The interaction between lung cancer metastases to the brain and their surroundings

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Abstract

Background and study purpose: due to the fact that an interrelation between metastases of lung cancer to the brain and surroundings may influence the prognosis, we made attempts to answer the following questions: 1) how is the border between the tumour and its surroundings formed? 2) are there any differences in the glial and vascular reactivity around different forms of lung cancer metastases to the brain?

Material and methods: a neuropathological examination has been done on 66 patients (27 women and 39 men) with lung cancer metastases to the brain. They were divided into three groups: group I – 18 cases of squamous cell lung cancer (sqclc), group II – 33 cases of lung adenocarcinoma (adl) and group III – 15 cases of small cell lung cancer (sclc).

Results: the most "aggressive" mode of metastatic infiltration into the brain was presented by sqclc. In many cases of the sqclc the end of neoplastic infiltration exceeded the area of the examined specimen, represented the material taken during the routine neurosurgical procedure (mean distance 153.8 μm compared to group II and group III cases: 56.10 and 26.09 μm respectively, p<0.05). The highest intensity of astroglial reaction was found around group III tumours (the mean number of astrocytes 48.15 SD±8.25 per measured area in comparison with 24.76 SD±10.54 and 19.75 SD±4.26 around group III and II metastases, respectively p<0.05). A remarkable fibrillary gliosis was also found around group III cases while the smallest one, comparable with normal nervous tissue glia immunoreactivity, within surroundings of group I metastases: group III : II p = 0.0098, group III : I p = 0.0000 and group III : control tissue p=0.0000). There were no significant differences in the mean number of vessels within the metastatic surroundings.

Conclusions: a dispersed mode of infiltration by sqclc metastases to the brain causes precise detection of the macroscopic border between the metastatic tumour and the nervous tissue to be much more difficult than in sclc, which is sharply demarcated from the surroundings. The poor prognosis in sqclc metastases to the brain may, among other, depend on metastatic remnants in the form of dispersed neoplastic cells within the seemingly uninvolved nervous tissue, not removed during neurosurgical procedures.

Key words: brain metastases, lung cancer, brain infiltration.

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Introduction

Lung cancer appears to be the most frequent neoplasm and cause of death in the world. It depends on the fatal influence of cigarette smoking on bronchial epithelium, which undergoes carcinogenic transformation to malignancy [27]. In the USA newly diagnosed lung cancers reach about 170,000 yearly [11]. The incidence permanently increases, predominantly among women, while in men the incidence rate decreased. The lung cancer is usually diagnosed in the fifth and sixth decades [11]. Distinct spread of lung carcinoma is a common phenomenon. The tumour usually metastasises to the adrenals (more than 50%), liver (30–50%), brain (20%) and bone (20%) [23].

On the other hand, primary lung cancer constitutes 30-60% of all brain metastatic tumours [5]. It is estimated that up to 65% of the patients with lung cancer will develop brain metastases [4,19]. The increasing number of brain metastases from lung cancer results from a longer time of exposure to the illness and thus, a higher risk of metastasising to the brain [1]. Increased duration of cancer survival may allow a longer window for detection of metastases, supported by better diagnostic procedures [6,29]. So far it has not been definitely established what type of lung cancer is a more predictive factor for brain metastases and poor neurological outcome. The interrelation between metastasis and the brain may depend on the biological aggressivity of the tumour and response of brain tissues surrounding the tumour.

Due to the fact that the interrelation between the metastatic neoplasm and surroundings may influence prognosis in metastases of lung cancer to the brain, we made attempts to answer the following questions: 1) how is the border between the tumour and its surroundings formed? 2) are there any differences in the glial and vascular reactivity around different types of lung cancer metastases to the brain?

Material and methods

Of the 246 individuals suffering from lung cancer a neuropathological examination has been done on 66 patients (27 women and 39 men) with metastases to the brain. They were divided into three groups: group I – 18 cases of squamous cell lung cancer (sqclc), group II – 33 cases of lung adenocarcinoma (adl) and

group III – 15 cases of small cell lung cancer (sclc). The age of patients ranged from 39 to 71 years. Uncommon types of lung cancer – i.e. large cell, bronchioloalveolar and combined carcinomas because of their infrequent occurrence were excluded from the analysis. Only metastases located within the brain hemispheres were taken into account. The clinical diagnosis of lung cancer verified histopathologically was done in each case. The tumours were taken during a routine neurosurgical procedure. The histological specimens were fixed in formalin for 18-22 hours and embedded in paraffin. They were stained with hematoxylin and eosin. The sections were also immunostained for GFAP, cytokeratin and for VIII-related antigen (FVIII rA – von Willebrand factor). All specimens were 2-3 micrometers thick and were stained and impregnated in the same conditions. An image-computerised analysis was made by means of the morphological material scanned in light microscope and a two-tube colour television camera. The illumination source was a 100 W halogen bulb. The Achroplan 20 x and Plan-Neofluar 40 x objectives were used for scanning and measurements. Each scanned image comprised 0.0096 mm^2 and 0.0048 mm^2 for magnification at 20 and 40x, respectively. Ten to fifteen images were scanned from each specimen. The morphometric analysis was done by means of KONTRON imaging system KS-100, v. 2.0 (license number 0100176). For analysis the images were defined on grey (brightness) scale. A grey value of 0 represented black – maximal density, a grey value of 255 represented white – minimal density. The following morphometric parameters were estimated: the DISTANCE between the edge of the solid part of the metastatic tumour and the most advanced neoplastic cells into surroundings (micrometers), COUNT of astrocytes within the vicinity of metastasis (per one visual field at magnification 40x), PROFILE i.e. the distribution of values in scanned areas along the x coordinate. The PROFILE curve consists of three true colour components: - red, green, and blue (RGB). Each colour component was also described by means of a corresponding numerical value (0-255) measured for every pixel between the first and the end point of the PROFILE. The PROFILE procedure allows us to estimate the density of measured area (amplitude of waves – the more elevated and separated colour components indicated less density of area being measured).

Statistical analyses were carried out using STATISTICA v. 6.0 program (licence number SN

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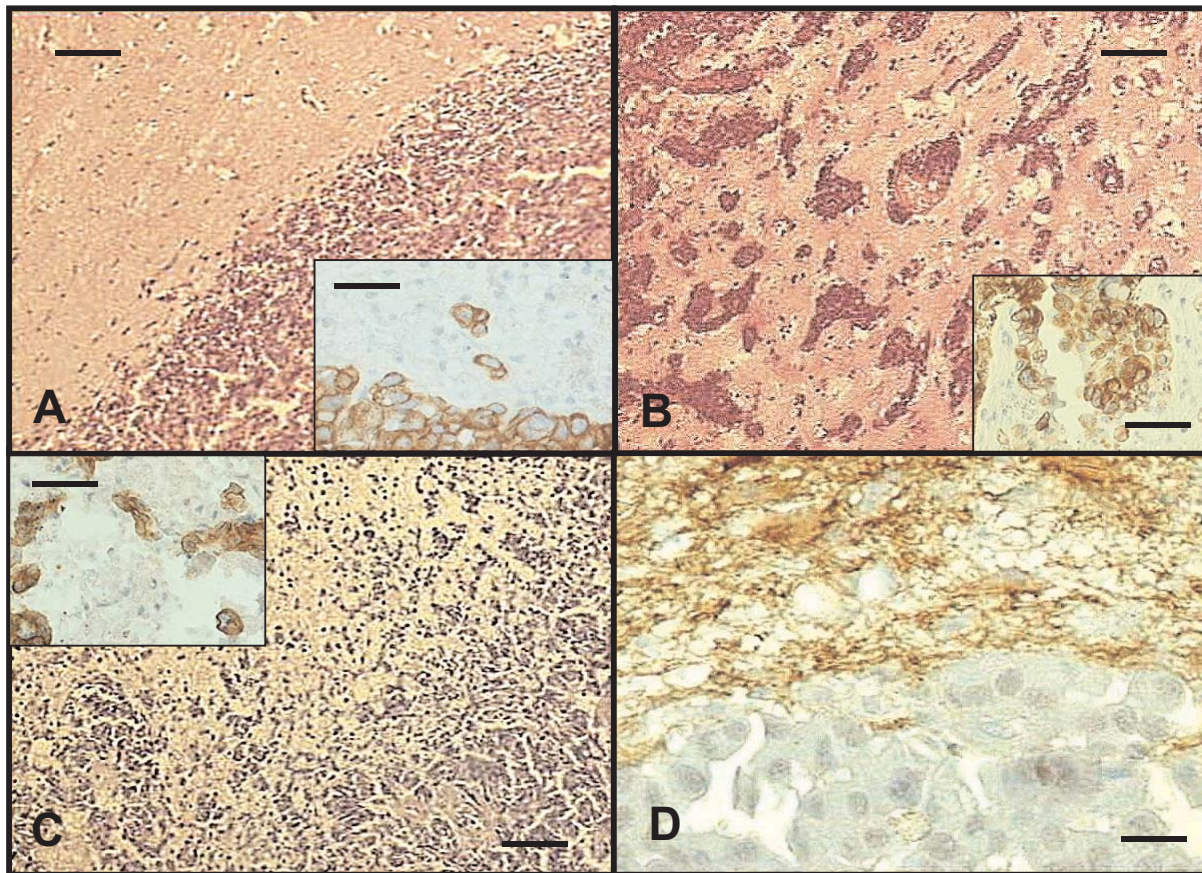
Fig. 1. Brain infiltration by the lung cancer metastases – predominant border between the neoplasm and vicinity: (**A**) sharp – when the metastasis edge was clearly defined as a unquestionable line, (**B**) irregular – when neoplastic cells penetrated into surroundings along the vessels or in form of "insets", (**C**) dispersed – when precise description of the neoplasm edge was difficult or impossible; the border was formed by many single neoplastic cells or small groups of cells (C). (**D**) GFAP-positive fibrillary gliosis around metastasis. Fig. A, B, and C – hematoxylin-eosin. Bars indicate: figs A, B and C – 80 μm; fig. D and figs in A, B and C corners – 20 μm

AXXP402C295823AR21). The following were used: the Mann-Whitney U Test, the Kruskal-Wallis one-way analysis of variance by ranks (KWANOVA). For all tests, a P value of less than or equal to 0.05 was considered as significant.

Results

Considering the mode of brain infiltration by the metastases we distinguished three forms of predominant border between the neoplasm and vicinity: sharp – when the metastasis edge was clearly defined as an unquestionable line (Fig. 1A), irregul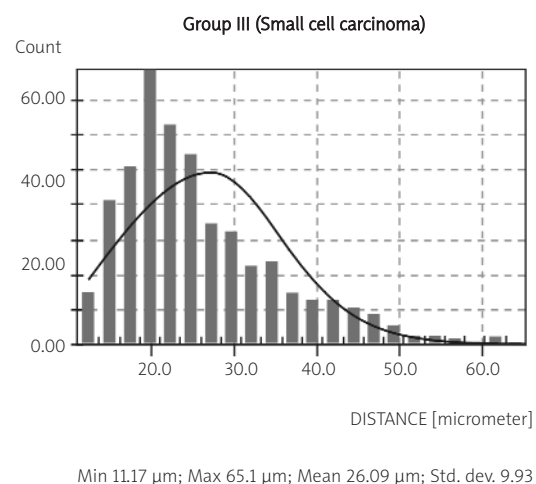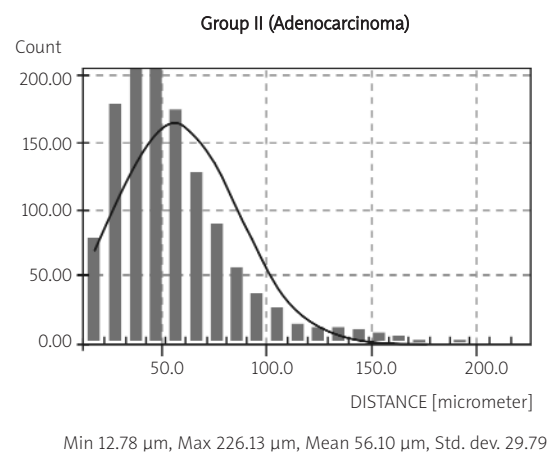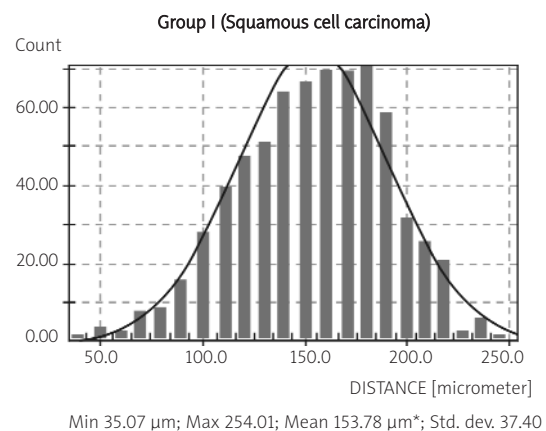ar – when neoplastic cells penetrated into surroundings along the vessels or in form of "insets" (Fig. 1B), dispersed – when a precise description of the neoplasm edge was difficult or impossible – the border was formed by many single neoplastic cells or small groups of cells (Fig. 1C). We recognised the border as predominant when at least 75-80% of visible part in histopathological specimen demonstrated one of three of the above listed forms of the metastatic edge.

The first form of border between the metastatic tumour and surroundings was predominantly found in sclc (58% of group III cases), the second form characterised majority of adl (64% of group II cases) while the third form of the metastatic edge was commonest for sqclc (55% of group I cases).
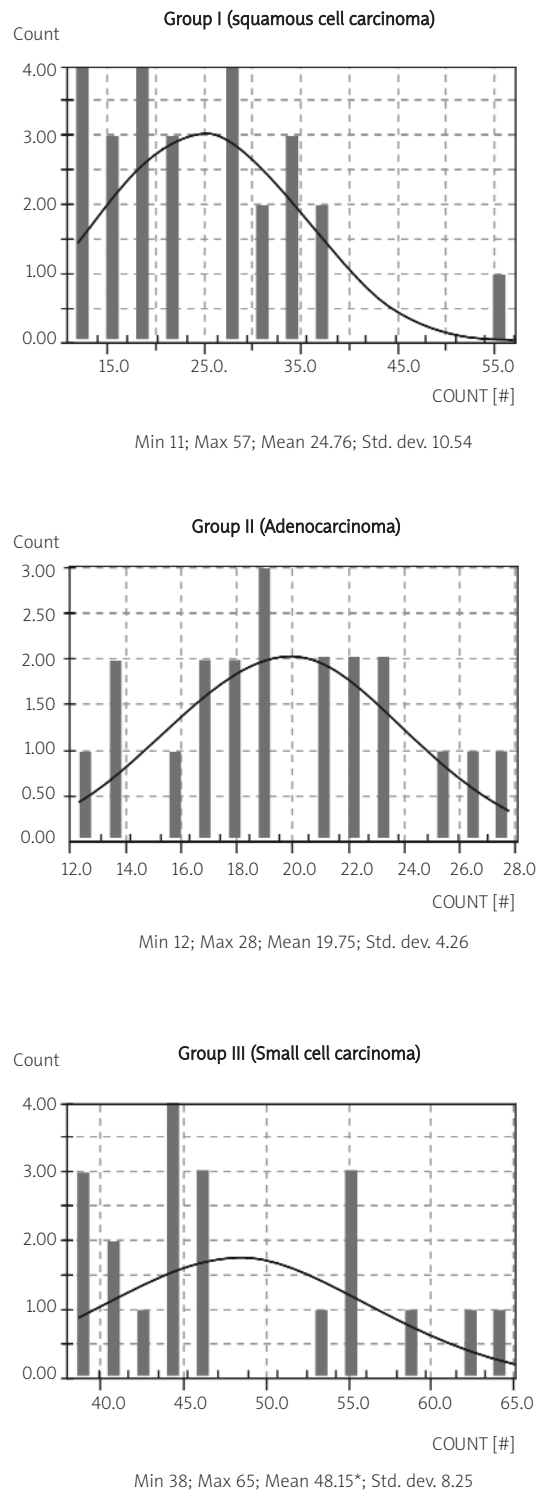
The most "aggressive" mode of metastatic infiltration into the nervous system appeared to be the third form (dispersed), common for group I. In many cases of this group the end of neoplastic infiltration exceeded the area of the examined specimen, representing the material taken during the routine neurosurgical procedure. Single neoplastic cells immunostained with cytokeratin were found 254 μm from the edge of group I metastases (mean value 153.8 μm compared to group II and group III cases: 56.10 and 26.09 μm respectively, p<0.05; Fig. 2).

The highest intensity of astroglial reaction was found around group III tumours (mean number of astrocytes 48.15 SD±8.25 per measured area in comparison with 24.76 SD±10.54 and 19.75 SD±4.26 around group I and II metastases, respectively p<0.05; Fig. 3). Remarkable fibrillary gliosis (Fig. 1D) was also found around group III cases, less evident around group II tumours while the smallest one, comparable with normal nervous tissue glia immunoreactivity, within surroundings of group I metastases. The morphometric PROFILE of fibrillary gliosis revealed the following numerical values: group III – 211.84 SD±22.8 red component, 193.43 SD±32.26 green component, 143.45 SD±29.79 blue component, group II – 218.64 SD±15.48 red component, 207.63 SD±16.39 green component, 160.73 SD±17.11 blue component, group I – 222.31 SD±6.34 red component, 219.20 SD±6.34 green component, 176.86 SD±6.67 blue component, control tissue – 229.43 SD±7.14 red component, 225.12 SD±6.63 green component, 184.12 SD±6.36 blue component. Group III : II p=0.0098, group III : I p=0.0000 and group III : control tissue p=0.0000 (Fig. 4).

An evident neovascularisation both in the white matter and in the cortex occurred around all types of metastatic tumours. In surroundings of 6.8% of metastatic tumours vascular glomeruloid structures were observed. In the majority of vessels endothelial cells demonstrated distinct immunoreaction against factor VIII related antigen. There were no significant differences in the mean number of vessels (3.5 SD±0.8; 4.1 SD±0.5; and 2.9 SD±0.7 per measured area), mean calibre (23.1 SD±7.2; 29.9 SD±9.7; and 26.8 μm SD±6.6 μm), and vascular wall thickness (4.3 SD±1.7; 5.7 SD±1.2; and 3.8 μm SD±1.4 μm) with respect to group I, II and III.

Min 35.07 μm; Max 254.01; Mean 153.78 μm*; Std. dev. 37.40

Min 12.78 μm, Max 226.13 μm, Mean 56.10 μm, Std. dev. 29.79

Min 11.17 μm; Max 65.1 μm; Mean 26.09 μm; Std. dev. 9.93

Fig. 2. Infiltrates of metastatic tumours into the nervous tissue (DISTANCE – micrometers; group I to group II and III p<0.05

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Group I (squamous cell carcinoma)

Count

Min 11; Max 57; Mean 24.76; Std. dev. 10.54

Group II (Adenocarcinoma)

Count

Min 12; Max 28; Mean 19.75; Std. dev. 4.26

Group III (Small cell carcinoma)

Count

Min 38; Max 65; Mean 48.15*; Std. dev. 8.25

Fig. 3. Intensity of the astroglial reaction around lung metastatic tumours into the brain (COUNT – the number of astrocytes; Group III to group I and II p<0.05)

Discussion

Brain metastases, including primary lung cancers, become an important clinical problem because of their poor prognosis. The central nervous system is one of the commonest sites of lung cancer metastases [9,28]. Brain metastases are a frequent feature of both: non-small cell lung cancer and small cell carcinoma [2,16,18]. So far the findings concerning poor neurological outcome are divergent. According to some authors the brain becomes one of the most common sites of lung metastases in non-small cell lung cancers [2,9,10,13,16,21]. The median survival in sqclc metastases to the brain was found to be shorter in comparison with adl of the lung metastases – 1.9 and 3.5 months, respectively [3]. Also other authors suggest that patients with IIIB sqclc may have a shorter 2-year survival rate of brain metastases than individuals with non-squamous lung cancer metastases to the brain [22]. The above listed data postulate the essential role of non-small cell lung cancers in poor neurological prognosis. Conversely, a tendency to earlier dissemination and more clinical aggressiveness of sclc than non-small cell lung cancers were described [7]. Approximately 10% of patients with sclc will develop brain metastases at the time of initial diagnosis of the cancer, while up to 50% of the cases will develop brain metastases later [19,24].

One of the factors which can influence the poor prognosis in the lung cancer metastases to the brain may be the mode of brain infiltration by neoplastic cells. Out of many mechanisms responsible for development of brain metastases we examined the ability and mode of both non-small cell and small cell cancer spread within the brain. In our findings sqclc metastases demonstrated dispersed surroundings infiltration, which appeared to be most invasive, compared to aclc and sclc metastases. It must be stressed that such mode of interrelation between metastasis and the brain does not mean the quickness of the neoplasm spread. As it was postulated above, sclc seems to involve the central nervous system quicker and is more aggressive than sqclc, because it probably grows faster. Probably sqclc is not so violent but its brain metastases behaviour more insidiously. It turned out that precise detection of
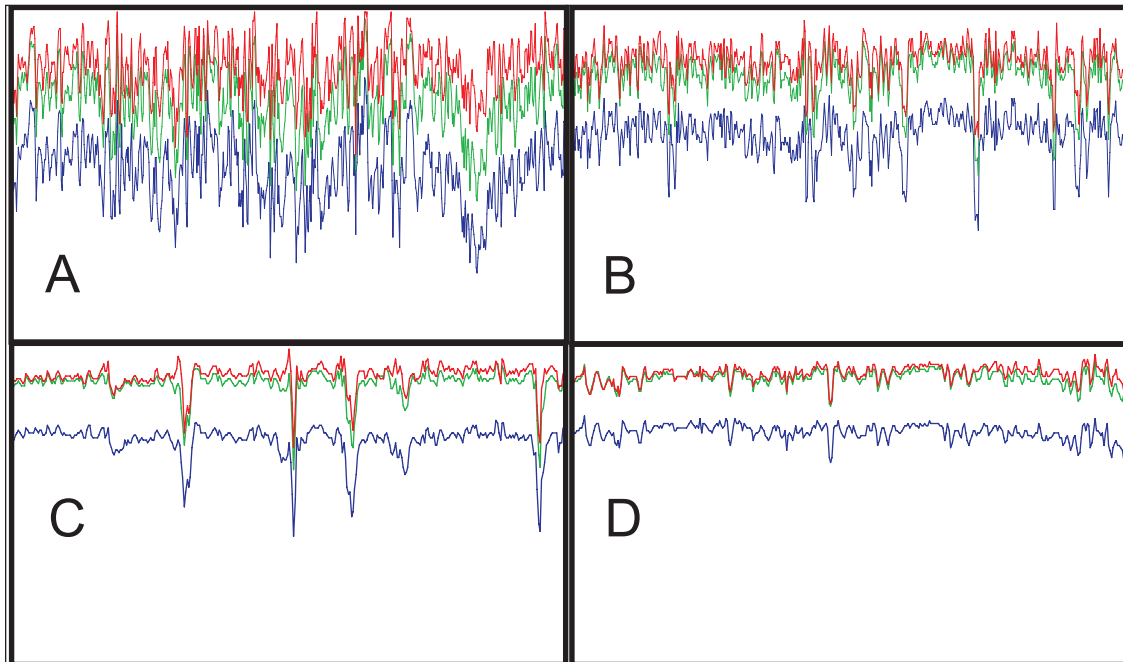
Fig. 4. The example of morphometric PROFILE of dense fibrillary astrogliosis around small cell carcinoma (**A**), less evident astrogliosis around adenocarcinoma (**B**) and slight astrogliosis around squamous cell carcinoma (**C**) similar to astrogliosis within the normal nervous tissue (**D**)

the macroscopic border between metastasis and the nervous system is much more difficult in sqclc than in adl and especially sclc, which appeared to be sharply demarcated from the surroundings. The poor prognosis in sqclc metastases to the brain may, among other, depends on remnants in the form of dispersed neoplastic cells within the seemingly uninvolved nervous tissue, which has not been removed during the neurosurgical procedure. Intrinsic resistance of metastatic clones of non-small cell lung cancers within the CNS as a mechanism responsible for high incidence of these neoplasm metastases within the brain is also postulated by Omuro et al [17].

Our results are in agreement with the molecular examinations, which support the concept on the bad prognosis of non-small cell lung cancer metastases to the brain. A disintegrin and metalloproteases in this group of lung cancer contribute to the cell-cell and cell-matrix interactions that are very important determinants of malignancy [8,30]. Especially metalloprotease 9 overexpression may enhance cell adhesion and invasion of non-small cell lung cancer, thereby promote metastatic capacity to the brain [25].

We have observed an evident astroglial reaction around sclc. It is hard to answer whether astrogliosis stimulates or rather limits the metastasis spread. However, cytokines produced by glial cells are suspected to contribute, in a paracrine manner, to the development of brain metastases by breast cancer cells [26], but it seems for us that, at least the fibrillary component of astroglial reaction, rather limits neoplastic cells penetration into surroundings. We found many neoplastic cells around sqclc metastases while fibrogliosis was slightly demonstrated within their surroundings.

The growth of tumours requires neovascularisation that occurs by angiogenesis, a process by which new capillaries are formed from the existing vessels [12,14,15,20]. The factors produced by tumour cells may increase the response of microvessels in the surroundings of the neoplasm, leading to neovascularisation [14]. No evident differences were found in our findings between intensity of neovascularisation around the examined types of lung cancer metastases to the brain, irrespective of neoplasm mode of spread.

Conclusions

1. The dispersed mode of brain infiltration by sqclc metastases causes precise detection of the macroscopic border between the metastatic tumour and the nervous tissue to be much more difficult than in sclc, which is sharply demarcated from the surroundings.
2. The poor prognosis in sqclc metastases to the brain may, among other, depend on metastatic remnants in the form of dispersed neoplastic cells within the seemingly uninvolved nervous tissue, not removed during neurosurgical procedures.

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