THE FEASIBILITY OF IMPRINT CYTOLOGY FOR ACCELERATING CANCER DIAGNOSIS

Ocena możliwości wykorzystania cytologii odbitkowej w szybkiej diagnostyce nowotworu złośliwego

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Abstract

Introduction and objective: Pathomorphological examination is one of the main pillars of cancer diagnosis, and the information obtained is important for making treatment decisions. Imprint cytology is a type of pathomorphological examination, where cells are obtained by moving a glass slide over a fresh cross-section of the tissue being examined, such as a cross-section of a tumour or a lymph node. The main objective of the paper was to investigate whether imprint cytology can be an alternative to intraoperative frozen section analysis. **Materials and methods:** The results of imprint cytology and intraoperative frozen sections performed in the Department of Thoracic Surgery General and Oncological Surgery at the Military Medical Academy Hospital in Łódź between 2020 and 2023 were analysed and compared with the final histopathological examination. A group of 58 patients undergoing elective surgery for malignant tumours of the lung, mediastinum, colon, stomach and gallbladder was included in the study. **Results:** Both frozen section analysis and imprint cytology showed 97% concordance with final histopathology. Imprint cytology yielded a false-negative result in two cases, with a malignant neoplasm confirmed in frozen section and the final histopathological examination. In two cases, a small-cell malignant neoplasm was diagnosed with imprint cytology, which was later confirmed in the final examination, while frozen section yielded a result inconsistent with the routine examination. **Conclusions:** Imprint cytology is a reliable method that can be used to accelerate the diagnosis of cancer. When intraoperative examination is not possible, imprint cytology allows for obtaining a rapid diagnosis, while at the same time the specimen taken does not have to be immediately transported to the pathomorphology department.

Streszczenie

Wprowadzenie i cel: Badanie patomorfologiczne stanowi jeden z głównych filarów diagnostyki w onkologii, a informacje uzyskane dzięki niemu mają istotne znaczenie w podejmowaniu decyzji leczniczych. Jednym z jego rodzajów jest cytologia odbitkowa, w której komórki są pozyskiwane poprzez przesuwanie szkiełka podstawowego po świeżo wykonanym przekroju badanej tkanki, np. przekroju guza lub węzła chłonnego. Głównym celem pracy było zbadanie, czy cytologia odbitkowa może być alternatywą dla badania śródoperacyjnego przeprowadzonego techniką mrożakową. **Materiał i metody:** W Klinice Chirurgii Klatki Piersiowej Chirurgii Ogólnej i Onkologicznej Szpitala im. Wojskowej Akademii Medycznej w Łodzi w latach 2020–2023 przeprowadzono analizę wyników badania cytologii odbitkowej oraz badania śródoperacyjnego wykonanego techniką mrożakową i porównano je z ostatecznym wynikiem badania histopatologicznego. Badanie przeprowadzono w grupie 58 pacjentów operowanych w trybie planowym, u których wykonano resekcję guza nowotworowego jelita grubego, pęcherzyka żółciowego, żołądka, płuca oraz śródpiersia. **Wyniki:** Zarówno badanie doraźne, wykonane techniką mrożakową, jak i odbitka cytologiczna okazały się w 97% zgodne z wynikiem ostatecznym badania histopatologicznego. W badaniu cytologii odbitkowej w dwóch przypadkach uzyskano wynik fałszywie ujemny, natomiast w badaniu doraźnym i ostatecznym badaniu histopatologicznym potwierdzono nowotwór złośliwy. W dwóch przypadkach w cytologii odbitkowej zdiagnozowano nowotwór złośliwy drobnokomórkowy, co później potwierdzono w badaniu ostatecznym, natomiast w badaniu doraźnym uzyskano wynik niezgodny z badaniem rutynowym. **Wnioski:** Cytologia odbitkowa jest wiarygodnym badaniem, które może być wykonywane w celu przyspieszenia rozpoznania choroby nowotworowej. W przypadku braku możliwości wykonania badania śródoperacyjnego cytologia odbitkowa daje możliwość szybkiego uzyskania rozpoznania, a jednocześnie pobrany preparat nie musi być natychmiast przetransportowany do zakładu patomorfologii.

Keywords: histological examination; intraoperative examination; imprint cytology

Słowa kluczowe: badanie histopatologiczne; badanie śródoperacyjne; cytologia odbitkowa

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Introduction

Pathological examination is one of the main pillars of cancer diagnosis. It delivers information of significant importance in making treatment decisions. Since histopathology, which is the basis for the diagnosis, is time-consuming, other methods, such as a frozen section analysis (FSA) and cytology, are utilised. Cytology, also known as a cytopathology, is a diagnostic method based on the assessment of cells under a light microscope. Body secretions, fluids from body cavities (exfoliative cytology), or cells retrieved by fine-needle aspiration may be used [1].

Exfoliative cytology involves microscopic evaluation of cells that have spontaneously shed from the surface of an organ or tissue, or have been collected as a smear. The harvested specimen may include, for example, sputum, bronchial secretions or washings, fluid from body cavities, cystic contents, or a cervical smear. The fluid should be placed in a clean container with a few drops of heparin and immediately sent unfixed to the pathology laboratory or facility. To perform a smear, specimens are harvested from the surface of a lesion or mucous membrane, and then applied directly to a microscope slide.

Fine needle aspiration biopsy (FNA) is a way to obtain samples for microscopic examination from sites suspected of pathology by puncture and aspiration of cells. Endobronchial ultrasound (EBUS) and endoscopic ultrasound (EUS) are special types of FNA. The obtained material is immediately spread on glass slides and fixed (cytology smears), or part or whole of the aspirated material is immediately fixed in a fixative, as in the case of tissue samples (cell blocks) [2]. A cell block is prepared by fixing the aspirated cells and embedding them in a paraffin block. Cell blocks allow for obtaining microscopic sections and thus for a greater number of additional tests necessary to reach the diagnosis and determine the predictive factors necessary for choosing the therapy. Crush preparations, where the sample is ground or crushed directly on a glass slide, can also be used. Imprint cytology (IC), in which cells are obtained by moving a glass slide over a fresh cross-section of the investigated tissue, e.g. a cross-section of a tumour or a lymph node, is another increasingly popular method. A similar specimen can be obtained by crushing a fragment of tissue on a glass slide [1, 3]. Further management depends on the type of material collected and the method of its preservation. Both fixed or unfixed specimens can be sent to the cytology laboratory. In the case of a specimen intended for a cell block, it is fixed in 10% buffered formalin with a pH of 7.2–7.4 or in 96% alcohol (50–70% in some cases). Crush and imprint specimens are fixed in alcohol [3].

Intraoperatively, the so-called frozen section analysis is usually performed. Intraoperatively collected, unfixed specimens are transferred to the pathology laboratory and assessed by freezing the specimens at low temperature, followed by their sectioning in a cryostat and staining with haematoxylin and eosin [3].

Objective

The main aim of the study was to assess whether imprint cytology may be an alternative to intraoperative FSA, which is considered the gold standard.

Materials and methods

We analysed the results of IC and intraoperative FSA and compared them with final histopathology. The study included 58 patients treated at the Department of Thoracic, General and Oncological Surgery, University Clinical Hospital of the Military Medical Academy between 2020 and 2023, who underwent elective surgeries for lung, mediastinal, colon, gastric or gallbladder cancer. Each patient underwent tumour resection, with cytological imprints taken, an intraoperative frozen section and a routine histopathology. The specimens harvested for intraoperative frozen section were not fixed in any way. The specimens collected for cytology were obtained by moving a glass slide over a fresh cross-section of the investigated tissue and fixed in alcohol. Regardless of the positive qualification, participation was voluntary. Each participant could refuse or withdraw consent to participate in the study at any time, without giving a reason and without any consequences, while maintaining the right to treatment in the same Department.

Results

The study included 58 patients treated surgically for cancers (Fig. 1). Malignancy was confirmed in 54 patients, benign lesions were diagnosed in 4 patients, as confirmed with all three methods (IC, intraoperative FSA and routine histopathology).

IC confirmed atypical or malignant cells in 52 cases and yielded a false negative cytological diagnosis in 2 cases, with malignancy confirmed in FSA and final histopathology. False negative results were obtained for lung tumours.

These were squamous cell lung cancer in one case, and a metastatic melanoma to the lung in the other case.

In two cases, IC diagnosed a small cell lung cancer, which was later confirmed in the final histopathology, while FSA suggested non-small cell lung cancer and anaplastic carcinoma.

Intraoperative FSA correctly diagnosed non-small cell carcinoma in 44 cases, whereas a more detailed diagnosis of squamous cell carcinoma and adenocarcinoma was obtained in 8 cases, whish was also consistent with histopathology (Fg. 2).

Both intraoperative FSA section and IC showed an efficiency of 97%.

Discussion

The advances in oncology have given rise to challenges for surgeons and pathologists, which aim at rapid, minimally invasive and accurate histopathological diagnosis allowing for immediate anticancer treatment. Several studies may be found in the literature that have confirmed the usefulness and effectiveness of cytology as an intraoperative diagnostic tool. According to these studies, the advantage of cytology is that it is much less time-consuming, easy to use and, apart from a microscope, does not require additional specialist equipment.

Compared to histopathology, cytology is less invasive and repeatable. Lower material costs and shorter duration of the test are also important. Inability to assess tissue topography, which means that cytopathological findings should be confirmed in doubtful cases by histopathology, which allows for assessing a greater number of microscopic image details, is a limitation [4].

Esbona et al. reviewed the literature on the assessment of excised tissue margins in patients who underwent breast-conserving treatment. Two intraoperative methods for margin assessment were used: FSA and IC. Although FSA was performed most frequently, it was shown to be associated with artifacts in the fatty tissue arising from the freezing and thawing process, which led to tissue loss. For this reason, IC has been proposed as an alternative. This technique for assessing margins in breast cancer patients undergoing breast-conserving surgery has been found to be sufficiently rapid and reliable to be utilised as an intraoperative aid. IC can effectively reduce the need for additional surgeries to achieve negative margins in these patient populations. Intraoperative IC took less than 15 minutes compared to 30 minutes for intraoperative FSA [5].

Ahuja et al. conducted a meta-analysis of studies on the diagnosis of lymph node metastases in breast cancer. Although the sensitivity of FSA was higher than that of IC in detecting micrometastasis, IC was found to be a rapid, inexpensive technique that can be used, for example, in the absence of a cryostat. The sensitivity of both techniques for detecting metastasis was comparable, making IC a useful tool for the rapid diagnosis of lymph node involvement. This meta-analysis highlighted the accuracy of IC and FSA in the diagnosis of lymph node metastases of breast cancer [6].

Jaswal et al. assessed 160 intraoperative imprints from 52 patients. These were patients with various cancer locations, mostly with head and neck tumours. The authors

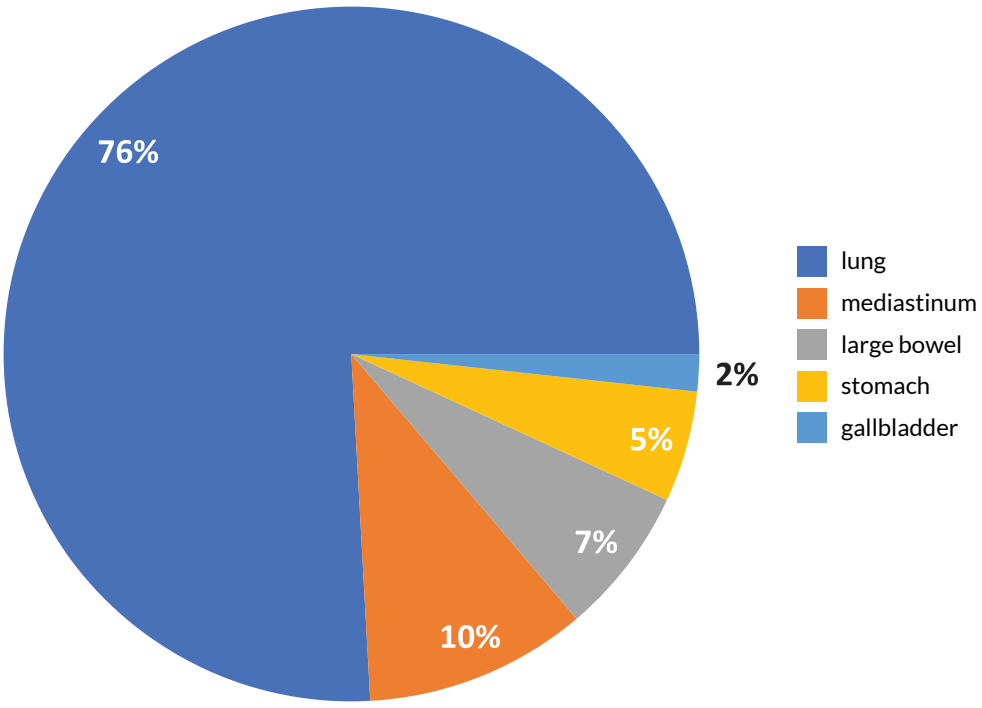

Figure 1. Tumour site

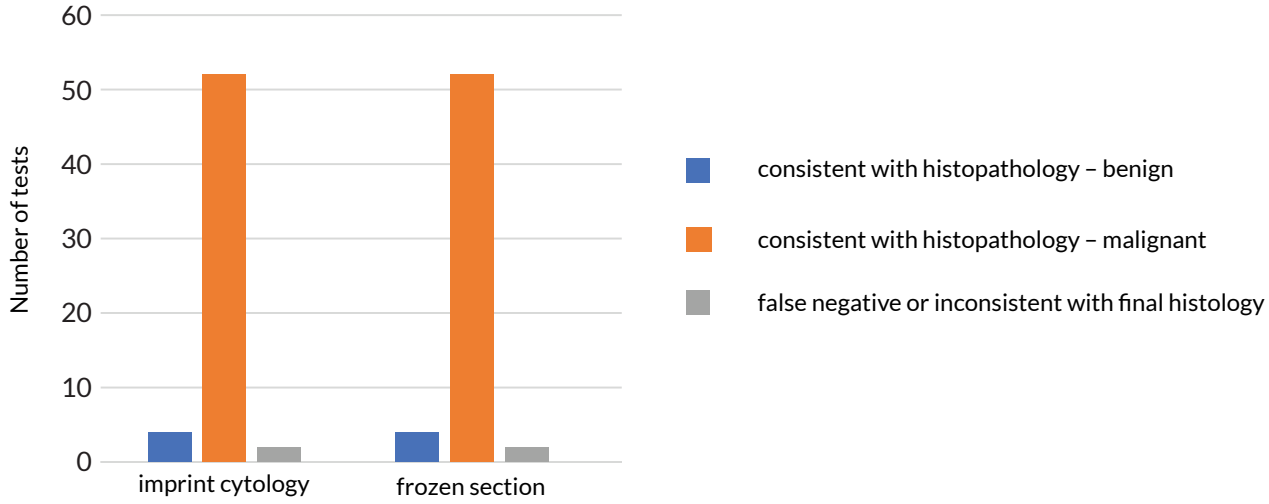

Figure 2. Concordance between imprint cytology and frozen section and the final histopathology

considered IC to be a rapid, inexpensive, and reliable diagnostic tool. The simplicity, speed, and cost-effectiveness of this technique, along with its ability to maximise cell recovery from very small tissue fragments, make IC a valuable tool. The limitations of intraoperative cytology are the same as those of cytology in general. These include sampling errors, inability to distinguish *in situ* malignancy from invasive lesions, inability to assess the depth of invasion in detail, and limited detection of micrometastasis [7].

Shubha et al. included 50 patients operated on for cancers of various locations in their study. The overall diagnostic accuracy was 94% for IC in various organs, and 98% for intraoperative FSA. The total diagnostic accuracy was 98%. The false negative and false positive rates for IC were 4% and 2%, respectively. The false negative rate for FSA was 2%. Of the three discordant IC cases, two were due to misinterpretation and one was due to an error during sample collection. The diagnostic accuracy of IC and FSA for malignancies was 96% and 98%, respectively [8].

Pallialil et al. assessed IC and frozen sections. A total of 157 tissue imprints were collected for the study and divided into specimens based on tumour type, tumour margin, and lymph node involvement. The overall diagnostic accuracy for tumour type detection was 97.9% for IC and 98.6% for FSA. The authors concluded that IC has many advantages, is technically simple, quick, and has a low learning curve. However, it also has some disadvantages, such as the inability to distinguish *in situ* carcinoma from invasive disease and to obtain information on the depth of invasion [9].

Biancosino et al. assessed the value of intraoperative IC. To this end, a total of 532 intraoperatively harvested specimens out of the 518 resected thoracic tumours from 360 patients were examined. The specimens were assessed using intraoperative IC, which was later compared with the final histology. The sensitivity and specificity of IC were 82% and 99%, respectively. The authors concluded that intraoperative IC is a rapid, accurate, and sensitive method allowing for intraoperative decision-making and is clearly a helpful alternative or adjunct for the thoracic surgeon, provided that they appreciate the potential limitations of this technique [10].

It seems obvious that neither an intraoperative frozen section nor imprint cytology will provide a precise diagnosis, but determining whether the lesion is benign or malignant and whether the tumour is small-cell or non-small-cell makes it much easier for the surgeon to make a decision on further treatment.

The importance of IC increases when a pathologist is not available at the time of the procedure. Since the collected specimen does not need to be immediately transported to the pathology department, the material can be collected in the afternoon, at night and on holidays, when ad hoc tests are typically unavailable. The importance of rapid diagnosis is of great importance in the era of fast-track oncology pathway. By obtaining a preliminary diagnosis immediately after the procedure, the patient can benefit from accelerated diagnostic process and prepare for further anticancer treatment.

Conclusions

- The analysis showed that imprint cytology is a reliable tool that can be used to accelerate the diagnosis of neoplastic diseases and can be an alternative to intraoperative frozen section analysis.
- Combining imprint cytology with frozen section helps achieve high diagnostic accuracy.
- If intraoperative examination cannot be performed, imprint cytology allows for rapid diagnosis, without the need to immediately transport the collected specimen to the pathology department.

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