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Contributions of cytology examination and methods in lung cancer diagnostic

Doprinos citoloških preiskav in metod v diagnostiki raka

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Background. *Lung cancer (LC) is still the leading cause of cancer death according to published data worldwide and confirmed also by the data obtained from the central Cancer Registry of Slovenia. Early detection of LC has an important impact on the long-term survival rate of the patients. In spite of a great advance in imaging technology for a better visualization and early detection of the neoplasms and a variety of screening tests, only cytopathology examination finally define the neoplastic lesion.*

Methods. *To evaluate the contribution of cytology examination in the diagnosis of LC we studied the cytology diagnoses, comparing them with histology reports in patients, who underwent the diagnostic procedure under suspicion of the LC during last 2 years.*

Results. *Of a total 772 patients, in 241 patients cancer was microscopically confirmed. The most frequent diagnoses were adenocarcinoma (36.9%), squamous cell carcinoma (26.6%), and small cell carcinoma (SCLC) (12.9%). There were 22% of neoplasms classified as non-small cell carcinomas (NSCLC). From the clinician point of view considering the therapy it is very important to distinguish NSCLC from SCLC. And in our study the cytology-histology correlation between these two major types of carcinoma was almost 100%. Based only on cytology, 68 (28.2%) patients received microscopic diagnosis of malignoma, and the specimens for this group of patients were obtained mostly from transbronchial or transthoracic fine needle aspiration biopsies.*

Conclusions. *Cytology is of great diagnostic value, a reliable and relatively non-invasive method for patients. Cytology specimens should be taken in cases where it is not possible to obtain samples for histology.*

Key words: lung neoplasms – cytology – pathology; biopsy

Izhodišča. *Od vseh vrst karcinomov prav pljučni rak, sodeč po objavljenih podatkih, povzroča v svetovnem merilu največjo umrljivost. To potrjujejo tudi podatki iz Registra raka za Slovenijo. Zgodnje odkritje pljučnega raka je ključnega pomena za dolgoročno preživetje pacientov. Kljub izrednemu tehnološkemu razvoju slikovne diagnostike za zgodnje odkrivanje raka in različnim presejalnim testom je za dokončno potrditev raka še vedno potrebna citopatološka preiskava.*

Metode. *Z namenom, da bi preučili vrednost in doprinos citoloških preiskav v diagnostiki pljučnega karcinoma, smo preučili citološke izvide in jih primerjali s histološkimi pri bolnikih, ki so bili v zadnjih dveh letih preiskani zaradi suma na pljučno neoplazmo.*

Rezultati. *Od skupno 772 bolnikov, je bila diagnoza pljučnega raka mikroskopsko potrjena pri 241 bolnikih. Izkazalo se je, da je najpogostejša oblika raka žlezni rak (36,9%), sledita ploščatocelični rak (26,6%) in drobnocelični rak (12,9%). 22% rakov je bilo opredeljenih kot nedrobnocelični rak. S kliničnega in terapevtskega stališča je ključno razlikovati nedrobnocelični od drobnoceličnega raka. V naši raziskavi je bila citološko-histološka korelacija med tema glavnima vrstama rakov skoraj 100%. Malignom je bil zgolj s citološkimi preiskavami potrjen kar pri 68 bolnikih (28,2%), in to predvsem s perbronhialno ali pertorakalno igelno biopsijo.*

Zaključki. *Citologija ima visok diagnostični pomen, je zanesljiva in sorazmerno neinvazivna metoda, primerna predvsem v primerih, ko tkivnih odvzemkov za histološki pregled zaradi različnih vzrokov ni mogoče zagotoviti.*

Ključne besede: pljuča novotvorbe – citologija – patologija; biopsija

Introduction

Lung cancer is still the leading cause of cancer death worlwide mainly for men. And this is confirmed also by the data obtained from the central Cancer Registry of Slovenia. The incidence of lung cancer in men remains in the first place, in women lung cancer appears after the cancers of the breast, skin, rectum and corpus uteri. Early detection of lung cancer, and screening tests especially in high-risk individuals rise significantly the 5-year survival rate and have as such an important impact on the outcome. It would be necessary to evaluate the most successful screening test for an early and accurate staging of the cancer. The screening test must provide benefits, and must be capable of detecting the disease at a point at which its course can be altered through treatment- because early detection can reduce lung cancer mortality. On the other hand, test should not be dangerous, harmful, nor time-consuming, nor should it have numerous false-positive results that cause necessitate invasive follow-up tests. The tests should with a high specifity predict and confirm also the benign lesions which might on chest-x-ray, or computed tomography (CT) look like cancerous lesions, and thus avoid surgical risk for a patients- such as thoracotomy.

In diagnostic work with patients suspected of having lung cancer, it is sometimes difficult to obtain adequate biopsy material for examination by pathologist. And cytology can be very helpful and diagnostic reliable method in such cases. From our experience cytology is of high diagnostic contribution in confirming the lung cancer.

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Table 1. The 241 (31.2%; 173 men, 68 women) of total 772 patients who received diagnosis of lung cancer based on cytology examination, with specification- subtyping of carcinoma

Subtype of carcinoma	No.	%
Adenocarcinoma	89	36.9
Squamous cell carcinoma	64	26.6
Small cell carcinoma	31	12.9
Non-small cell carcinoma	53	22.0
Carcinoid	4	1.6

Methods

Into the 2-year study (2004-2005) were included all the patients with a suspicion of lung cancer, visible as a nodule or an infiltrate on x-ray or CT. The patients underwent diagnostic procedure with bronchoscopy or transthoracic fine-needle aspiration biopsy. In cases, when the tumour was visible during bronchoscopy, specimens were taken for histology, and also cytology, by means of bronchial brushings, smears or bronchial lavage. When the tumour was not visible at bronchoscopy, transbronchial sampling was performed: for histology and for cytology. Transbronchial fine needle aspiration (TBFNA) for cytology sampling were defined and obtained at different sites, as: paratracheal, tracheobronchial, hilar, bronchial, peripheral. In some cases, when a pulmonary nodule cannot be reached by bronchoscopy, samples were taken percutaneously (by percutaneous transthoracic fine-needle aspiration biopsy – PTTFNAB), for cytology examination only. All the samples for the histological and cytological examination were processed in a routine fashion. For the study we examined all the cytology specimens taken by bronchial/transbronchial or PTTFNAB procedures, but we excluded aspirates, sputum and pleural fluids specimens. We evaluated the cytologic material and classified as: neoplastic, nonneoplastic disease, suspicious for malignancy, unclassified, unadequate material for diagnosis. All the neoplastic lesions were further differentiated into subgroups according to WHO classification if possible. The diagnoses obtained from cytologic material were compared with histologic diagnoses.

Results

A total of 772 patients were examined (72% men, 28% women). The diagnosis of lung cancer was confirmed in 31,2% patients. The patients' age ranged from 24-87 in men (mean age 64.8 years), and 41-88 years in women (mean age 61.8 years). There were 13 men and 5 women aged 50 years or less, which represents 7.5 % of the men and 7.2% of the women.

Of total 241 patients with neoplastic disease, cytology examination subclassified the cancers (Table 1) as adenocarcinoma (AD) in 89 patients (in 25.5% in men, 12.3% in women), squamous cell carcinoma (SC) in 64 patients (20% in men, 5.4% in women), small cell carcinoma (SCLC) in 31 patients (10.8% in men, 3.1% in women), and carcinoids in 4 patients. A diagnosis of non- small cell lung carcinoma (NSCLC) was based in 53 patients (16.9% in men, 3.8% in women) because on cytological basis it wasn't possible to subclassify them any further. In women and in men the most frequent carcinoma was AD (confirmed in 51.4% of all women and in 34% of all men). In this group classified as AD were includ-

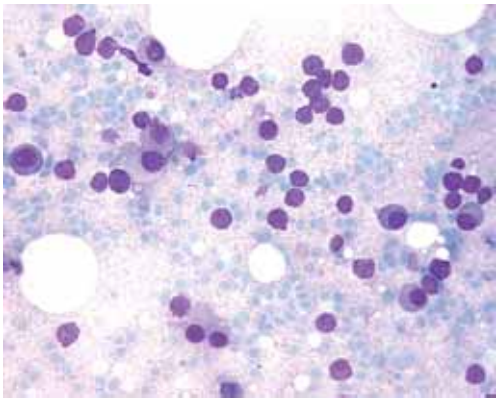

Figure 1a. Cytology smear obtained by PTTFNAB demonstrated atypical plasma cells, consistent with plasmacytoma (MGG, x 400), radiologically and clinically mimmicking carcinoma with local invasion into the rib.

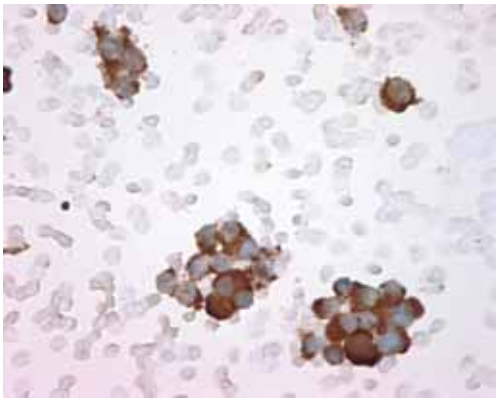

Figure 1b. Immunocytochemistry on cytospin preparations revealed monoclonal expression of kappa-light immunoglobulin chain (alkaline phosphatase, x 600).

ed also metastatic AD from breast (4 cases), colon (3 cases), kidney (2 cases).

On the second place was SC (in 26.8% of all men and in 22% of all women). SCLC was confirmed in aproximatelly 13%, in both men as well in women. 5% (12 cases – 9 AD and 3 SC) were uncorrectly classified in correlation to histological diagnoses. From the group with cytology diagnosis as NSCLCs, aprox. 40% were subclassified by histological examination (AD 14 cases, 7 SC), the remainder were further histologically classified only after surgical lung resection.

Based only on cytology, 68 patients (28.2%) received microscopic diagnosis of lung carcinoma. In all these cases histology was negative or material inadequate or specimens for histology examination not

Table 2. Cytologic-histologic correlation of diagnoses obtained from a total of 772 patients examined under suspicion of lung cancer

Type of C-H correlation	No.	%
C neg - H neg	493	63.9
C pos - H pos	165	21.3
C pos - H neg	68	8.8
C susp -H pos	19	2.5
C neg - H pos	10	1.3
C pos - H susp	8	1.0
C susp - H neg	4	0.5
C uncl - H pos	3	0.4
C susp - H susp	2	0.3

Abbreviations: C = cytology; H = histology; neg = negative for malignancy; pos = positive for malignancy (cancer); susp = suspicious for malignancy; uncl = unclassified

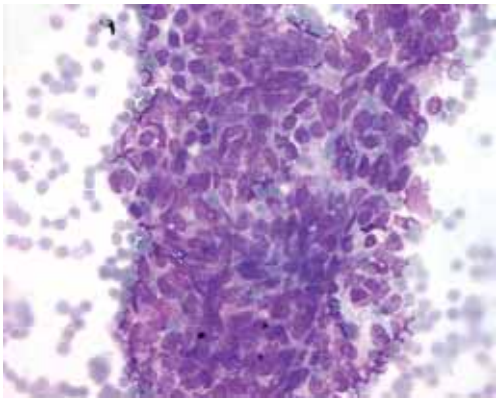

Figure 2a. Metastasis of skin basal cell carcinoma-BCC (MGG, x 400), clinically presented as primary lung carcinoma with solitary nodule. Cytology specimen, obtained by PTTFNAB.

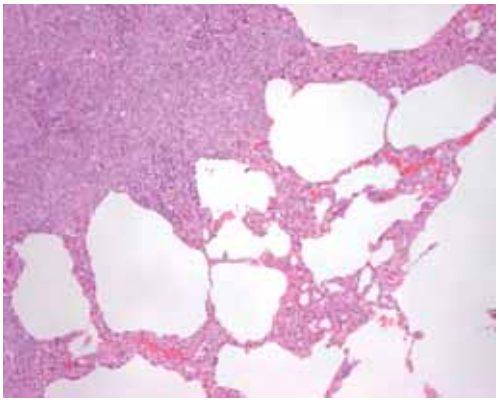

Figure 2b. The same tumour, BCC, confirmed by histology examination after wedge resection of the lung (HE, x 100).

taken because the lesions weren't possible to be reached by bronchoscopy forceps. In some cases a broad spectrum of immunocytochemical stainings was used to elucidate the lesion (Figures 1a, 1b), in others only very detailed additional clinical data were clue to final diagnosis (Figures 2a, 2b). This high percentage (of 28.2%) therefore prove that cytology specimens are of great diagnostic value. In such cases the most useful type of cytology specimens were taken by TBFNA and PTTFNAB. In all these cases (except in two patients) the diagnoses of malignancy were confirmed and further histologically subclassified by the specimens of resected lung. In the remainder two cases (0.8%) the cytology was false-positive and misinterpretated due to heavy reactive inflammation changes and atypical adenomatoid hyperplasia which were disclosed after wedge lung resection.

Of all the diagnoses given (for the whole material of 772 cases) by cytological examination only 3.6% (28 cases) were unclassified or diagnosed as 'suspicious for malignancy'. Of these cases histology confirmed the malignoma in 22 cases (78,6%).

In Table 2 are summarized observations from cytologic-histologic correlation.

Discussion

In lung carcinoma, cell typing of tumour is important in determining prognosis and often in influencing therapy. Therefore, it is highly desirable to obtain a correct morphological diagnosis. Clinicians are sometimes reluctant to rely on cytological procedures. But many times it is difficult to obtain adequate biopsy material for examination by the pathologist. That could be due to a tumour location (peripheral location) or practical difficulties with bronchoscopy procedure (patient's dyspnoe), and in this cases specimens for histology examination may not be representative or even false negative. Cytological examinations in such cases are very helpful. Thus, when the tumour is not clearly visible by bronchoscopy, the samples may be taken percutaneously by PTTFNAB, guided by CT or ultrasound (US). US-PTTFNAB makes accurate access to lesions in pleura, peripheral lung, anterior mediastinum, bone and soft tissue and thus unnecessary open biopsy (thoracotomy) can be avoided. By this procedure a false-positive diagnosis of pulmonary malignancy is exceedingly rare, and are estimated to around 1.5%.[1]

The specifity and positive predictive value of PTTFNAB in malignancy are extremely high. However, the rate of definite benign diagnosis by PTTFNAB is low, ranging from 4-14% depending on the prevalence of lung cancer in the population studied.[2,3] So, a negative cytologic report by this procedure may not represent a truly benign diagnosis, but merely a failure to reach a malignant one. A repeat biopsy reduces the rate of false-negative result. The interpretation of the received samples by the cytologist must be careful, to inform the clinicians about the adequacy of the specimen, and even recommend a repeat sampling for additional special stainings or immunocytochemical analysis to avoid misinterpretation of the specimen and to reduce false-negative results. It is important to clarify that false-negative interpretation of the cytology specimen has many causes. Size and location of the lesion are important. Also, in cases, where the lesions are very hemorrhagic, a bloody aspirate with very few viable malignant cells can be obtained. On the other hand, some lesions are almost entirely necrotic or associated with significant amount of fibrosis, etc. Besides, PTTFNAB, provides the material for bacteriological study in patients with pulmonary infections.

However, it is noteworthy to mention that this method (PTTFNAB) obtained over 21% of all lung cancers for our study.

Concerning the value of cytology, several reports have shown also a good correlation between cytologic and eventual histologic diagnoses.[4-7] And our results support those observations. In our series, cytologic-histologic correlation in SC and AD is aprox. 85%, in SCLC more than 97%, and in poorly differentiated carcinoma less than 60%. Factors that can affect the correlation between the cytologic and histopathologic examination include beside the adequacy (representativity) of sampling also the degree of the cellular differentiation of the tumour and the observer variations in interpretation. Anyway, it is noteworthy, that from the therapeutic implications it is of utmost importance to distinguish with a high diagnostic accuracy NSCLC from SCLC. And in our experience, cytological specimens have almost 100% efficiency of correct diagnosis of SCLC. Probably, the most important finding was that in only one SCLC diagnosis was inconclusive where differential diagnosis included also malignant lymphoma.

However, it is true that cytological specimens might sometimes be inconclusive, and in other cases merely give diagnostic indications that need confirmation by histology. In our study, in 3.6% of all cases the cytologic diagnosis was inconclusive or 'malignancy suspected', and in this cases subsequent histology examination (sometimes with open biopsy- mediastinoscopy /thoracotomy) was necessary to evaluate the process. Histology confirmed the malignant process in 78.6% of those cases.

Concerning the value of cytology, difficulties arise (in a very high percentage – 85%) when the clinicians need the specification of a benign process (i.e. inflammation, fibrosis, granulomatous lesions, hamartoma, etc) where the cytologic diagnosis is interpreted only as 'negative'. In this setting, histology examination is much more specific and valuable.

On the other hand, the diagnosis of lung carcinoma was obtained by cytology specimens alone in 28.2%. In such cases, the majority of cytological specimens were performed by TBFNA and PTTFNAB, and were obviously of high diagnostic value.

Furthermore, it is also important to be able to sort out a tumourous mass in the lung which is a metastasis from primary cancers elsewhere. Conventional morphologic evaluation of a patient's tumours may not accurately define the relationship

between multiple tumours in the same individual and may possibly lead to inappropriate treatment. Of course, the cytologic-histologic correlation in confirming the metastatic tumours can significantly drop when there is no material for an extra immunocytochemical stainings. On the other hand, even traditionally used immunohistochemistry as a method itself has some limitation. For example, difficullty may be encountered when attempting to differentiate squamous cell carcinomas of head and neck and pulmonary origins.[8] But the new insights of molecular techniques illustrate the possible practical diagnostic utility using the material obtained by fine needle aspiration biopsy (FNAB) in assessment of primary and metastatic malignancies. It has been shown that that synchronous and metachronous neoplasms are genetically different. Evaluation of primary and metastatic neoplasms may be enhanced by genomic analysis of loss of heterozygosity (LOH). This methodology can be used on a very small amounts of material, even on a cytologic smear. Panels of defined genetic targets are used to asses specific tumours and the relationships between primary and metastatic sides. LOH analysis provides also insight that additional genetic alterations may be observed in the metastatic foci, which are not seen in the primary origin, suggesting genetic progression of the neoplasm. Thus, LOH analysis may play a significant role in evaluating treatment efficacy and is useful and practical adjunct to conventional cytologic diagnosis, as only small numbers of cells from a cytologic smear are required.[9,10]

Because the patient's outcome is closely associated with the stage at diagnosis of cancer, there has been persistent interest in testing the methods for early detection of lung cancer. It is wellknown that the current staging test for lung cancer uses the TNM system, where the system relies on the pathological evaluation of the primary tumour (T), regional nodes (N) and distant metastases (M). The most common sites of metastases are the mediastinal lymph nodes. There are several technologies available for evaluation of mediastinal lymph nodes (LN). LN larger than 1cm in diameter can be detected by CT and are usually pressumed to contain metastatic disease. According to published data CT misses the LN metastasis in 13% and is false positive in 50%.[11] Because of this limitation, mediastinoscopy is accepted as 'golden standard' but requires general anaesthesia and expertize to perform safely, and therefore not universally used. Recently, positron emission tomography (PET) with its sensitivity and specifity[12] is suggested as non-invasive method for evaluating cancer stage, but cannot 100% distinguish between inflammation and cancer. In the final diagnosis histological analysis is used to determine the presence/absence of mediastinal LN metastases. However, the presence of micrometastases has a significant impact on long-term survival even among patients with histologically 'normal' LN. Namely, survival statistics indicate, that histology is inadequate, because the 5-year survival rate in patients with pathological stage I disease (without histological evidence of LN metastasis) is only 62%, and in patients with metastasis in hilar LN (but not mediastinal LN) 42%.[13] Obviously, histological examination can miss metastasis if not used wih serial sectioning and immunohistochemical stainings which are expensive for routine basis. To reduce unnecessary preoperative invasive methods and to increase correct preoperative staging, US (as minimally invasive method) with FNAB is used as safe and accurate method for detection LN metastasis, and thus for staging. The molecular characterization of LN tissue is a newly emerging field. Micrometastasis cannot be detected with standard cytologi-

cal and histological methods. Tumour cell can be detected using molecular techniques such as immunohistochemistry or reverse transcriptase-polymerase chain reaction (RT-PCR).[14,15] Using this method several markers can be identified, as telomerase and KS1/4, which can detect metastatic disease in a cytopathology-negative LN. [16,17] Telomerase can represent a unique target for molecular staging in lung cancer. Evidence of telomerase overexpression was noted in both pathologically positive and negative mediastinal LN from patients with NSCLC after US-FNAB. And according to published studies telomerase expression is absent in all normall LN.[18,19] But some new data have put into question the specifity of telomerase as a marker of malignancy[20], and in recent publication the effect of EGRF (epidermal growth factor receptor) expression in NSCLC appears promising.[21]

There are several experiments and attempts to identify the specific gene which would be highly expressed in NSCLC. Iwao et al[22] showed that lunx is highly expressed in NSCLC but not in normal LN. Another molecular high specifity marker for NSCLC is KS1/4, a gene that encodes a glycoprotein expressed on epithelial cells.[23] Because cytokeratin genes are normally expressed in epithelial but not lymphoid cells, the presence of cytokeratin mRNA in a LN suggests the presence of metastatic cells of epithelial origin. RT-PCR is a highly sensitive technique and is reported to be capable to detect one cancer cell per one milion normal cells.[24] Recently, RT-PCR for p53 and K-ras was shown to improve detection of occult LN metastasis.[25]

There are some controversial publications whether the examination of induced sputum is of use in detecting an early lung cancer. Thunnissen[26] in a review analysed the new technical aspects of conventional sputum cytology. Nuclear image analysis, the measurement of heterogeneous nuclear riboprotein A2/B1, p53, and DNA examination appear to be promising for the detection of early lung cancer, especially in a high-risk patients. K-ras analysis could be useful in detection of pulmonary adenocarcinoma. To date, microsattelite alteration have been detected also on specific cells scraped from a sputum sample, which confirms that chromosomal abnormalities (LOH) are present also in the cytologically abnormal sputum cells. However, this approach is not practical for screening tests.

In conclusion, cytology is of great diagnostic value, a reliable and relatively noninvasive method for patients. Eventhough, that cytology has sometimes less 'defined' diagnosis than histology, cytology specimens should be taken in cases where it is not possible to obtain samples for histology. Also, our results confirm that there is a good correlation between cytology and histology diagnoses, especially concerning the malignant lesions. In the prospect, there are also promising some molecular studies for an early detection, evaluation, and staging of lung cancer.

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