibrosing alveolitis (CFA) and extrinsic allergic alveolitis or hypersensitivity pneumonitis, but not sarcoidosis. Moreover, in CFA an increasing percentage of BALF PMNs correlated with serum LDH levels, whereas in sarcoidosis the percentage of BALF lymphocytes correlated with serum LDH, suggesting different sources of the LDH activity [10].

Determination of the LDH isoenzymes might be of additional value to establish the origin of the elevated LDH activity. Moreover, alkaline phosphatase (AP) activity has been observed histochemically in type II pneumocytes [22, 23, 40], which have been shown to proliferate following exposure to a pulmonary toxicant and to

replace the damaged type I pneumocytes [41]. Therefore, increases in AP levels in BALF were noted as a marker of type II cell damage and/or proliferation [5, 22]. It is well-known that the type II pneumocyte is important in the repair of alveolar epithelium after injury and responds to oxidant stress (such as hyperoxia). It was suggested that adaptive responses to oxidant injury occur in type II pneumocytes after exposure to minerals. Future studies are needed to clarify the possible relationship between the LDH and AP activity and outcome in patients suffering from pulmonary diseases, as well as the role in the follow-up regarding prognosis and evaluation of medical treatment.

Serum LDH isoenzyme patterns in pulmonary disease

The isomorphic pattern of LDH isoenzymes in serum - showing LDH-2 levels greater than LDH-1 levels, with relatively lower levels of LDH-3, LDH-4 and LDH-5 - is common. In addition, diagnostically more specific patterns are identified. Several studies have shown the diagnostic use of the measurement of LDH activity in pulmonary diseases. However, the further role of LDH isoenzymes has not been extensively explored. The lung pattern is characterized by proportional increases in isoenzymes 3, 4 and 5, compared to the isoenzyme patterns in normal serum [9, 42]. It has been suggested that increased plasma LDH-3 activity reflects acute lung injury causing cell damage and cell death, as LDH-3 was to be found elevated in the plasma when pulmonary embolism occurred [9, 42]. On occasions, a raised LDH level may be the only evidence to suggest the presence of a hidden embolus. The serum LDH-3 level is probably elevated because of the massive destruction of platelets after the formation of an embolus. Additionally, it is proposed that plasma LDH-3 isoenzymes are released into the circulation from injured alveolar capillary endothelial cells.

Therefore, LDH-3 may be a useful biochemical index of acute immunological antibody-mediated lung injury,

with potential diagnostic and prognostic value in pulmonary disease [9]. Release of LDH-3 activity from injured pulmonary parenchyma into the circulation presumably produces the observed rise in plasma LDH-3 activity. However, during acute rejection of a pulmonary graft in man, serum LDH-4 and LDH-5 increased [43]. BANSAL and KAW [6] found that AMs contained all five LDH isoenzymes, LDH-5 being the most prominent. Despite the reported predominance of certain LDH isoenzymes related to particular pulmonary disorders, fractionation of serum LDH often revealed the isomorphic, *i.e.* normal serum pattern, whereas fractionation of pleural effusion or BALF showed a more specific increase in LDH isoenzyme fractions. To clarify the discrepancy between these isoenzyme patterns, it is important to compare the ratio of each isoenzyme/albumin in the respective fluid to the corresponding ratio in serum.

Some of the unusual LDH isoenzymes, such as LDH-6, may have diagnostic importance, however, the majority are laboratory curiosities. The presence of LDH-6 in serum carries a very poor prognosis, all earlier reported cases were critically ill. KETCHUM *et al.* [44] described nine patients with LDH-6, of whom eight died during their hospital stay. In another study of 18 patients who showed the LDH-6 band, 15 died. However, no common cause was apparent for the LDH-6. At the time that LDH-6 was observed in the serum, all patients had hypotension and the great majority had documented episodes of hypoxaemia just preceding the LDH isoenzyme assay [44–47].

LDH in BALF

Although serum LDH, serum AP and serum protein cannot be used as indices of pulmonary disease, measurement of these parameters in BALF is a more specific alternative. LDH, known as a cytoplasmatic enzyme, occurs extracellularly in BALF only in the presence of damaged cells [23, 31]. Therefore, analysis of BALF LDH is a potentially useful tool for evaluating lung tissue damage. Cellular as well as noncellular constituents have been extensively studied. The analysis of soluble components in BALF, along with the cellular components, has been used to rank the toxicity of respirable toxicants in the lung, and to determine the level of exposure to such toxicants that is required to produce a detectable inflammatory response in the lung [4, 7, 14].

Some of the most commonly analysed soluble components of BALF are total protein (or albumin) concentrations, and the activity of enzymes, such as LDH, AP, and acid hydrolytic enzymes, such as β-glucuronidase. There are measurable levels of these components in BALF from nontreated animals [7]; increases in background levels can be used as a measure of the degree of the inflammatory response and, in some instances, are associated with a particular type of toxicant (table 2) [4]. In general, however, the changes observed are not toxicant-specific, but indicate the degree of the inflammatory response to any respirable toxicant. The parameters are also useful in determining the response of the lung to systemic toxicants that are known to damage the lung [13].

In several experiments, LDH, AP and protein were used as biochemical indices of pulmonary damage in

Table 2. – Most sensitive changes in bronchoalveolar lavage fluid (BALF) in response to different types of pollutants

Pollutant	Components of BALF increased
Oxidant gases (NO_2, O_3)	Neutrophils
	Protein
Soluble metal salts	Neutrophils
	Protein
	Lactate dehydrogenase
Insoluble toxic particles, including metal salts	Neutrophils
	Protein
	Lactate dehydrogenase
	Acid hydrolytic enzymes

(Modified from HENDERSON and MUGGENBURG [4]).

BALF, *e.g.* in rat models, they were found to be correlated with the degree of inflammatory cell influx in BALF from lungs exposed to pathogenic particulates [4, 22, 32] (table 2).

Lysosomal enzyme activities, such as β-glucoronidase (BG), N-acetylglucosaminidase, or acid proteinase activities, provide a measure of macrophage activation or lysis [4, 5]. AP activity in BALF has been associated with increased secretory activity of alveolar type II cells, or injury of these cells [22–24]. Other mediators of inflammatory response, such as tumour necrosis factor, various arachidonase metabolites, proteases and antiproteases, and interleukins may also be of help [4, 18, 19]. However, for screening, the analysis of BALF can be limited to a relatively small number of parameters. The total and differential cell counts, LDH, AP, and perhaps one lysosomal enzyme activity, along with the protein content of the BALF, are sufficient to measure the degree of the inflammatory response [4].

Changes in serum LDH do not reflect changes in lavage LDH [11]. However, looking at lavage LDH and its isoenzymes may be extremely helpful in elucidating pathogenic mechanisms in diffuse pulmonary diseases. Although total lavage LDH activity represents only about 0.1% of the total LDH activity available in lung tissue, the histological changes suggest that the lungs are a likely contributor to the increase in LDH activity observed in cell-free BALF in rats with induced lung injury [7].

SMITH *et al.* [33] noted markedly increased total BALF LDH levels in *Pneumocystis carinii* pneumonia patients which were independent of BALF cell populations. The latter group also found that the BALF LDH/albumin ratio was significantly higher than the same ratio in serum [33]. This observation suggests that the lavage fluid LDH originates from pulmonary tissue, rather than reflecting transudation from blood to alveoli.

In the rat model, induced injury to the basolateral aspect of endothelial cells would be expected to release LDH into the alveolar interstitium, and a compromised alveolar epithelial barrier would allow access of the enzyme into alveolar spaces. SCHULTZE *et al.* [14] suggested that the increased LDH activity in cell-free BALF found after experimentally-induced lung injury arises from lung tissue. HENDERSON and co-workers [31] also suggested that the source of LDH in BALF was most likely damaged lung cells. Thus, it appears that the increased serum LDH in several pulmonary disorders associated with inflammation and cell damage originates from the lung interstitium and flows back across a more permeable alveolocapillary

membrane. Thereafter, the serum LDH level increases, as has been reported, *e.g.* in *Pneumocystis carinii* pneumonia [33–35], pulmonary alveolar proteinosis [11], desquamative interstitial pneumonitis [10, 37–39], and extrinsic allergic alveolitis [10] (table 1). An analogous situation is seen in sarcoidosis - characterized by the formation of noncaseating epitheloid cell granulomas, probably antigen driven - where increased amounts of immunoglobulin G (IgG) produced at pulmonary sites of disease activity are considered responsible for the observed serum hypergammaglobulinaemia [48].

LDH isoenzymes in BALF

When homogenates of human pulmonary tissue were assayed for LDH, a particular and identifying isoenzyme pattern was found. Analysis of lung washing as a probe to detect early responses of the lung has used LDH activity in the airways of rats to detect the effect of a toxicant in the lung. Moreover, in animal studies, LDH isoenzyme patterns have been used to differentiate between various types of pulmonary injury and have helped to identify the sites of injury at a cellular level [49].

Analyses of the relative ratios of LDH isoenzymes in BALF and serum indicate that cationic isoenzymes 3, 4 and 5, are preferentially retained in the air spaces. Thus, despite increased permeability, it has been suggested that the alveolocapillary membrane continues to function as a sieving membrane, discriminating between proteins of equal molecular weight on the basis of electrical charge. Selective backflow of elevated BALF LDH, in particular isoenzyme 2 to the serum, may be responsible for the isomorphic LDH pattern seen in the serum of patients with *Pneumocystis carinii* pneumonia. In pulmonary alveolar proteinosis, there are a number of cells capable of releasing LDH into the alveolar space, including type I and type II alveolar epithelial cells, AMs, and even bronchiolar epithelial cells. HOFFMAN and ROGERS [11] found an isomorphic isoenzyme pattern in serum of pulmonary alveolar proteinosis patients. This was in marked contrast to the LDH isoenzyme pattern observed in the lavage effluent, which showed a lower percentage of LDH-1 and LDH-2 and a higher percentage of LDH-3, LDH-4 and LDH-5. Moreover, the LDH elevation found consistently in the alveolar fluid points to this as the source of the serum LDH. The large quantities of LDH found in BALF and the isoenzyme pattern of the lavage LDH suggest that pulmonary cell death occurs to a considerable extent in pulmonary alveolar proteinosis.

SCHULTZE *et al*. [14] reported that an intravenous injection of a small dose of monocrotaline pyrrole (MCTP), a putative, toxic metabolite of monocrotaline, causes injury of lung tissue, but not of other organs. Reported changes in LDH activity in cell-free BALF were characterized by increases in the isoenzymes LDH-4 and LDH-5 and an elevated LDH-4/LDH-5 ratio in BALF only. The induced changes in the LDH isoenzyme pattern suggest that the increased LDH activity of cell-free BALF arises from lung tissue. Inflammatory cells in the lungs (such as AMs and PMNs) and leakage of plasma into the interstitium and alveolar spaces may slightly contribute to the increase of cell-free BALF LDH activity, in particular to the activity of LDH-5. However, these sources alone cannot explain the alterations in LDH-isoenzyme patterns that occur in cell-free BALF after treatment of rats with MCTP [14].

LDH and its isoenzymes in pleural effusion

LDH activity has been extensively used in the analysis of pleural effusion, especially in distinguishing between transudate and exudate, and also between malignant and nonmalignant effusions [50–57]. However, few studies reporting the analysis of LDH isoenzymes in pleural effusion were found. The first results of studies of the diagnostic value of pleural fluid LDH-isoenzyme patterns have been conflicting. RICHTERICH and BURGER [58] reported that the LDH isoenzyme pattern of benign effusions reflected the serum pattern, whereas malignant effusions contained more LDH-4 and LDH-5. In contrast, others [57] have reported that malignant effusions were characterized by maximal enzyme activity in LDH-2, LDH-3 and LDH-4, whereas benign effusions were characterized by maximal enzyme activity of LDH-4 and LDH-5.

A study of pleural fluid of 122 patients at the Johns Hopkins Hospital in 1971 showed the following results [54]. In general, transudative pleural effusion - having a total LDH lower than 200 U·L^{-1} or 60% of the serum value - showed a slightly higher percentage of LDH-4 and LDH-5 compared to the serum values.

Studies of the exudative effusions showed that some malignant effusions had an LDH isoenzyme pattern different from that of the benign exudates. The fraction of LDH-2 was unusually high in approximately one third of the malignant exudative effusions, with a corresponding reduction in the LDH-4 and LDH-5 fractions. No relationship was found between the histological pattern of the malignancy and the pleural fluid isoenzyme pattern. Moreover, no significant differences were found in the pleural fluid LDH isoenzyme patterns between the various groups of benign exudative effusions [54].

VERGNON *et al*. [59] found an increase in LDH-5 isoenzyme level to be a good marker of malignant pleural effusion, except when the pleura is involved by malignant lymphoma or small cell lung carcinoma. The latter group [59] further concluded that the follow-up of the LDH-5 isoenzyme level in pleural fluid appears to be an accurate marker of the evolution of malignant pleural effusion. DEV *et al*. [60] found a significant difference in total LDH, LDH pleural fluid/serum ratio and LDH isoenzymes; minimum in cardiac failure and maximum in empyema. The value was intermediate in malignancy and other exudative conditions. The LDH-5 isoenzyme activity ratio between pleural fluid and serum tended to be higher in pleural effusions of mesothelioma origin than in those from nonmesothelial tumours.

In conclusion, comparison of LDH electrophoretic patterns found in serum and pleural fluid, is a valuable tool in the diagnostic work-up of pleural effusions, especially in the differentiation between malignant and nonmalignant origin of the pleural fluid.

In conclusion, LDH, a cytoplasmic enzyme, present in essentially all organ systems is thought to be released only after cell death. Various cell types are frequently characterized by different LDH isoenzyme profiles. Therefore, LDH isoenzyme activity patterns can be used to

localize the cellular injury. In BALF, LDH activity and its isoenzyme pattern give a direct indication of pulmonary cell damage. However, future research should focus on the specific pulmonary cells that contribute to the local LDH increase, and the impact of intra-alveolar LDH on serum LDH. The screening of BALF by relatively simple, well-established biochemical assays has proved to be useful as an indicator of the lack of lung injury and of value in damage evaluation, especially in animal studies. Further studies are required to define the limits of usefulness of BALF screening for detecting acute inflammatory responses in the lung, and to assess the link between the LDH activity and the severity and prognosis of pulmonary diseases.

Furthermore, it will be necessary to determine whether the alterations in the lactate dehydrogenase isoenzyme composition in pleural effusion and BALF, observed in earlier studies, are restricted to particular lung diseases or whether they represent a more general pattern of lactate dehydrogenase isoenzyme profile abnormalities.

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