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PRACA PRZEGLĄDOWA

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Rapid pathologic and molecular diagnosis of lung cancer patients based on telepathology techniques

Szybka diagnostyka patomorfologiczna i molekularna chorych na raka płuca na podstawie techniki telepatologii

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ABSTRACT

Telepathology is an emerging form of diagnostic process introducing digitalization of slides prepared from formalin-fixed paraffin-embedded materials and stained cytological smears. The use of whole slide imaging (WSI) systems could accelerate and improve the diagnosis of malignant neoplasms without the need of on-site pathologist or transporting diagnostic material in-between different locations. The implementation of endobronchial ultrasound (EBUS) and endoscopic ultrasound (EUS) with fine needle aspiration (FNA) in diagnostic process of advanced lung cancer creates a new field for telepathology utilization. In selected patients, pathomorphological and genetic diagnosis may take less than a week and therapeutic decisions can be made in a short time. There are several important issues that concern the use of telepathology and WSI in everyday clinical environment. This short review presents the pros and cons of this technology and its applicability in rapid diagnosis of lung cancer, its utilization in connection with novel sampling methods and molecular analysis.

Key words: lung cancer, telepathology, molecular tests

STRESZCZENIE

Telepatologia to nowa forma procesu diagnostycznego wprowadzająca cyfryzację preparatów przygotowanych z bloczków parafinowych lub wybarwionych rozmazów cytologicznych. Wykorzystanie systemu skanowania całych preparatów może polepszyć i znacząco przyspieszyć diagnostykę nowotworów złośliwych bez wymaganej obecności patomorfologa w miejscu wykonywania diagnostyki oraz bez potrzeby transportowania pobranego materiału pomiędzy różnymi lokalizacjami. Wprowadzenie biopsji aspiracyjnej cienkoigłowej (FNA) przeprowadzanej pod kontrolą przezoskrzelowego lub przezprzełykowego USG (EBUS lub EUS) do procesu diagnostycznego zaawansowanego raka płuca tworzy nowe pole do zastosowania telepatologii. U wybranych chorych diagnoza patomorfologiczna i genetyczna może trwać poniżej tygodnia, a decyzje terapeutyczne mogą zostać podjęte w krótkim czasie. Istnieje kilka istotnych kwestii związanych z wykorzystaniem telepatologii oraz skanowania całych preparatów w codziennej praktyce klinicznej. Ten krótki przegląd ma na celu przybliżenie wad i zalet opisanej technologii i ich możliwości wykorzystania w szybkiej diagnostyce raka płuca w połączeniu z nowoczesnymi metodami pobierania materiału i koniecznością prowadzenia diagnostyki genetycznej.

Słowa kluczowe: rak płuca, telepatologia, testy molekularne

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Introduction

Telepathology is an emerging form of diagnostic process introducing digitalization of stained cytological smears and slides prepared from formalin-fixed paraffin-embedded (FFPE) tissues or cells (ethanol-fixed paraffin-embedded cells and fresh-frozen tissues are also acceptable) and its further analysis without the need of on-site pathologist or transporting diagnostic material in-between different locations. The use of novel techniques involving whole slide imaging (WSI) systems and remote robotic microscopy allows real-time consultation and diagnosis significantly accelerating further steps of treatment. It is especially important in case of areas without a skilled pathologist available, without a pathology laboratory or when a quick consult is needed. Such techniques might also be useful in education and archiving of interesting cases, as well as in quality control of pathological analysis [1].

Whole slide imaging is a computer-based technique involving scanning of glass slides and converting them into digital images that may be accessible through special software and viewed later from any location in the World as long as it has Internet access. The image viewing software allows for previewing, zooming in and out or panning the whole picture, frequently with possibility of its analysis, adding annotations and comments on the image [1, 2].

WSI is still an expensive method, as it is a novel and freshly implemented technology. One of the main drawbacks of WSI is the image size and analysis problems, as the scanned picture is created out of many high resolution and high magnification photos that create the whole slide picture. A common problem is also the speed of Internet connection and its capacity. Large size images need to be stored on servers and sent via fast streaming in order to maintain quality and speed of diagnosis. The other issue is the thickness of the slide which in case of cytology smears depends on skills of person performing the procedure and does not allow the analysis of dense clusters of cells, which may have the effect on diagnosis result. Highly specialized technicians should carefully prepare the slides (including material processing procedures and staining of cells or tissues). However, the analysis is a domain of a skilled pathologist. There are also no proper standards and validation options for this kind of technology and for different types of materials which makes it difficult to use in clinical setting. On the other hand scanning glass slides allows precious material to be prevented from transporting and risks of losing or destruction [2].

Apart of whole slide imaging there are other techniques used in telepathology such as static image analysis or remote live video microscopy which allows pathologists to view the glass slides from a remote location without the need of personal appearance in the place where the procedure is being performed. Of course, a trained technician is necessary to operate the on-site microscope. The advantage is the possibility of live focusing on different plains of the smear, which is not possible in case of WSI. Its limitations are mostly concerning the viewed image, as its choice in both methods depends on the person who operates the microscope and this persons' judgment, hence — training. In contrast to WSI the image sent from the microscope represents only a part of the whole slide and a proper selection is necessary in order to prevent misdiagnoses [1].

In lung cancer patients, pathological examination becomes very fast, which allows genetic diagnostics and rapid therapeutic decision. This is particularly important for patients with advanced lung cancer qualified for systemic therapy. The acceptable time from the start of the lung cancer diagnosis to start first-line treatment should not exceed one month. Ideally, the time to therapeutic decision should be less than two weeks. Trained technicians prepare hematoxylin and eosin (H+E) stained smears, scan the slides and share them with pathologists. In the absence of cancer cells in analyzed material, pathologist could immediately notify diagnosing physician. However, H+E stained slides are often sufficient to make a preliminary lung cancer diagnosis. Pathologist may order the use of histochemistry and immunohistochemistry (IHC) staining in case of diagnostic problems and in the event where quantity and quality of material archived in cell blocks is sufficient. In selected cases (non-squamous non-small cell lung cancer), in scanned H+E images, pathologist evaluates the percentage and localization of tumor cells and marks them for the purpose of macrodissection and molecular tests (*EGFR* gene mutations) [3].

Telepathology is also a way of archiving and creating large databases of scanned images for educational purposes and comparison analysis as the availability of normal and specific cases is usually a problem. Therefore, the scanned images could be consulted by several pathologists, including those who specialize in unusual or rare cases [4].

Telepathology and telecytology, when compared to rapid on-site evaluation (ROSE) procedure, seem to have similar diagnosis results and are believed to be cost effective methods. The number of repeated procedures and the cost of maintaining on-site pathologist are reduced, as well as minimizing the time needed for material transportation between the place of procedure and pathology lab. If the team performing the endoscopy/biopsy is trained in producing cytologic preparations it also reduces the time to preliminary diagnosis [5].

The use of WSI in Polish clinics is not common, as there are only two multicenter consortia that use this technology regularly. Although many hospitals and

universities already possess microscopic slide scanners, they are used for scientific purposes (computer image analysis, morphometry), didactic activities, archiving and consultation of rare or difficult cases. Pulmonary Hospital in Zakopane was the first center in Poland that utilized online diagnosis of digital microscopic slides in routine diagnostics. Since 2007, microscopic slides (initially only from intraoperative diagnoses, subsequently from fine-needle aspiration biopsies of tumors and metastatic lymph nodes) were examined by a team of five pathologists from Szczecin, Gliwice, Warszawa, Zabrze and Rzeszow. Similar system was implemented in Pulmonary Hospital in Bystra in 2010. Zakopane and Bystra centers utilized both scanners and dynamic microscopes. Same technology will soon also be implemented in Department of Pneumonology, Oncology and Allergology in Lublin in conjunction with above mentioned centers. The Gliwice Branch of the Maria Sklodowska-Curie Memorial Cancer Center and Institute of Oncology leads the organization of the biggest telepathology project, meant to connect several Silesian hospitals. Implementation of rapid diagnostic and treatment of malignant neoplasms enforces the use of telepathology techniques in Poland. It would allow for a diagnosis and treatment decision during one patients' stay in hospital [6].

Use of WSI in clinic

The WHO classification of lung tumors was created on the basis of histological analysis of resected tissues, however in case of non-small cell lung cancer the majority of diagnosed cases present late stages of the disease, hence there are very limited surgery options and not many possibilities of acquiring histologic material. Therefore the preliminary diagnosis is mostly given based on small biopsies and their picture, often accompanied by cytologic smears.

The current WHO classification of lung tumors puts a particular emphasis on the evaluation of small slices and cytology samples as a material which is the basis for lung cancer diagnosis. According to these new criteria, the diagnosis of adenocarcinoma (ADC) or squamous cell carcinoma (SCC) based on morphological criteria in such samples can be made only when glandular differentiation or intracellular bridges and keratinization are visible. All other cases require additional IHC staining. The WHO recommends using anti-TTF-1 antibodies to detect adenocarcinoma and anti-p40 for squamous cell carcinoma, however in ADC cases a co-expression of TTF-1 or napsin with squamous differentiation markers (p40, p63, CK5/6) is possible, whereas SCC cannot be described when the tumor cells present TTF-1 expression. Furthermore, a NSCLC NOS (non-otherwise specified) diagnosis should be presented when different cell populations presents both TTF-1 and p40 expression, with the annotation of adenosquamous carcinoma. It should also be noted that the IHC analysis in small biopsies only favors the diagnosis of the tumor type. The classification also contains recommendations to prepare 10 microscopic slides to avoid loss of material during subsequent treatments of paraffin blocks in the microtome. These preparations may be used for standard pathological, IHC or molecular (also fluorescent in-situ hybridization — FISH) analysis. The panel of molecular tests included in the classification is very broad and goes beyond routinely used in Poland *EGFR* mutation testing. It is stressed that a confrontation of pathological and molecular diagnosis with radiologic image is crucial. There are also new recommendations for the pathological diagnosis of surgically resected lung cancer samples. In case of ADC the classification includes: preinvasive lesions (atypical adenomatous hyperplasia (AAH) and adenocarcinoma in-situ (AIS) (≤ 3 cm); minimally invasive ADC (MIA) (≤ 3 cm with ≤ 5 mm invasion) and invasive ADC (G1: lepidic, G2: acinar, papillary and G3: micropapillary, solid). The classification limits the large cell carcinoma (LCC) diagnosis only to the cases when the morphological image is concomitant with negative mucin, TTF-1 and p40 staining. Some rare types of lung cancer are reclassified as ADC or SCC based on their IHC staining status. Among SCCs a basaloid carcinoma is introduced, which apart of characteristic morphological image and lack of keratinization, presents positive staining for anti-p40 antibodies [7].

The implementation of endobronchial ultrasound (EBUS) and endoscopic ultrasound (EUS) with fine needle aspiration (FNA) in rapid diagnostic process of neoplasms creates a new field for telepathology utilization. The ideal steps of the process would be (Figure 1):

1. Acquisition of sample material from EBUS/EUS-FNA.
2. Simultaneous, collection of cells to perform cells blocks (alcohol or formalin-fixed paraffin-embedded) and creating glass smears.
3. Archiving cell blocks for further histochemistry and IHC examination as well as molecular tests (*ALK* gene rearrangement).
4. H+E staining of glass smears.
5. Scanning of the H+E stained smears and its analysis by a pathologist in remote location.
6. Preliminary diagnosis by the pathologist that would allow (if sufficient data is available) further diagnostic steps or beginning of treatment to be taken almost instantly after the first diagnostic procedure.
7. In the case of diagnosis of non-squamous non-small cell lung cancer, evaluation of tumor cell percentage and localization (virtual marking) on H+E stained and scanned smears by pathologist.

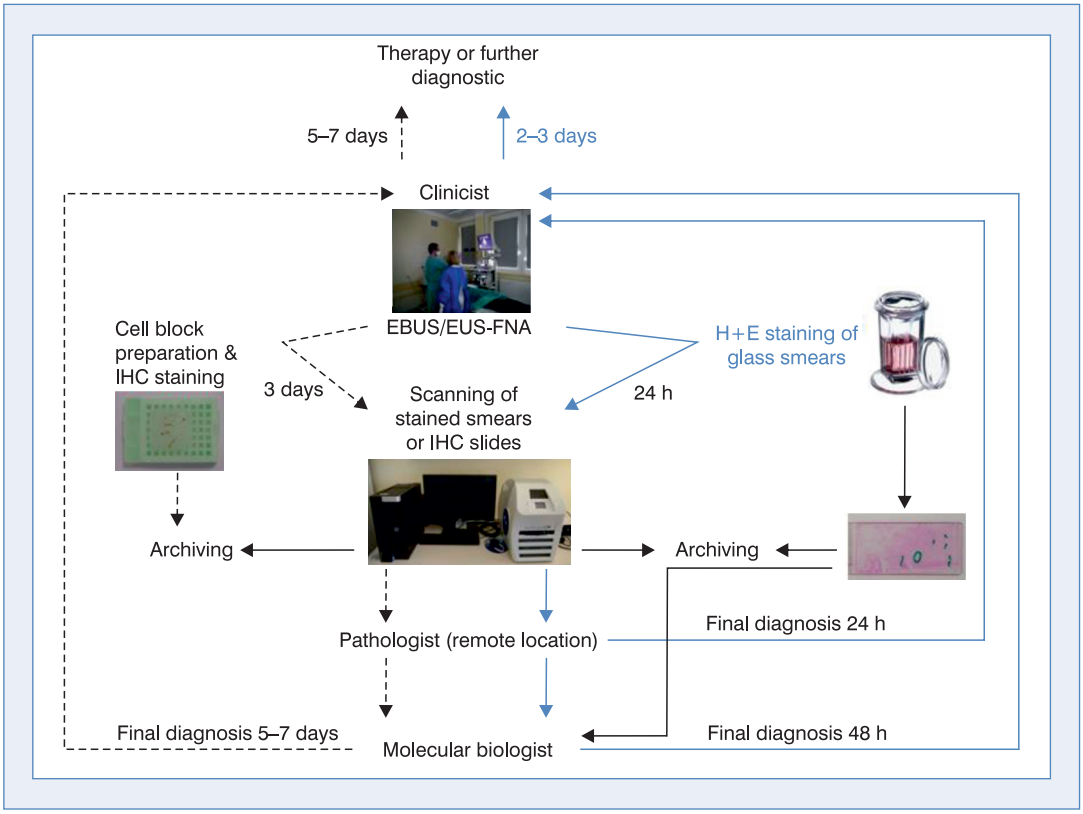

Figure 1. Algorithm for the diagnosis of lung cancer using EBUS/EUS FNA and telepathology procedures as well as genetic tests

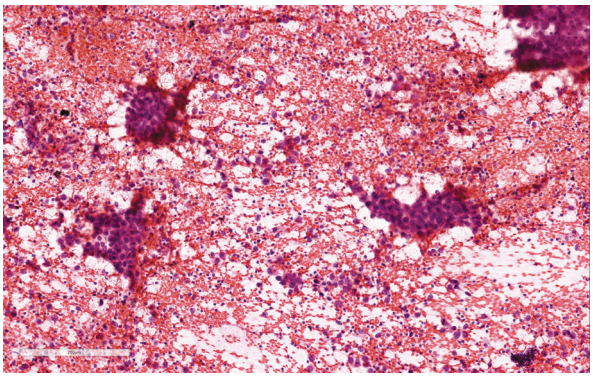

Figure 2. Properly made cytology smear from EBUS FNA material

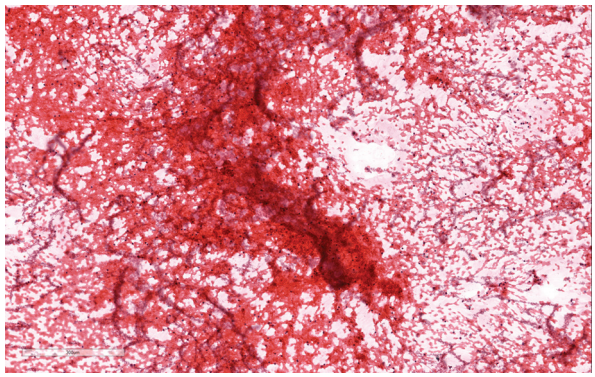

Figure 3. Incorrectly made cytology smear from EBUS FNA material

8. Transferring H+E stained slides to genetic laboratory.
9. Tumor cells macrodissection based on scanned images.
10. DNA isolation from tumor-cell-enriched material.
11. *EGFR* gene mutations testing that would allow (if sufficient data is available) for further diagnostic or beginning of treatment steps to be taken 3–4 working days after the first diagnostic procedure.
12. Further examination of cell blocks in case of diagnostic problems and necessity of further testing (including *EGFR* gene mutations and *ALK* gene rearrangement testing or testing for qualification to novel therapies in clinical trials).

The main advantage of this kind of rapid diagnostics is short time period between each decision being made and in the end a lot quicker treatment qualification of the patient. However it is important to note that in order

to this kind of diagnostic path to work properly a valid and coherent system of communication needs to be implemented between the technician, the pathologist, the surgeon/clinician/oncologist and molecular biologist. Obtaining high quality cytology smears depends on the skills of highly trained bronchoscopist (Figure 2 and 3). The samples gathered during EBUS/EUS-FNA procedure also need to be fully pathologically analyzed after the procedure and the results need to be compared with the preliminary remote pathological diagnosis.

A very important issue is the amount of diagnostic material that is being obtained from the biopsy, as it has to be divided between few diagnostic stages — first of all the pathologic diagnosis, and later on the molecular analysis. Current knowledge and availability of molecularly targeted therapies make it an important concern in case of NSCLC patients. The amount of cancer cells in the obtained material is crucial for molecular studies and the accuracy of diagnosis, which is why pathologists should pay attention to the amount of used material. Glass smears that are used to present a preliminary diagnosis may be the only source of cancer cells that are available for molecular analysis; hence the process of their digitalization is very important.

Legal issues of telepathology

Telepathology, mainly concerning whole slide imaging, is more and more popular around the World with specific guidelines being created in order to carry viable diagnostic, consultation and education processes. The American Telemedicine Association (ATA) created such guidelines concerning technical, staff and Food and Drug Administration (FDA) issues. Similarly Canadian Association of Pathologists and British Royal College of Pathologists created such sets of rules and suggestions. These guidelines however do not consist of specific recommendations in methodology, as there are no validation data on the available equipment and no standards or best practices for specific equipment manufacturers. Each of the presented guidelines suggests validation of the procedure inside the performing institution [8–10].

In Polish conditions a recently presented plan of rapid oncological diagnosis creates a niche where telepathology fits perfectly. In order to give a quick and relevant diagnosis the presented techniques should be used on daily basis quickly connecting pathologists with molecular biologists and clinicians performing diagnostic and therapeutic procedures. In large hospitals, where pathology labs are swarmed with samples from different clinics and of different origin, it is difficult to obtain quick answer to questions that arise during the surgical or diagnostic procedures that would allow easier and faster qualification of the patient for specific treatment, which in case of not only lung cancer but also other neoplasms is crucial.

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