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# High Lactate Levels Predict Likelihood of Metastases, Tumor Recurrence, and Restricted Patient Survival in Human Cervical Cancers[1]

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## ABSTRACT

**Pathophysiological parameters such as vascular density and tissue oxygen pressure can influence tumor malignancy and patient survival. Observations from our group showed that metastatic spread of carcinomas of the uterine cervix and of head and neck cancers was closely correlated with the lactate concentration in the primary lesion. Because these results were obtained in a low number of patients, the present investigation was performed to verify such a correlation in a larger population. Cryobiopsies were taken at first diagnosis of cervical cancer from 34 patients. Tissue concentrations of ATP, glucose, and lactate in viable tumor regions of these biopsies were measured microscopically using the technique of imaging bioluminescence. There was no correlation between stage or grade and any of the metabolic parameters measured. ATP and glucose concentrations were not significantly different in metastatic and nonmetastatic primary tumors ($P > 0.05$). However, lactate concentrations were significantly higher ($P = 0.001$) in tumors with metastatic spread (mean ± SD, 10.0 ± 2.9 μmol/g; $n = 20$) compared with malignancies in patients without metastases (6.3 ± 2.8 μmol/g; $n = 14$). The majority of patients who suffered a recurrence of the disease (17 of a total of 22 patients) or died (15 of 20) within the observation period of up to 8 years belonged to the metastatic, *i.e.*, high lactate group. A Kaplan-Meier analysis of the data showed that the overall and disease-free survival probabilities of patients having low tumor lactate values were significantly higher compared with patients with high tumor lactate concentrations ($P = 0.015$ and 0.014, respectively). We conclude that tumor lactate content may be used as a prognostic parameter in the clinic. Furthermore, these findings are in accordance with data from the literature showing that the presence of hypoxia in cervical tumors is associated with a poorer patient survival.**

## INTRODUCTION

Solid malignant lesions are characterized by severe disturbances of the microcirculation that occur already at an early stage of tumor growth (1, 2). The abnormalities of the tumor vasculature include a loss of the natural hierarchy of blood vessels, changes in the vascular density, increases in microvascular permeability, and loss of the physiological regulation of blood perfusion. Perhaps the most striking difference between solid tumors and healthy organs is the emergence of pronounced spatial and temporal heterogeneities in tumor blood supply (3, 4). As a consequence, solid tumors often exhibit regions with insufficient blood perfusion adjacent to areas with ample blood supply which leads to corresponding heterogeneities in the metabolic milieu, *i.e.*, with regard to tissue oxygenation or acid-base status (5). Unlike metabolic gradients in healthy organs, such metabolic heterogeneities are arranged in irregular patterns that are not related to the physiological function of the tissue.

Previous studies on experimental tumors and on cancer in patients have shown that several pathophysiological parameters, such as local oxygen pressure, blood perfusion, energy status, or concentration of metabolites, can modulate tumor growth and therapeutic sensitivity. Global ATP content of various experimental tumor entities was positively correlated with tumor oxygenation (6) or with regional blood perfusion (7). Human tumor xenografts with high blood flow grew faster than xenografts with low blood flow (4), and oxygen appeared to be growth limiting in murine RH carcinomas of the mouse (8). The significance of tissue oxygen pressure as predictive of patient survival after radiation therapy has been shown for metastasis of head and neck squamous cell carcinoma (9, 10), for cervical cancer (11–13), and for soft tissue sarcoma (14). On the other hand, recent studies that imaged ATP, glucose, and lactate with quantitative bioluminescence in human melanoma xenografts have documented that the concentrations of these metabolites were not related to the largely variable intrinsic radiosensitivities of these tumors, but rather mirror the relatively uniform vascular density within the population of melanomas investigated (15). It may be concluded from these data that the metabolic micromilieu in malignant tumors mainly reflects the efficiency of tumor microcirculation. Metabolic imaging thus may be useful in relation to those phenomena in clinical oncology that are correlated with the function of microvessels in tumors.

Preliminary observations on metabolites in human cervical cancer (16) and in squamous cell carcinomas of head and neck (17) have indicated a correlation between the lactate concentration in tumor tissue and the incidence of metastasis, but the numbers of patients in both of these studies were too low, *i.e.*, 10 and 12, respectively, to allow for firm conclusions. On the basis of these findings, the present investigation was performed to evaluate possible correlations between metabolite distributions in primary tumors of the human cervix and the incidence of regional lymph node metastasis and patient survival including a total of 34 patients.

## MATERIALS AND METHODS

Thirty-five tumor specimens from 34 patients with cervical cancer were obtained from the Norwegian Radium Hospital in Oslo. The biopsies were rapidly frozen in liquid nitrogen and transferred on dry ice to our laboratory, where metabolic measurements were made. The specimens were parts of biopsies taken from patients mostly in stage II or stage III (Fédération Internationale des Gynaecologistes et Obstetristes) at first diagnosis of the disease. Experiments were approved by the local ethics committee, and informed consent was obtained from all patients involved in this study. The biopsies, which were randomized with regard to the anatomical site of removal, were taken from the tumors with a curette before conventional radiation treatment. Relevant patient data, such as treatment protocol, disease recurrence, patient survival, incidence and location of metastasis, were documented during pretherapeutic staging in the clinic. This information was not made accessible before metabolic imaging had been performed to avoid a possible bias in measurements.

The spatial concentrations of ATP, glucose, and lactate in cryosections of tumors were obtained using the method of imaging bioluminescence (for more details, see Refs. 18–20). This technique allows for the histographic mapping of metabolite concentrations in tissue sections at a high spatial resolution. For

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measurement, ATP, glucose, or lactate are enzymatically linked to the light reaction of bioluminescence enzymes, leading to light emission with the intensity being proportional to the tissue content of each metabolite.

Cryostat sections were made from the frozen tumors and were subsequently adhered to the upper side of a coverglass. The coverglass was laid upside down on a glass slide with a casting mold. The mold was filled with a liquid reaction solution containing various enzymes to link the substrate of interest to the luciferase light reaction. Different mixtures of enzymes and luciferases were used for the detection of ATP, glucose, and lactate; however, the use of 20-$\mu$m thick serial sections allowed for the determination of the different metabolites at quasi-identical locations within the biopsies. The casting mold carrying the coverglass and the tissue section was transferred to a microscope stage in an air-conditioned environment. The temperature of the array was adjusted to 22°C ± 1°C, resulting in reproducible kinetics of the enzyme reactions. The spatial distribution of the bioluminescence intensity within the tissue section was registered directly using an appropriate microscope (Axiophot; Zeiss, Oberkochen, Germany) and an imaging photon counting system (Argus 100; Hamamatsu, Herrsching, Germany). The light intensity was calibrated by appropriate tissue standards so that density distributions were obtained that represented the distribution of ATP, glucose, and lactate in weight-related tissue concentrations ($\mu$mol/g wet weight). These values were routinely validated by independent measurements with high-performance liquid chromatography and enzymatic standard assays, respectively.

The digitized images of the different substrate distributions as well as of an adjacent tissue section stained with H&E were transferred to a personal computer with commercial image software (Optimas; Media Cybernetics, Silver Spring, MD). By optical overlay of the metabolite distributions with the image of the adjacent histological section, we evaluated metabolites separately in tumor regions with densely packed viable cancer cells, in areas with necrosis, and eventually in stromal tissue elements. Furthermore, a computer algorithm allowed for the pixel-to-pixel correlation among the images of the different substrates (7, 21). Between seven and nine sections for each metabolite and for histological analysis were made from each tumor. Pixel values of each section and region of interest, respectively, were summarized for individual tumors into one distribution histogram. From this histogram, mean values (± SD) and additional statistical parameters were calculated.

A difference between two populations was considered significant at $P < 0.05$ (two-sided) using the Mann-Whitney test. To compare the intratumoral variations of the metabolites with the variability of the measured values between the tumors (intertumoral variance), we used a hierarchical ANOVA. Overall and disease-free survival probabilities were calculated with the Kaplan-Meier life table method. Differences between survival probabilities were analyzed using the log-rank test.

## RESULTS

Metabolite distributions in all of the tumors investigated were extremely heterogeneous, but the distribution patterns of ATP, glucose, and lactate that were acquired in serial sections showed obvious similarities. This was reflected in most of the cases by positive correlations between the distribution patterns of the three metabolites, *i.e.*, there was a high concentration of ATP at a location with high glucose and lactate and *vice versa*. As a representative example for the evaluation of data acquired in one tumor biopsy, metabolite correlations and distribution histograms from a cervical carcinoma are depicted in Fig. 1. The correlations between corresponding pixel values of the different substrates measured in viable tumor regions are shown in Fig. 1, *left panels*. The quality of each correlation was quantified by Spearman's correlation coefficient, $r_s$, which was typically between 0.2 and 0.5 and reached values of up to 0.7 for the best correlations obtained.

Representative frequency distributions of measured concentrations of ATP, glucose, and lactate in viable tumor areas are shown in Fig. 1, *middle panels*. ATP distributions were mostly tilted toward low values in a range of 0–3 $\mu$mol/g, and glucose distributions were nearly Gaussian, ranging from 0 to 8 $\mu$mol/g. In contrast to this relatively invariable behavior, frequency distributions of lactate values were very variable, ranging from left-tilted shapes (with values exclusively below 10 $\mu$mol/g) to multimodal distributions that may be extended from 0 to >40 $\mu$mol/g.

Pronounced concentration differences were obvious within each tumor, preferentially between vital and necrotic tumor regions, which is demonstrated in Fig. 1, *right panels*. As a general observation, lactate was high next to the necrotic zones, but dropped within these areas to levels far below those found in viable tumor regions. Nevertheless, concentration differences between tumors (intertumoral variance) were even more pronounced than the intratumoral variability of the metabolites investigated. This was verified by hierarchical

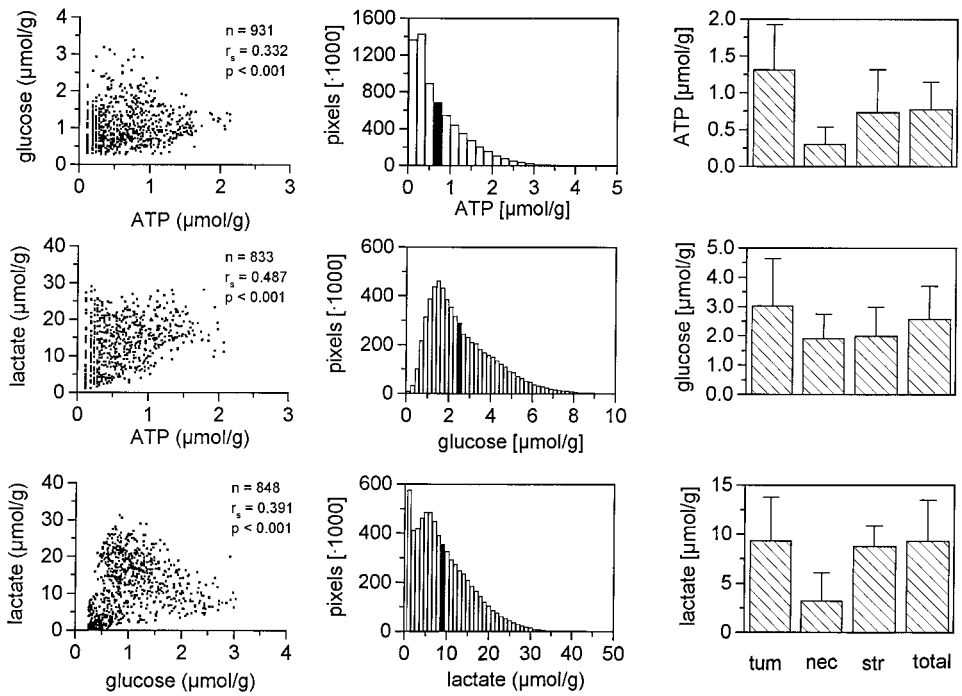


Fig. 1. Representative data evaluation for one biopsy of a human carcinoma of the uterine cervix. Data represent pixel-to-pixel correlations among local ATP, glucose, and lactate concentrations (*left panels*), frequency distributions of measured pixel values of the three metabolites (*middle panels*), and structure-related concentrations (mean + SD) of these parameters (*right panels*). The data in the *left* and *middle panels* were measured in viable tumor regions. *Black columns*, mean class; *tum*, viable tumor area; *nec*, necrosis; *str*, stroma; *total*, overall value.

variance analysis, which showed that 75% of the total variance was based on intertumoral differences and only that 25% was due to intratumoral variations.

Patients were grouped into two categories: (*a*) a group of 14 patients (nm-patients) who had no detectable metastases; and (*b*) a group of 20 patients (m-patients) with metastatic spread of the disease when entering this study. For these two groups, patient data and mean (± SD) values of the measured tumor metabolites are summarized in Table 1. The data show that recurrence of the disease after treatment occurred only in 5 of the 14 nm-patients, whereas 17 of 20 m-patients had a regrowing lesion after radiotherapy. In addition, a total of 20 patients died within the observation period of up to 100 months. A majority of these, 15 patients, belonged to the m-group, whereas only 5 patients of the nm-group died.

Fig. 2 shows the respective means (+ SD) of individual tumors and patients, respectively, for the nm- and m-groups in increasing order. Patients who died in the course of the follow-up are indicated by plus signs in Fig. 2. Recurrence of the disease is indicated by "R." Despite a considerable overlap of the lactate concentration values in the two groups, there was a statistically significant difference between the two populations of data. Corresponding statistical parameters are shown in Fig. 3. Mean lactate concentrations (± SD) averaged over viable tumor regions were significantly higher ($P = 0.001$) in tumors of patients with para-aortal and abdominal lymph node metastases (10.0 ± 2.9 μmol/g) compared with malignancies of metastasis-free patients (6.3 ± 2.5 μmol/g), as illustrated in Fig. 3. No such differences were found for mean intratumoral ATP or glucose concentrations, which are also included in Fig. 3.

The Kaplan-Meier life table method and the log-rank test were used to further analyze the relationship between lactate content in viable tumor areas and patient survival probabilities. The mean lactate concentrations of all tumors were grouped into a high- and a low-lactate class compared with the median value of the overall data. As shown in Fig. 4, statistically significant differences were found for both the overall (Fig. 4*a*) and disease-free survival probabilities (Fig. 4*b*). The mean (± SD) overall survival of patients in the low-lactate group was 70.9 ± 9.7 months, which is significantly higher ($P = 0.015$) compared with the high-lactate group, which had a mean survival time of only 31.0 ± 5.2 months (Fig. 4*a*). The same finding holds true for the disease-free survival probabilities, showing values of 60.5 ± 10.3 and 22.1 ± 5.4 months for the low- and high-lactate groups, respectively ($P = 0.014$; Fig. 4*b*).

## DISCUSSION

The technique of imaging bioluminescence allowed for the quantitative assessment of tissue concentrations of ATP, glucose, and lactate at quasi-identical locations, and thus, spatial correlations between the respective distribution images could be established. In most of the cases, positive correlations between corresponding pixel values

Table 1 *Patient data and tissue content of ATP, lactate, and glucose in carcinoma of the human uterine cervix in patients with no clinically detectable metastases, and patients with diagnosed metastases*

| | | | | | Metabolite concentrations[b] (μmol/g) | | |
|---|---|---|---|---|---|---|---|
| Patient | Recurrence | FIGO[a] | Survival | KI | ATP | Lactate | Glucose |
| Nonmetastatic tumors (nm) | | | | | | | |
| nm 1 | Yes | IVa | No | 100 | 1.1 ± 0.5 | 2.7 ± 1.7 | 4.8 ± 1.5 |
| nm 2 | No | IIIb | Yes | 100 | 0.4 ± 0.3 | 2.9 ± 1.2 | 3.5 ± 0.6 |
| nm 3 | No | IIb | Yes | 100 | 0.4 ± 0.2 | 3.7 ± 2.3 | 2.2 ± 1.3 |
| nm 4 | No | IIb | Yes | 100 | 0.4 ± 0.3 | 3.9 ± 3.1 | 3.3 ± 2.1 |
| nm 5 | No | IIb | Yes | 100 | 0.9 ± 0.9 | 4.7 ± 2.9 | 3.7 ± 1.8 |
| nm 6 | Yes | IIb | No | 100 | 1.0 ± 0.7 | 5.0 ± 2.8 | 1.7 ± 0.6 |
| nm 7 | No | IIb | Yes | 80 | 1.0 ± 0.7 | 5.1 ± 3.2 | 1.9 ± 1.0 |
| nm 8 | Yes | IIb | No | 100 | 1.6 ± 0.6 | 6.4 ± 4.1 | 1.3 ± 0.7 |
| nm 9 | No | IIb | Yes | 100 | 1.4 ± 1.0 | 6.7 ± 4.3 | 1.9 ± 0.7 |
| nm10 | No | Ib | Yes | 100 | 0.8 ± 0.4 | 7.2 ± 4.2 | 1.6 ± 0.9 |
| nm11 | Yes | IIb | No | 80 | 0.6 ± 0.6 | 9.0 ± 5.3 | 0.7 ± 0.4 |
| nm12 | No | IIb | Yes | 100 | 1.6 ± 0.9 | 9.2 ± 5.9 | 1.4 ± 1.0 |
| nm13 | Yes | IIIb | No | 100 | 0.4 ± 0.3 | 10.7 ± 5.9 | 1.0 ± 0.7 |
| nm14 | No | IIa | Yes | 100 | 0.4 ± 0.2 | 11.2 ± 5.8 | 4.4 ± 0.7 |
| Total (mean ± SD) | | | | | 0.9 ± 0.4 | 6.3 ± 2.8[c] | 2.4 ± 1.3 |
| Range | | | | | 0.4–1.6 | 2.7–11.2 | 0.7–4.8 |
| Metastatic tumors (m) | | | | | | | |
| m 1 | No | IIIb | Yes | 90 | 0.7 ± 0.8 | 5.4 ± 3.3 | 2.2 ± 1.2 |
| m 2 | Yes | IIb | Yes | 100 | 1.1 ± 0.6 | 5.7 ± 3.6 | 1.7 ± 0.9 |
| m 3 | No | IIb | Yes | 100 | 0.6 ± 0.4 | 6.9 ± 4.8 | 4.0 ± 2.6 |
| m 4 | Yes | IIb | No | 100 | 2.2 ± 1.3 | 7.3 ± 3.3 | 1.6 ± 1.1 |
| m 5 | Yes | IIb | No | 100 | 0.4 ± 0.2 | 7.4 ± 4.1 | 2.7 ± 1.5 |
| m 6 | Yes | IIb | No | 90 | | 7.4 ± 4.1 | 2.3 ± 1.4 |
| m 7 | Yes | IIb | No | 100 | 0.5 ± 0.4 | 7.8 ± 3.6 | 2.5 ± 0.6 |
| m 8 | Yes | IIIb | No | 80 | 0.5 ± 0.6 | 8.8 ± 5.1 | 1.8 ± 0.9 |
| m 9 | Yes | IIb | No | 100 | 1.2 ± 0.6 | 8.8 ± 4.8 | 3.0 ± 1.0 |
| m10 | Yes | IIb | No | 80 | 0.2 ± 0.03 | 9.5 ± 4.5 | 1.5 ± 0.9 |
| m11 | Yes | IIIb | Yes | 80 | 0.9 ± 0.9 | 11.2 ± 4.7 | 2.7 ± 1.5 |
| m12 | Yes | IIIb | No | 70 | 0.4 ± 0.3 | 11.2 ± 5.1 | 1.4 ± 0.8 |
| m13 | Yes | IIb | No | 100 | 1.1 ± 0.7 | 11.6 ± 5.0 | 4.4 ± 1.9 |
| m14 | Yes | IIb | No | 100 | 1.3 ± 0.6 | 11.8 ± 5.5 | 3.1 ± 1.2 |
| m15 | Yes | IIb | No | 90 | 1.0 ± 0.4 | 12.3 ± 5.4 | 1.2 ± 0.7 |
| m16 | Yes | IVa | No | 100 | 0.6 ± 0.4 | 12.4 ± 5.8 | 1.1 ± 1.0 |
| m17 | No | IIb | Yes | 100 | 0.6 ± 0.4 | 12.5 ± 5.1 | 2.1 ± 1.0 |
| m18 | Yes | IIIb | No | 90 | 1.2 ± 0.5 | 12.8 ± 5.5 | 0.5 ± 0.3 |
| m19 | Yes | IIIb | No | 100 | 0.5 ± 0.4 | 13.7 ± 8.9 | 1.5 ± 0.8 |
| m20 | Yes | IIIb | No | 90 | 1.2 ± 0.6 | 15.6 ± 8.0 | 3.8 ± 1.8 |
| Total (mean ± SD) | | | | | 0.8 ± 0.5 | 10.0 ± 2.9 | 2.2 ± 1.0 |
| Range | | | | | 0.2–2.2 | 5.4–15.6 | 0.5–4.4 |

[a] FIGO, Fédération Internationale des Gynaecologistes et Obstetristes; KI, Karnofsky's index.
[b] Mean ± SD.
[c] $P = 0.001$.

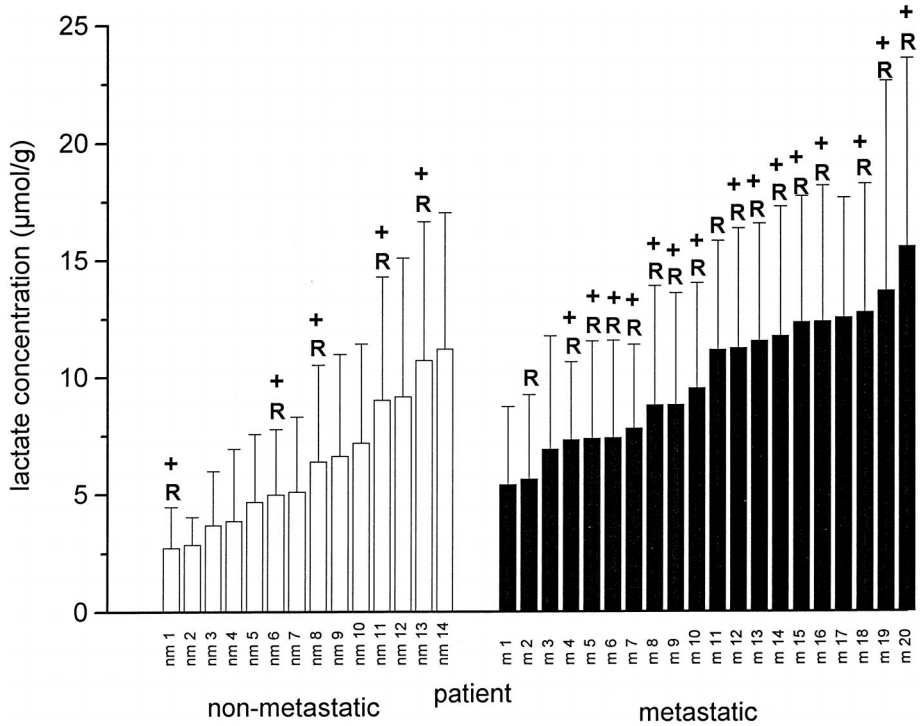


Fig. 2. Mean (+ SD) lactate concentrations in viable tumor tissue in all individual patient tumors in this study, with increased ranking for each the nonmetastatic (*open columns*) and the metastatic (*closed columns*) group. +, patient died within the observation period; *R*, recurrent disease within observation period.

of the three metabolites were obtained. This indicates that ATP and lactate levels are high at locations where glucose concentrations are high, and *vice versa*, and it may indicate that ATP is generated mainly from glucose associated with an extensive release of lactate by the tumor cells. Nevertheless, it should be kept in mind that this is only one possible interpretation of the steady-state concentration profiles measured, and that metabolic turnover rates cannot be derived from the data obtained.

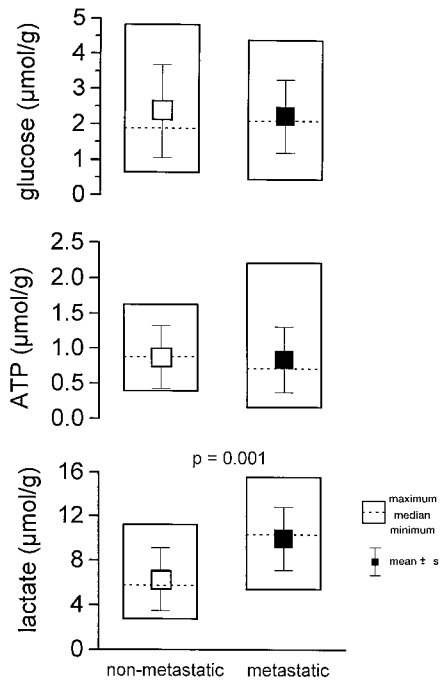


Fig. 3. Mean (± SD), maximum, minimum, and median values of local concentration values of glucose, ATP, and lactate in viable areas of cervical tumors of patients with metastatic spread (■) and of patients without clinically documented metastasis (□). Differences were statistically significant only for lactate ($P = 0.001$).

The regional evaluation in selected tissue areas demonstrates that concentrations of metabolites can be obtained in relation to the histological architecture of the tissue. Thus, tumor-adjacent normal tissue, viable tumor areas, and infiltrated and necrotic regions can be evaluated separately. As expected, ATP and glucose were less in tumor regions with more necrosis compared with viable "tumor cell nests." Stromal tissue that was adjacent to or incorporated in viable tumor tissue showed lower ATP and lactate concentrations than malignant cell areas. In contrast, glucose distributions did not exhibit such consistent differences between normal and tumor tissue, with glucose being higher or lower in normal compared with tumor tissue in some instances.

In the present study on human cervical cancer, there was no correlation between clinical staging or pathohistological grading of the tumors and any of the metabolic parameters measured. However, there was a striking difference between tumor lactate content in patients with and without metastasis. This difference was statistically highly significant, with a probability of error of $<1\%$. Additionally, the overall and the disease-free survival probabilities of patients having low tumor lactate values were significantly higher compared with patients with high lactate values in the viable tumor tissue. These findings indicate that high local levels of lactate within cervical cancers may be associated with a high risk of incidence of metastasis and a bad prognosis for survival. As one possible explanation, among others, such spots with unfavorable metabolic conditions within tumor tissue may enhance neovascularization, which may be true for both blood vessels and lymphatic vessels (22, 23). Such immature, newly formed vessels may "harvest" tumor cells from the primary lesion and thus may increase the probability of metastasis.

Although still under debate, investigations on various tumor entities in patients, including carcinomas of breast, head and neck, lung (non-small cell), and prostate, have shown that vascular density is correlated with the incidence of metastasis (24, 25). Preliminary data, however, from the German laboratory and recent findings of the Norwegian group (26) on vascular density in part of the cervical cancers did not show any correlation between vascularity and lactate

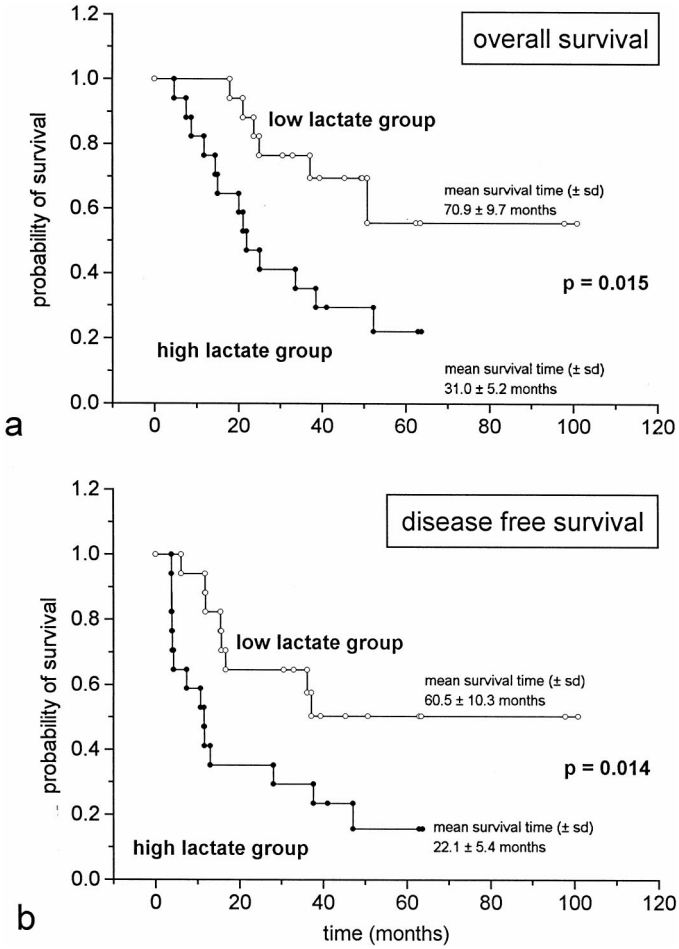


Fig. 4. Kaplan-Meier plots showing overall (*a*) and disease-free (*b*) survival probabilities of patients with high or low tumor lactate values measured in viable tumor areas of the biopsies. Lactate values were grouped into high- and low-lactate classes relative to the median value of the overall population. In each case, a significant difference of patient survival was obtained.

or the incidence of metastases. On the other hand, oxygen tension measurements in cervical tumors showed that tumor hypoxia was correlated with a high incidence of metastasis (26).

The correlations between metabolic milieu, likelihood of metastases, and patient survival were documented by our data despite the restriction that only a limited proportion of the total tumor volume could be taken into consideration when biopsies of roughly $3 \times 3 \times 3$ $mm^3$ in size were used. Although by far not proven, this suggests that macroscopic heterogeneities may not be as pronounced as heterogeneities in microscopic dimensions in these malignancies. As a consequence, biopsy material as used in this study may be, at least to some extent, representative of the entire tumor mass. This interpretation of the data is supported by previous findings from animal tumors comparing data registered with the bioluminescence technique with those from nuclear magnetic resonance spectroscopic measurements (6). Accordingly, it has been shown that the use of only a few biopsies of one animal tumor for measuring the fraction of hypoxic cells with nitroimidazole was sufficient for statistically reliable quantification of hypoxia of the whole tumor (27–29). Together with our findings, these data indicate that the heterogeneity of at least some physiological parameters in viable areas of solid tumors are expressed mainly at a microscopic level.

The present findings are in accordance with studies measuring oxygen tensions in several entities of patient tumors relating hypoxia to therapeutic outcome and patient survival (9–12, 14). In particular, Brizel *et al.* (14) showed that tumor oxygenation predicts the likelihood of distant metastases in human soft tissue sarcomas, and Hoeckel *et al.* (12) demonstrated that tumor hypoxia is a predictor of malignant progression in advanced cancer of the uterine cervix. These data may, at least partially, reflect the influence of oxygen on the expression of the malignant phenotype via mutation of p53, as determined by Graeber *et al.* (30). In this discussion, it is not necessarily anticipated that the distribution of lactate in tumor tissue is inversely correlated with that of oxygen. Preliminary measurements of oxygen partial pressures in some of the cervical tumors in this study indicate that there is no correlation between the mean values of these two parameters when averages over relatively large tumor or biopsy volumes are considered. Nevertheless, both parameters seem to predict the probability of metastases.

It is obvious that the correlation between lactate and metastasis should be challenged in more tumor entities. For example, the preliminary data obtained in squamous cell carcinoma of the head and neck are very striking and promising (17), and currently are being verified in a larger number of patients. In the case of successful verification, tumor lactate content may serve as a prognosticator of the likelihood of metastasis at the time of the first diagnosis of the malignant disease. Metabolic imaging in human cancer may therefore give important information to the oncologist who must decide how aggressive a potentially curative therapy should be.

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