

Usefulness of Monitoring β -Glucuronidase in Pleural Effusions

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Background: The objective of the study was to evaluate the additional value of β -glucuronidase (BGD), a lysosomal enzyme in the analysis of transudative and exsudative pleural effusions, especially between malignant and non-malignant effusions.

Design and methods: Pleural fluid samples obtained from four respective diagnostic groups: transudates parapneumonic effusions, malignant effusions or pleuritis carcinomatosa, and empyema were evaluated.

Results: Beta-glucuronidase was significantly different between transudative and exsudative effusions ($p < 0.001$) as well as between parapneumonic and malignant effusions ($p < 0.03$), parapneumonic effusions and empyema ($p < 0.002$), and malignant and empyema ($p < 0.002$), respectively. Logistic regression analysis yielded a weak discrimination between the parapneumonic and malignant groups.

Conclusions: Beta-glucuronidase activity differed between pleural effusions of various origin. However, including BGD in the biochemical work-up of pleural effusions did not reveal discriminatory value in the assessment of the classification of these effusions. Copyright © 1999 The Canadian Society of Clinical Chemists

KEY WORDS: beta-glucuronidase; BGD; pleural effusions.

Introduction

The pleural fluid lactate dehydrogenase (LDH) activity together with glucose and total protein concentrations, are used in the discrimination between transudative and exsudative effusions (1–6). However, these parameters are of no clinical value in the classification of the various subtypes of exsudative effusions, such as malignant from non-malignant effusions (1,3–9). There is a need for clinical parameters easy to assess, like enzymes, useful to distinguish exsudative effusions of malignant and infectious etiology.

Enzymes are easy to monitor and are more stable

than for example cytokines. Recently, we found that LDH isoenzymes have no additional value in the initial classification of pleural effusions either for transudate and exudate, or for discriminating between parapneumonic and malignant effusions (10). Besides LDH, other indicators of cell damage or death have been identified. Hydrolytic enzymes are a major constituent of phagocytic cells, such as alveolar macrophages (AMs) and polymorphonuclear neutrophils (PMNs) (11,12). These cells have shown to be involved in many aspects of the inflammatory response. Beta-glucuronidase (BGD) is known to be a membrane bound lysosomal enzyme, necessary in the hydrolysis of glucuronides, localized in the endoplasmatic reticulum and in lysosomes (13). Release occurs from inflammatory, phagocytotic cells, such as AMs or PMNs, as a result of increased cell membrane permeability, before the actual lysis of the cell. So lysosomal enzymes, such as BGD, are useful to detect phagocytic activity or lysis of phagocytic cells. In contrast LDH—a cytoplasmic enzyme—is released only after cell lysis and is used to detect cell death. Therefore, one might suppose, that BGD could be of additional value in distinguishing between the various causes of exsudative pleural effusions.

The aim of this study was to evaluate the possible diagnostic value of BGD in the analysis of pleural effusions. First interest was the differentiation between transudative and exsudative effusions. Second interest was the differentiation between two different causes of exsudative effusions; parapneumonic (effusions caused by a pneumonic infection with negative bacterial cultures of the pleural effusions) and malignant effusions (effusions caused by malignant involvement of the pleura).

Methods

PATIENTS

During a 1-year period, retrospectively, all patients referred to the pulmonary ward because of

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pleural effusion diagnosis were studied. For this study, only diagnostic thoracenteses were considered, and, when more than one was performed only data of the first were studied.

MATERIALS

On all pleural fluid samples, the following analyses were performed: glucose, protein, LDH, BGD, cell count, amylase, bacterial and fungal culture, acid-fast bacilli smear and culture and cytology. Simultaneously, a sample of serum was obtained to measure biochemical parameters. The pleural effusions were individually classified in transudate or exudate after careful evaluation of all clinical and biochemical data, mentioned above, with respect to the criteria of Light (14). According to Light, exudative pleural effusions meet at least one of the following criteria, whereas transudative effusions meet none:

1. Pleural fluid protein divided by serum protein greater than 0.5
2. Pleural fluid LDH divided by serum LDH greater than 0.6
3. Pleural fluid LDH greater than two-thirds the upper limit of normal for serum LDH.

A transudative effusion is caused by congestive heart failure. Exudative effusions represent all other effusions with different causes. Out of the exudates, parapneumonic, malignant effusions and empyema were selected. The diagnosis was based on biochemical, cytologic, and bacteriologic examination of the fluid. An effusion was considered parapneumonic when this effusion was associated with a pneumonia, pulmonary abscess, or bronchiectasis and when the pleural fluid demonstrated a predominance of polymorphonuclear leucocytes, but negative bacterial cultures. A malignant effusion was considered when malignant cells were demonstrated in the pleural fluid, pleural biopsy specimen, or at autopsy. Empyema were diagnosed by positive gram stain and/or bacterial culture. The following were excluded for this study: effusions of undetermined origin, effusions with more than one possible cause, tuberculosis and hemothorax. Finally, pleurafluid samples of 75 patients (age 69 [25–91] years) were used for the present study.

CONTROLS

A group of 48 healthy control subjects (age 58 [22–90] years)—without relevant medical history—was used to assess reference values of serum BGD. Serum values of LDH, gamma-glutamyl transferase (GGT), alanine amino transferase (ALT), creatine kinase (CK), creatinine and protein were within normal ranges.

LABORATORY TESTS

The pleural fluid was immediately centrifuged, or if necessary, stored at 4° C and centrifuged within 2 h at 1000g for 5 min. The supernatant was collected and BGD activity was measured at 37° C using p-nitrophenyl- β -D-glucuronide as a substrate. The assay was run in an acetate buffer on an automatic plate reader (Cambridge 7520 Microplate Reader, Cambridge Technology, Inc., Watertown, MA, USA). The LDH activity was measured on a Beckman Synchron CX-7 system (testkit No 442660) according to the recommendations of the Deutsche Gesellschaft für Klinische Chemie (DGKC-recommendations). For determination of total protein a Synchron CX-7 analyser and testkits from Beckman instruments were used. The amylase activity was measured by an enzymatic rate method on a Beckman Synchron CX-7 system (testkit 442775) and glucose by a time endpoint method (testkit 442640). The total cell count was done by a “Koulter” impedance technique.

STATISTICAL METHODS

Data are expressed as median and ranges in parenthesis. In order to detect statistically significant differences between more than two patient groups, for each of the discriminatory variables separately, data were analysed by the Kruskal-Wallis one-way analysis of variance (ANOVA) test. The Mann-Whitney *U* test was used for pairwise comparisons. Because 5 comparisons were made, a probability value smaller than 0.05/5 being 0.01 was considered statistically significant (Bonferroni's correction).

Logistic regression analysis was used to test the discriminatory effect of explanatory variables simultaneously. Primary interest was in discriminating group I from group II, III, and IV combined; second interest was in discriminating group II from group III. In these analyses likelihood ratio (LR) tests were used; variables with a *p* value of < 0.05 were included in the model and variables with a *p* value > 0.1 were left out of the logistic regression models. The results are presented by means of log odds ratios, observed versus predicted group membership, and receiver operating characteristics (ROC) curves (15). For discriminating group II from group III by only the variable BGD, predicted probabilities are calculated per quartile (16).

Results

Of the 75 patients studied, 21 of the obtained pleural effusions were classified as transudative effusions (group I), 9 as parapneumonic effusions (group II), 31 as malignant effusions (group III), and 14 as empyema. The diagnosis of the malignant effusions in group III were: adenocarcinoma (*n* = 18), non-small-cell carcinoma (*n* = 7), small-cell carcinoma (*n* = 3), mesothelioma (*n* = 2), and

TABLE 1
 Biochemical Characteristics and β -Glucuronidase (BGD) in Pleural Effusions Obtained From the Studied Groups, as well as in Serum Obtained From a Healthy Control Group

	<i>n</i>	Leukocytes (10 ⁹ /L)	Glucose (mmol/L)	Protein (g/L)	LDH (U/L)	BGD (U/L)
Controls ^a	48				367 (219–475)	0.252 (0.000–2.785)
Transudative effusions (I)	21	0.6 (0.1–2.9)*†	6.8 (4.8–11.0)*††	25.3 (5.9–44.4)**††	179 (90–376)**††	0.063 (0.000–1.386)††
Parapneumonic effusions (II)	9	2.2 (0.7–6.7)	6.0 (4.0–7.4)	45.5 (36.5–66.1)	482 (205–2194)	0.252 (0.000–0.922)†
Malignant effusions (III)	31	1.3 (0.2–5.1)	5.6 (0.2–11.9)	46.0 (19.9–56.1)	784 (200–5104)	0.680 (0.000–2.558)
Empyema (IV)	14	4.2 (1.9–86.2)*††§	3.3 (0.6–8.5)*†§	43.0 (3.3–70.7)§	10117 (289–94150)*†§	5.834 (0.126–51.030)**††§
<i>p</i> value ^b		<0.0001	<0.003	<0.0001	<0.0001	<0.0001

Data are expressed as median with range in parenthesis.

^aSerum lactate dehydrogenase (LDH) and β -glucuronidase (BGD) of the healthy control subjects.

^bKruskal-Wallis ANOVA test (between group I–IV); *p* value < 0.01 statistically significant (Bonferroni's correction). NS = not significant.

**p* < 0.01 Mann-Whitney versus group II.

***p* < 0.001 Mann-Whitney versus group II.

†*p* < 0.03 Mann-Whitney versus group III.

††*p* ≤ 0.002 Mann-Whitney versus group III.

§*p* < 0.001 versus group I.

lymphoma (*n* = 1). The causes of empyema in group IV were: *Staphylococcus aureus* (*n* = 5), *Streptococcus pneumoniae* (*n* = 5), *Streptococcus milleri* (*n* = 2), and *Escherichia coli* (*n* = 2). Some biochemical parameters are detailed in Table 1. The BGD activity was significantly higher in the exudate pleural effusions (groups II, III, and IV) compared to the transudate effusions (group I) (*p* < 0.001) as well as between the parapneumonic and malignant effusions (*p* < 0.03), parapneumonic effusions and empyema (*p* < 0.002), and between malignant effusions and empyema (*p* < 0.002). The BGD activity between all four groups (I–IV) was also significantly different (*p* < 0.0001) (Table 1).

In empyema the BGD activity, like the LDH activity, was very high. Because of the possible statistical influences of the extremely high BGD values of group IV in the total exudative group we choose to compare group I with II+III with group IV (Table 1 and Figure 1) and group I with II+III without group IV (Tables 2 and 3). Logistic regression analysis yielded a strong discrimination, as expected, between group I and II+III+IV, simultaneously using the LDH and the BGD activity as explanatory variables (Figure 1). This good discrimination was already found using LDH alone and including BGD in the logistic regression analysis yielded no statistical additional value in this comparison. Using only the BGD activity as explanatory variable between transudates and exudates revealed a sensitivity of $100 \times (10/21) = 47.6\%$ and a specificity of $100 \times (46/53) = 86.8\%$, area under the ROC curve 0.7803. The cut-off point is the BGD activity where the probability of either outcome is 0.50; this is for BGD equal to 0.045 (Figure 1).

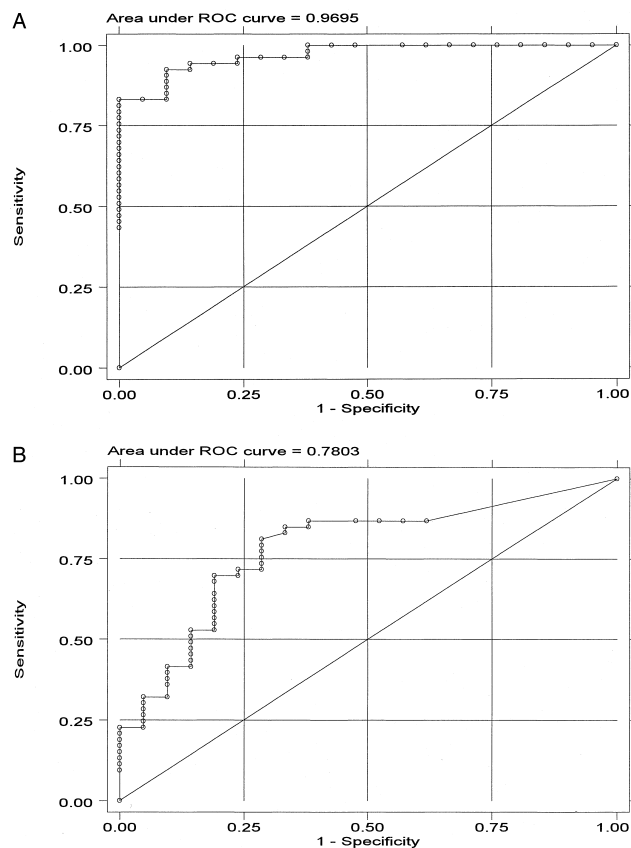


Figure 1 — Receiver-operating characteristic curve of the linear predictor score using lactate dehydrogenase (LDH) (A) and β -glucuronidase (BGD) (B) as predicting variable: transudative effusions (group I) versus exudative effusions (group II+III+IV). LDH: SE(area) = 0.0162, 95% CI area under curve = 0.938–1.00. BGD: SE(area) = 0.0592, 95% CI area under curve = 0.663–0.896.

TABLE 2

Logistic Regression Analysis for Discriminating Between Group I (Transudative Effusions; $n = 19$) and Groups II (Parapneumonic) + III (Malignant Effusions) Combined ($n = 39$)

Explanatory Variable (unit)	Log Odds Ratio	Likelihood Ratio Test p Value
Lactate dehydrogenase (U/L)	0.0442	0.0000
β -glucuronidase (U/L)	6.1915	0.0052
(constant:	-14.8462)	

Results are expressed as log odds ratios of groups II (parapneumonic) + III (malignant effusions) versus group I (transudative effusions) per unit increase of the explanatory variables (3 missing values); likelihood ratio tests used.

However, between group II and III a weak discrimination was found given the variable BGD. The BGD activity in the logistic regression analysis revealed better discrimination than the LDH activity alone (see Tables 4, 5 and Figure 2). All other independent variables were far from significant when added to the model (p value > 0.10).

Table 6 gives the predicted probabilities of belonging to group III rather than to group II per quartile of the variable BGD. These predicted probabilities are calculated from a logistic regression model only containing the variable BGD. The high prior probability of belonging to group III rather than to group II (*i.e.*, 31/39) does not vary much across the BGD quartiles (0.58 to 0.94): it is still as high as 0.58 in the first quartile.

It has to be mentioned that the predictability of the model is only checked in the same data set as from which the model was estimated. Hence, the results in the predicted versus observed group membership tables (*e.g.*, as in Table 3) may be (slightly) too optimistic.

Discussion

This study shows that the BGD activity is significantly different between transudative and ex-

TABLE 4

Logistic Regression Analysis for Discriminating Between Parapneumonic Effusions (Group II, $n = 8$) and Malignant Effusions (Group III, $n = 31$)

Explanatory Variable (unit)	Log Odds Ratio	Likelihood Ratio Test p Value
Lactate dehydrogenase (U/L)	0.0003	0.4248
Glucose (mmol/L)	-0.0320	0.8631
β -glucuronidase (U/L)	2.1436	0.0336

Results expressed as log odds ratios of group III versus group II per unit increase of the explanatory variable (1 missing value); likelihood ratio tests used.

The effect of β -glucuronidase as single variable was: log odds ratio = 2.0217 with $p = 0.0357$

udative pleural effusions. Moreover, in exudative effusions, the BGD activity differed between parapneumonic, malignant effusions, and empyema. Further statistical analysis indicated that BGD has no additional value in distinguishing between exudative effusions of malignant and non-malignant etiology.

To the best of our knowledge, this study is the first to evaluate the possible role of BGD in the diagnostic work-up of pleural effusions. Several animal studies on bronchoalveolar lavage fluid reported elevated BGD activity after exposure to particles associated with pulmonary cell inflammation of damage (17-21). Release of BGD, because of increased lysosomal membrane permeability, is useful to detect phagocytotic cell activity, already before the actual lysis of the cell. Therefore, increase of BGD activity is likely to occur before the increase of LDH activity. In line with this, recently, we found increase of the serum BGD activity even in ex-coalminers with a normal serum LDH compared to a healthy control group (22).

In the group of patients, suffering from empyema, the BGD activity was significantly increased similar to the LDH activity. However, monitoring both enzymes added no additional diagnostic usefulness as

TABLE 3

Observed Versus Predicted Group Membership Following From the Estimated Logistic Regression Model in Table 2

Observed membership	Predicted Group Membership (n)		
	Transudative Effusions (I)	Parapneumonic Effusions (II) + Malignant Effusions (III)	Total
Transudative effusions (I)	18	1	19
Parapneumonic effusions (II) + Malignant effusions (III)	1	38	39
Total	19	39	58

Sensitivity = portion of predicted parapneumonic effusions + malignant effusions among observed parapneumonic effusions + malignant effusions = $100 \times (38/39) = 97.4\%$. Specificity = portion of predicted transudative effusions among observed transudative effusions = $100 \times (18/19) = 94.7\%$.

TABLE 5
Observed Predicted Group Membership Following From the Estimated Logistic Regression Model in Table 4

Observed group membership	Predicted Group Membership (<i>n</i>)		
	Parapneumonic Effusions (II)	Malignant Effusions (III)	Total
Parapneumonic effusions (II)	0	8	8
Malignant effusions (III)	0	31	31
Total	0	39	39

Sensitivity = portion of predicted malignant effusions among observed malignant effusions = 100 (31/31) = 100%. Specificity = portion of predicted parapneumonic effusions among observed parapneumonic effusions = 100 (0/8) = 0%.

empyema already was confirmed by positive gram stain and/or bacterial cultures.

Pérez-Arellano *et al.* (12) found significantly higher BGD activity in BALF obtained from a group of patients with lung infiltration due to adenocarcinoma compared to a control group. Many tumors showed enhanced BGD activity and many studies in

the past have been focused on the use of the reactivity of glucuronides for tumor cell killing therapy (23,24). This interest is relevant to the theory that malignant cells elaborate enzymes that catabolize glycosaminoglycans (the compounds that are largely responsible for imparting viscosity of the intercellular ground substance) to low-molecular mass, low-viscosity subunits (24). Accordingly, malignant cells may thus infiltrate a medium, so radically altered as to present a much reduced mechanical barrier to invasion. The BGD activity was high in the parapneumonic effusions as a result of the inflammatory cell activity, but the BGD activity was even higher in the group of malignant effusions, which is in agreement with the knowledge of increased BGD activity in tumor cells (13,25). Future prospective studies should focus on the role of BGD in the diagnostic work-up of pleural effusions and should aim to clarify the clinical relevance, especially in discriminating between malignant and parapneumonic effusions.

Although the BGD activity differed between transudative and exudative effusions, including BGD, besides LDH, in the biochemical work-up of these pleural effusions did not reveal additional discriminatory value. To establish the possible discriminatory power of BGD between parapneumonic and malignant effusions, future prospective studies are recommended.

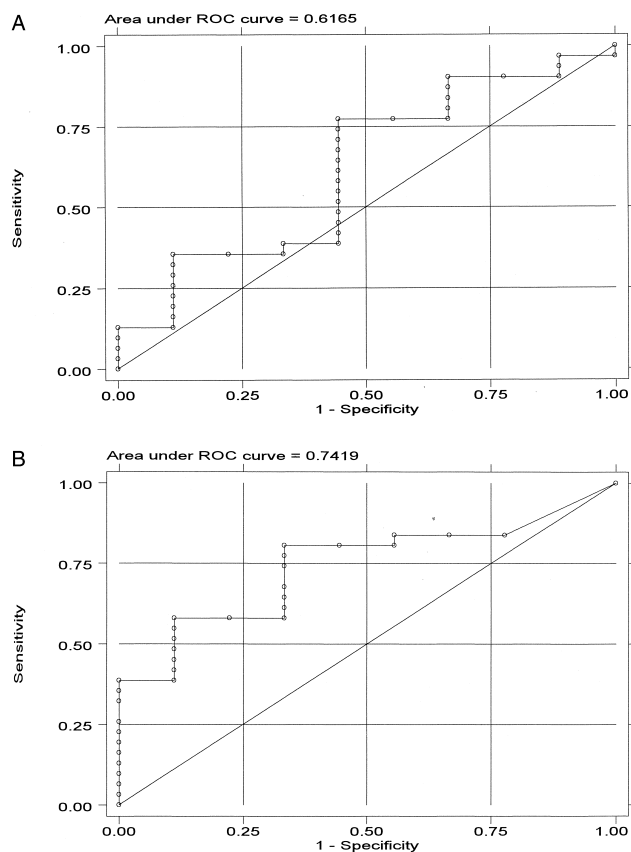


Figure 2 — Receiver-operating characteristic curve of the linear predictor score using lactate dehydrogenase (LDH) (A) and β -glucuronidase (BGD) (B) as predicting variable: parapneumonic effusions (group II) versus malignant effusions (group III). LDH: SE(area) = 0.115, 95% CI area under curve = 0.3911–0.842. BGD: SE(area) = 0.084, 95% CI area under curve = 0.577–0.907.

TABLE 6
Predicted Probability of Malignant Effusions (Group III) Versus Parapneumonic Effusions (II) in Quartiles of the Explanatory Variable β -Glucuronidase (BGD), No Other Variables Considered

Variable	Quartile	Predicted Probability of Malignant Effusions (III)
BGD (U/L)	1. <0.0065	0.5793
	2. 0.0065–0.365	0.6661
	3. 0.365–0.8515	0.8205
	4. >0.8515	0.9362

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