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TOTAL TISSUE LACTATE DEHYDROGENASE ACTIVITY IN ENDOMETRIAL CARCINOMA

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ABSTRACT

Lactate dehydrogenase (LDH) is essential for continuous glycolysis necessary for accelerated tumor growth. The aim of this study was to reconsider if assay of total tissue activity of this enzyme could be useful as marker for endometrial carcinoma.

Activity of LDH was measured spectrophotometrically in homogenate supernatants of uterine tissue samples of 40 patients (10 normal endometria, 27 normal myometria, and 33 endometrial carcinoma), including 30 matched pairs. Data obtained were analyzed in relation to clinical and histopathological findings, and compared with our previously published results on the tissue levels of the same enzyme in ovarian cancer and on the proteolytic activity of dipeptidyl peptidase III (DPP III) in endometrial carcinoma (suggested biochemical indicator of this malignancy).

Significantly increased (1.8 - 3.0 times, $P<1x10^{-4}$) LDH activity was observed in endometrial carcinoma samples, if compared with normal uterine tissues. This rise was not related to the clinico-pathological findings, however. In contrast to previous results on LDH in ovarian carcinomas, a significant rise in LDH activity was found already in grade 1 endometrial carcinoma. Using the cutoff value of 1.06 U/mg, diagnostic sensitivity of 82%, specificity of 100% and accuracy of 91% for total tissue LDH assay have been calculated. A correlation of tissue's LDH and DPP III activities was found, and their combined assay for endometrial carcinoma showed increased diagnostic sensitivity (94 %) and accuracy (96 %).

INTRODUCTION

Cancer of the corpus uteri is the eighth most common malignant neoplasm in women worldwide, and endometrial cancer constitutes about 95% of all malignant lesions of uterine cavity[1]. The prevailing form of endometrial cancer is endometrial carcinoma (EC), tumor originating from the glandular epithelium of uterine endometrium. Endometrial carcinoma arises through a series of precursor lesions, which are thought to develop and be promoted in response to unopposed and prolonged stimulation by estrogen. On the other hand, some types of endometrial carcinoma are estrogen-independent[2]. Endometrial carcinoma is usually postmenopausal disease with peak incidence between age 50 and 60. Prognosis of endometrial carcinoma is fairly good, since overall 5-year survival rate is 83% and for the early stage of the disease, about 90%[1]. This is mainly due to early diagnosis indicated by abnormal bleeding and based on mandatory endometrial biopsy which is strengthened by transvaginal ultrasonography, hysterescopy, vaginal and endometrial cytology and biochemical clinical tests. One among these lasts, assay of lactate dehydrogenase (LDH), is still under clinical evaluation in gynecological oncology.

Lactate dehydrogenase, (EC: (S)-lactate:NAD^+ oxidoreductase, 1.1.1.27) is one of the major glycolytic enzymes that catalyzes the last step of glycolysis, conversion of pyruvate to lactate. It is a tetrameric protein composed of two immunologically distinct subunits, “A” or “M” (muscle) and “B” or “H” (heart) type, which combine to form five isoenzymes[3].

LDH is ubiquitous cytosolic enzyme present in all tissues, wherein shows origin- and tissue-specific isoenzymatic pattern[4]. Routine serum measurement of this enzyme is of clinical use in the diagnosis and monitoring of certain diseases including cancer, but is of low diagnostic value for gynecological malignancy[5] . In response to the need for more specific diagnostic and prognostic tools, attempts have been made also to measure LDH in

other body fluids[6,7], cavity washings[8], and uterine tissues[9–14]. Since data for these last are still scarce and our recently published results on ovarian carcinoma[15] indicated correlation of total tissue LDH activity with histological epithelial tumor grade, we wanted to extend our research to uterine tissue. Therefore, we assayed total LDH activity in the extracts of normal and malignant endometrial tissue, and correlated it with histopathological and clinical data. In addition, the diagnostic value of the total tissue LDH for endometrial carcinoma was calculated and compared to that of proteolytic enzyme dipeptidyl peptidase (DPP III) which was previously shown by us to be a biochemical indicator for endometrial and ovarian cancer[16, 17].

MATERIALS AND METHODS

Patients and Samples. This study covered 40 patients undergoing surgical treatment at the Department of Obstetrics and Gynecology, School of Medicine, University of Zagreb, Croatia. The mean age of patients was 60.6 ± 1.7 (mean ± SEM) years. The consecutive specimens of uterine tissues obtained at surgery or biopsy comprised 10 samples of normal uterine endometrium (NE), 33 samples of endometrial carcinoma (EC), and 27 samples of normal uterine myometrium (NM). Among them were 30 matched pairs (endometrial carcinoma/normal uterine tissue) originating from the same patient, and having normal uterine endometrium (n = 5, “true” pairs) or normal uterine myometrium (n = 25, “virtual” pairs) as endometrial carcinoma’s counterpart. Patients were untreated for endometrial carcinoma before the sampling.

Histopathologic classification of endometrial carcinoma samples was based on the International Federation of Gynecologists and Obstetricians (FIGO) staging system Additional histopathological characteristics included determination of the degree of

leukocyte infiltration. Clinical informations were obtained after completion of biochemical assays. The protocol was approved by the Ethics of the Research Committee at the School of Medicine, University of Zagreb.

Tissue Sampling and Processing. Samples of uterine tissue were frozen within 10 min in liquid nitrogen and kept at -196° C until use. For biochemical assays, tissues were minced, suspended in a buffer (50 mM Tris·HCl, 250 mM sucrose, 134 mM KCl, pH 7.6) and homogenized on ice (Ultra-Turrax T 25 homogenizer, Janke&Kunkel, Ika-Labortechnik, Germany) for three 5 s bursts. Supernatants obtained after centrifugation (4° C, 15 min, 15,000 x g) were used for analysis.

Biochemical assays.Total tissue LDH activity was determined by following initial rate of pyruvate reduction to lactate, using slightly modified procedure[18]. Assay mixture of 1 mL was buffered by 100 mM potassium phosphate pH 7.0, and contained finally 0.096 mM pyruvate and 0.060 mM NADH. Reaction was started by the addition of enzyme sample (up to 20 μL supernatant of tissue homogenate), and followed spectrophotometrically at room temperature (25º C) for 3 min by measuring decrease in absorbance of NADH at 340 nm. Initial velocity was calculated using the linear regression method. The specific activity of LDH was expressed in units per mg (U/mg) of the sample protein. One unit of enzyme activity was defined as the amount of enzyme which transforms (reduces pyruvate or oxidizes NADH) one μmole of substrate in one minute at 25° C and pH 7.0.

Specific activitiy of the DPP III was determined as described elsewhere[16]. Protein concentrations were measured by the protein-dye binding assay[19].

Statistical Analysis. The results were analyzed statistically using the STATISTICA (StatSoft Inc., 1984–1995, Version 5.0) software, by evaluating groups consisting of at least five pieces of data. Mainly normal or normalized distribution of the data prompted us to apply methods of parametric statistics for their evaluation – (in)dependent t-tests for the

analysis of differences between the groups, and simple linear regression analysis, for the correlations among parameters assayed, have been used. In a few other cases however, distribution-free methods were employed – differences between groups of independent samples were analyzed by Mann-Whitney U-test, those between dependent (paired) samples using Wilcoxon's paired samples test, and correlations among parameters assayed, by Spearman "rho" method. Two tailed probability values of less than 0.05 were considered to be significant. Calculation of diagnostic parameters for LDH- and DPP III-assay was performed according to Schneider et al.[8], using cut-off values of enzyme activities as mean + 2 SD of the control group (normal uterine tissue consisting of endometrium plus myometrium) sample.

RESULTS

Figure 1, Table1 and Table 2, present the results of total LDH activity determination in samples of normal uterine tissues and endometrial carcinoma. These data show that normal uterine myometrium had the lowest LDH enzymatic level (mean = 0.544 U/mg, n = 27) which was about 30% lower than that found in normal uterine endometrium (mean = 0.808 U/mg, n = 10).

When all endometrial carcinoma samples have been compared with all normal uterine tissues, considerably higher levels (1.8–fold to 3-fold) of LDH activity (mean = 1.525 U/mg, n = 33) have been observed in malignant than in normal uterine tissue (Figure 1, Table 1). Similar values were also obtained when matched pairs of endometrial carcinoma and their normal counterpart tissue were compared (Table 2). There was no overlapping in total tissue LDH activity based on 95% confidence intervals (Table 1); thus the enzyme level was clearly distinguishable in each type of uterine tissue, and

significantly higher in the malignant one. Total LDH activity of endometrial carcinoma tissues apparently did not depend on the age of patients and was not related to the clinical stage, tumor grade, histological type of tumor or presence of tissue's inflammation (Table 1).

In order to further examine diagnostic utility of total tissue LDH assay, we correlated activity of this enzyme in the normal uterine tissues and endometrial carcinoma with levels of DPP III determined earlier by us[16] (Table 3). Moderate (Pearson's "r" ~ 0.5) but significant association of LDH activity with DPP III ($P = 1x10^{-3}$) activity was found. For both of these assays cutoff values have been determined, and diagnostic parameters for separate and combined tests have been calculated (Table 4). Both assays showed similar specificity, positive predictive value and diagnostic accuracy, but DPP III assay seems to be superior to the LDH test concerning sensitivity and negative predictive value. Combined results of the assay of these two enzymes improved diagnostic parameters of LDH measurement alone, resulting in at least 96% reliability to discriminate endometrial carcinoma from normal uterine tissue.

DISCUSSION

LDH is a ubiquitous cytoplasmic enzyme, and its appearance in body fluids is recognized as a pathological manifestation that can be used as a measure of cell or tissue injury. The determination of serum LDH, routinely used for diagnostic purposes for at least thirty years, was established as relevant in the diagnosis of myocardial infarction (late detection), hemolytic anemia, ovarian dysgerminoma and testicular germ cell tumor[20].

Being necessary to enable continuous glycolysis for accelerated growth rate of

malignant tissue, LDH has been subject of many investigations in tumor metabolism, and its clinical use as a possible tumor marker has been suggested. Increased total serum LDH activity and isoenzymatic "shift" toward "M" isoforms are reported for most of malignancies[21, 22]. Assay of LDH activity has been evaluated and in the diagnosis and monitoring of gynecological malignancies, contributed mostly by serum data and much less by other body fluids or tissue extracts. Increased total LDH activities have been reported for sera of the patients with ovarian[23] or cervical[24] cancer, and some tumors of uterine cavity[25]. Similar increase has been observed also for other body fluids and cavity washings of patients with gynecological malignancies – vaginal[7], uterine[6], and peritoneal fluid[8]. With exception for ovarian dysgerminoma where increased levels of total serum LDH activity and its "H" isoforms have been well documented and accepted as useful in the managing of this disease[20], relevant diagnostic utility of LDH assay is not firmly established however, since observed changes are not enough sensitive and specific. All that is mainly due to different rates of clearing of LDH isoenzymes from the circulation[21], which consequently does not reflect true tissue enzyme activity.

Normal uterine tissue, cyclically influenced by sex hormones, differs in LDH level during menstrual cycle. Total LDH activity of normal uterine myometrium remains stable and is altered only by prolonged hormonal stimulation in pregnancy or post-menopause and in the malignancy[26]. On the contrary, total LDH activity of normal uterine endometrium gradually increases in almost linear fashion over the entire period of menstrual cycle[27], being lowest in early proliferative phase and highest in the late secretory phase[28].

Our results of measurement of total LDH activity in matched normal and malignant uterine tissues corroborate that neoplastic transformation of human endometrial tissue significantly increases activity of this important glycolytic enzyme. Endometrial

hyperplasia, which is considered as premalignant neoplasm[1], is characterized by 2-4 fold higher total tissue LDH level than that found in normal secretory endometrium[11]. Even higher activities of this enzyme occasionally have been reported for limited number of endometrial carcinoma samples studied up to now - the four to eight fold rise has been observed in (totally) 17 endometrial carcinomas studied previously[9, 12, 14], and these findings were confirmed later[10, 11, 13] on additional several tenths of similar samples. This rise was proposed to represent an adaptation mechanism of energy supply and glycolysis to an increased demand for energy at a time when the normal capacity of oxygen consuming pathways becomes inadequate to satisfy the needs of proliferating malignant cells[10].

To improve the knowledge on this tumor biology and biochemical diagnosis of endometrial carcinoma, few other molecular markers are under study. Recent findings suggest possible use of some enzymes among which are glutathione S-transferase[29] which is involved in detoxification system, and proteases that participate in the degradation of the basement membrane and digestion of extracellular matrix in the course of invasion and metastasis. These last comprise collagenase[1] and cathepsin D[30] which were found to be elevated not only in malignant endometrial tissue but even in the hyperplastic[1] one, when these have been compared with normal or benign tissues[1, 30]. The increased level of another proteolytic enzyme, dipeptidyl peptidase III (DPP III) has been reported in endometrial carcinomatous tissue, but its role in malignant growth is not elucidated[16].

Increased glycolysis of the malignant tissue with the pronounced role of LDH together with the convenience of its measurement, makes this enzyme still current in the evaluation as a tumor marker in gynecological malignancy. In spite of that, diagnostic utility of LDH assay has not been (except for ovarian dysgerminoma) yet firmly established. Therefore, we intended to contribute to these attempts by measuring total LDH enzymatic activity in homogenates of normal uterine tissues and endometrial

carcinoma, and correlating obtained data with routine clinical and histopathological findings and with tissue levels of DPP III, a suggested marker of endometrial carcinoma. Due to marked variability within human normal and neoplastic tissues which requires evaluation of paired specimens[31], we also analyzed separately 30 "matched pairs" in which malignant tissue and normal counterpart originated from the same patient. Determination of LDH by continuously monitoring consumption of NADH while pyruvate is converted to lactate is generally accepted by most of the European Societies for Clinical Chemistry[21] and was used in this study. Our results on assay of total LDH enzymatic activity in uterine tissues are consistent with earlier findings, yet with a few distinctions.

Firstly, similarly to the findings of others, we observed significant increase in total tissue LDH activity when normal endometrium underwent malignant transformation to endometrial carcinoma. This elevation was however, far from some extreme values reported earlier[9-11, 14], and the difference could be probably result of the analytical and/or sampling methods applied. Secondly, thorough analysis of the obtained data showed no correlations with clinico-pathological findings, irrespectively of which endometrial carcinoma samples (paired or unpaired) were examined. This indicated that total tissue LDH assay may not be of prognostic value for endometrial carcinoma. High values of its diagnostic parameters (Table 4) suggest however, that this assay could be useful in the diagnosis of endometrial carcinoma.

In precedent study [15] we found that grade 1 ovarian carcinoma, contrary to the G2 and G3 ovarian tumors, did not differ in total tissue LDH activity from normal ovarian tissue. In the present study, significantly enhanced total tissue LDH activity was measured in endometrial carcinoma samples of G1 and G2 grade, and no difference was observed between these two subgroups. Our results on well-differentiated endometrial carcinomas

point to the complexity and the difference in regulation of this glycolytic enzyme in malignant gynecological tissues.

No single marker has proved sufficient to meet the full requirements of clinical application, and therefore many workers have reported that a combination of more than one marker would prove more effective than any single assay[24]. In an attempt to establish such a tissue-based biochemical index for the diagnosis of endometrial carcinoma, we compared the levels of LDH and DPP III, two cytosolic enzymes, and found a significant correlation of LDH with DPP III in normal as well as in transformed uterine tissues (Table 3). Further attempts have been focused on the comparison of diagnostic value of LDH and DPP III assays (Table 4). Obtained results imply that the first one is somewhat more specific and the second one, more sensitive; thus, a biochemical index consisting of LDH and DPP III assays showed diagnostic reliability of at least 96% to detect endometrial carcinoma.

The data presented in this study establish the cutoff value between LDH activity in normal and malignant endometrial tissue and show high diagnostic value of this assay in endometrial carcinoma, very close to those of a new tumor marker, DPP III. Therefore, total tissue LDH activity measured by simple, fast and inexpensive assay which showed high values of diagnostic parameters (sensitivity of 82%, specificity 100% and accuracy of 91%) might be an additional marker for endometrial carcinoma.

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Table 1.

Total tissue LDH activity in normal uterine tissue and in primary endometrial carcinomas: distribution according to the clinical and histopathological findings

Findings	*n*	Total tissue LDH (U/mg protein)			
		Mean	SD	Median	95% Confid. Int.
Normal uterine tissue:	37	0.616	0.222	0.580	0.542 – 0.690
endometrium:	10	0.808	0.145	0.800	0.704 – 0.912
myometrium:	27	0.544	0.203	0.480	0.464 – 0.625
Endometrial carcinoma:	33	1.525	0.633	1.460	1.300 – 1.749
- Clinical stage:					
Ia	4		*N/A*[a]		
Ib	13	1.710	0.694	1.500	1.290 – 2.130
Ic	16	1.467	0.577	1.480	1.159 – 1.775
- Tumor grade:					
G1	16	1.584	0.727	1.425	1.196 – 1.971
G2	15	1.478	0.544	1.500	1.177 – 1-779
G3	2		*N/A*[a]		
- Carcinoma type:					
Endometrioid	23	1.441	0.508	1.600	1.221 – 1.661
Mixed	8	1.694	0.967	1.810	0.885 – 2.502
Others[b]	2		*N/A*[a]		
- Inflammation:					
Present	16	1.680	0.584	1.680	1.369 – 1.991
Absent	17	1.379	0.660	1.240	1.040 – 1.718
- Age of patients:					
< 50 years	5	1.356	0.411	1.250	0.845 – 1.867
> 50 years	28	1.555	0.666	1.480	1.297 – 1.813

[a] *N/A*, numbers too low for statistical analysis

[b] Clear cell carcinoma 1, undifferentiated carcinoma 1

Table 2

The comparison of total tissue LDH activity in malignant versus normal uterine tissues

Sample	(*n1/n2)*	Ratio[a]	*P*[b]
I. Endometrial carcinoma versus :			
- Normal endometrium	33/10	1.825	0.000148**
- Normal myometrium	33/27	3.042	$< 10^{-6*}$
- Normal endometrium plus normal myometrium	33/37	2.517	$< 10^{-6**}$
II. Paired[c] samples:			
- "True" pairs	5	2.128	$< 10^{-6*}$
- "Virtual" pairs	25	2.775	$< 10^{-6*}$
- "All pairs"	30	2.626	$< 10^{-6*}$

[a] Ratio of mean or median values of LDH activity

[b] Calculated by "*t*-test" (*) or by "*U*-test" (**)

[c] Endometrial carcinoma versus normal tissue sampled from the same patient, where counterpart is normal endometrium ("True" pairs), normal myometrium ("Virtual" pairs), or normal endometrium plus normal myometrium ("All" pairs)

Table 3

Correlation[a] of total tissue LDH activity with activity of tissue dipeptidyl peptidase III (DPP III)[b]

	DPP III		
Uterine tissue			
	n	*r*	*P*
Normal endometrium and myometrium	37	0.543	0.001
Endometrial carcinoma	33	0.546	0.001

[a] Pearson's product-moment correlation, at significance level of 0.05

[b] Calculated from the published data[16]

Table 4

Diagnostic value of the total tissue LDH and tissue DPP III assays for detecting endometrial carcinoma

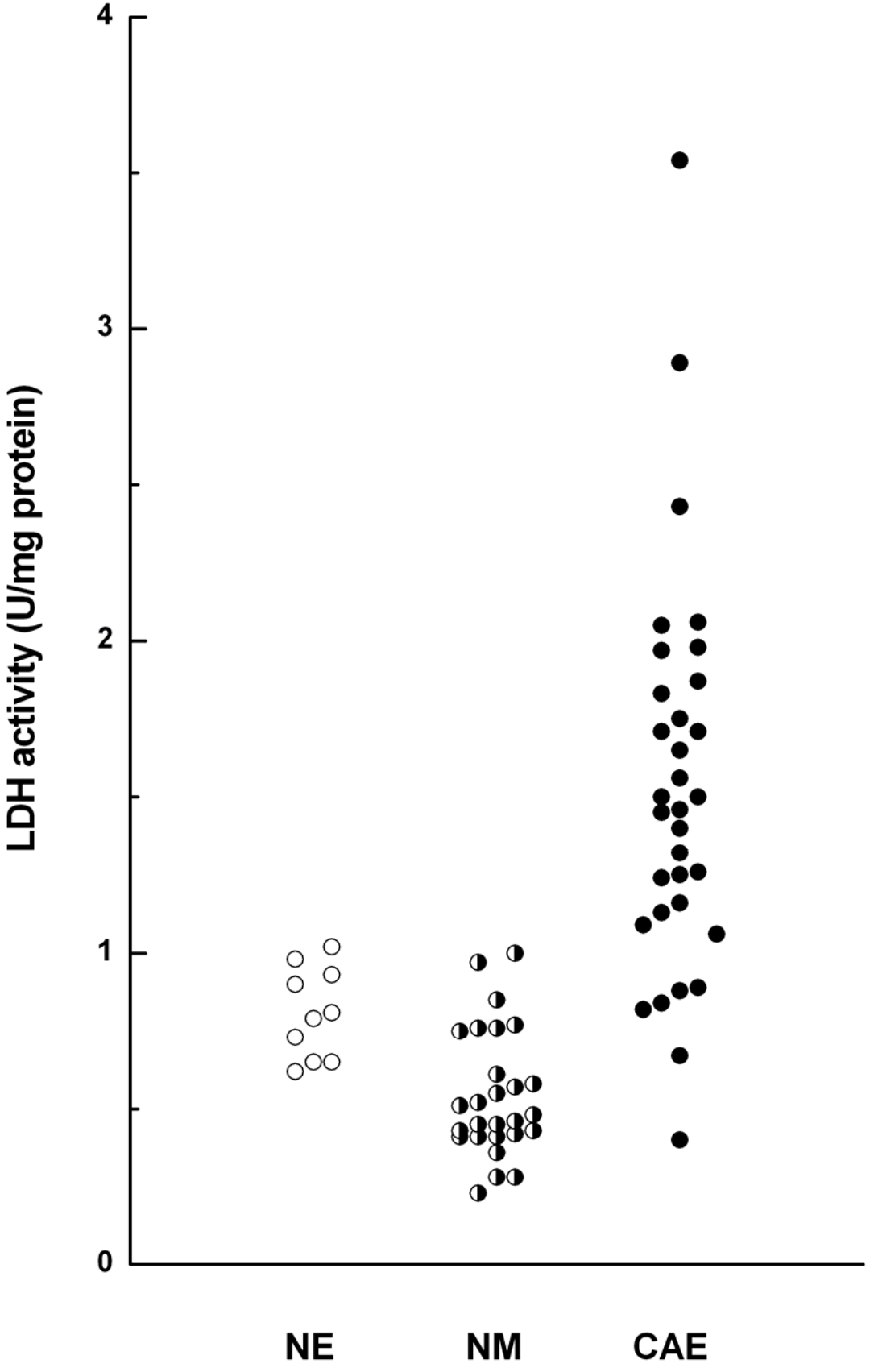
Assay	Cutoff value[a]	Sensitivity (%)	Specificity (%)	Positive predictive value (%)	Negative predictive value (%)	Diagnostic accuracy (%)
LDH	1.06 U/mg	81.8	100	100	86.0	91.4
DPP III	20.60 mU/mg[b]	90.9	97.3	96.8	92.3	94.3
LDH + DPP III	-	93.9	97.3	96.9	94.7	95.7

[a] Calculated on the basis of "all normals" (normal endometrium plus normal myometrium) sample, as described under "Material and Methods"

[b] Calculated from the published data[16]

Legend for Figure 1

Fig. 1 Total LDH levels in normal and malignant uterine tissue. NE = Normal endometrium (*n* = 10), NM = Normal myometrium (*n* = 27), CAE = Endometrial carcinoma (*n* = 33). Enzyme activity was measured as described under "Materials and methods".


LDH activity (U/mg protein)
4
3
2
1
0
NE
NM
CAE