

## Tissue prints for the rapid diagnosis of malignancy in lung cancer

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### Abstract

Rapid diagnosis of malignancy during oncological surgery is crucial for making decisions related to the extension of the resection. The tissue prints, used initially for plant biology but also for prostate or breast cancer diagnosis, might be useful as a rapid cytological diagnosis. *Materials and Methods:* Tissue prints were done from freshly sectioned excised tissue fragments in patients operated between March 2010 and February 2012 in the Department of Surgery for cancer or benign lesions. Tissue prints were examined by a cytologist and considered as malignant or benign. Same fragments were then processed in the pathology laboratory using the typical paraffin-embedding method. All slides were examined by the same pathologist and considered the golden standard for malignancy and histological type. *Results:* Three hundred and eleven fragments were examined, obtained from lung masses, lymph nodes, pleura and mediastinal masses, pathology showed 208 malignant and 103 benign. Tissue prints identified 227 malignant and 84 benign. For identifying malignancy, tissue prints had a sensibility of 0.91, specificity 0.64. Positive predictive value was 0.86 and negative predictive value 0.78. For lymph nodes, the specificity was better. In lymphomas and adenocarcinomas, tissue prints identified also the histology type in most cases. *Conclusions:* Tissue prints are rapid, easy to perform, cheap, with high sensibility but specificity lower than literature data on frozen sections. This might be improved by a better selection of cases where tissue prints are used for rapid diagnosis.

**Keywords:** tissue prints, lung cancer, rapid diagnosis.

### Introduction

The technique of "tissue printing" was described by Varner & Ye, in 1994, in *FASEB Journal*, in a series of articles focusing on plants infrastructure [1]. The tissue prints were initially used for plants studies, for better describing the structure of their extracellular matrix, the presence of various proteins or enzymes or identifying plants pathogens [2–4]. This technique was used also in several trials for specific diagnosis techniques of cancer in breast [5] or prostate [6]. These trials were not aiming to the simple cytological description of tumors or lymph nodes, but were more prone to immunocytochemical diagnosis or to identify specific proteins or biomarkers in the tissue prints. The technique involved using a nitro-cellulose membrane for printing, and not a glass slide. Currently, tissue printing is not used as a routine clinical tool, still needing trials to prove the benefit of this technique as a cytology diagnostic tool.

During oncological surgery, the surgeon needs to decide the extension of the resection, and on-site information about the involvement of regional lymph nodes or true extension of the lesion is crucial for these decisions. The cryosections are typically used to certify the malignancy of the operated tumor, to verify if the margins of the resected area are free of cancer cells, and to identify the lymph nodes involved (N staging and sentinel node

procedure). This technique is rapid (about 10 minutes), but the quality of the sections is much lower than the traditional histology technique with paraffin embedding. Cryosections have some limitations, besides the poor quality of the sections, frozen artifacts might be misinterpreted (ice crystals, frozen nuclear chromatin changes), special stains cannot be used to differentiate subtypes of tumors mimicking other lesions, and last but not least, the skills of the technician for obtaining good slides for an accurate diagnosis is very important. Obtaining quick answers, in real time, regarding the extension of a tumor or involvement of lymph nodes, is crucial for making decisions in the operating room, therefore the responsibility of the person performing such a rapid technique is very high [7].

Tissue prints can also be prepared and interpreted in a short time. If tissue prints prove to be confident in identifying malignancy in a tissue, as compared to the paraffin-embedding technique, they might be an alternative to cryosections.

This paper aims to compare the malignancy and histology type of lung tumors as described by the cytology of a tissue print, to the classical pathology examination of tissue slides, considered as the golden standard, in tissue fragments from patients operated in a Department of Thoracic Surgery.

## ☐ Materials and Methods

In patients who underwent surgery for lung cancer or other indications in the Department of Thoracic Surgery of “Marius Nasta” Institute of Pneumophthisiology, Bucharest, Romania, between March 2010 and February 2012, tissue prints of freshly sectioned fragments of tumor or lymph nodes were examined by the same cytologist in all cases. Same fragments were then processed by the classical technique of paraffin embedding and examined by a single pathologist in the Laboratory of Pathology of the Institute. The results of the cytology of tissue prints were compared to the classical histology examination, considered the golden standard.

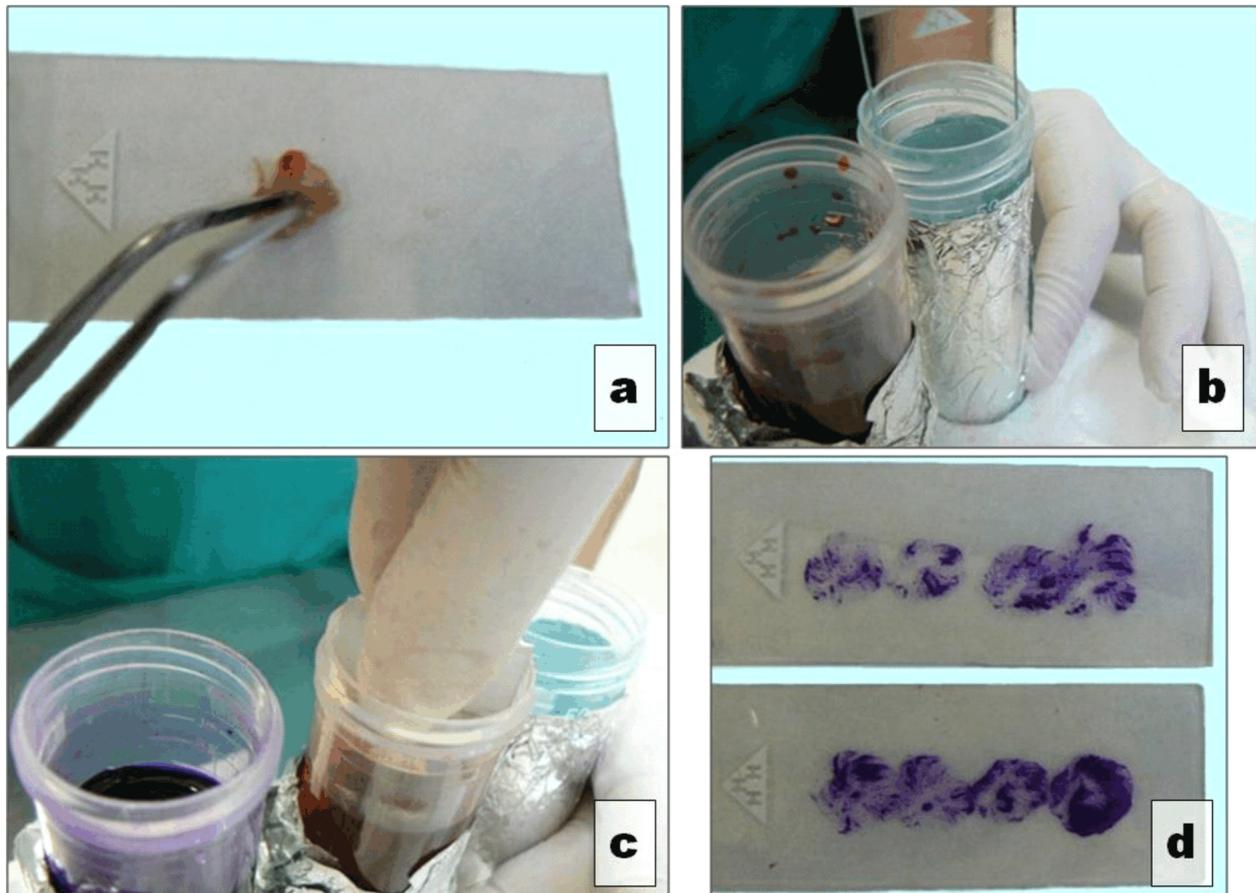
We included fragments obtained from patients who underwent open lung surgery, thoracoscopy or mediastinoscopy. Tissue fragments from lung nodules or masses, lymph nodes, mediastinal masses and pleura were examined.

Patients were operated for mediastinal or hilar lymph node enlargement, for lung nodules, suspected lung meta-

stasis from a remote primary tumor, mediastinal tumors, chest wall tumors, pleural effusions. In most patients with cancer, the diagnosis was already known before surgery, confirmed by other methods: bronchoscopy with bronchial biopsy, computed tomography (CT) scan. Also, patients with suspected benign lesions (*i.e.*, sarcoidosis) were included, in order to build up a large group of benign fragments to allow us to assess the capability of tissue prints to identify benign lesions. In some cases, the diagnosis was unknown before surgery, this being performed for diagnostic purposes. The results of tissue printing included in this study were not used to make decisions during surgery, being used only for research.

### Tissue printing technique

The tissue fragments were sectioned immediately after surgical excision. The sectioned surface was gently pressed over a glass slide (Figure 1a). The slide was fixed by immersion in 95% alcohol (Figure 1b) and then stained with Hematoxylin–Eosin (HE) (rapid staining) (Figure 1c).



**Figure 1** – (a) Printing a freshly sectioned tissue fragment on a glass slide; (b) Fixation in 95% alcohol; (c) Rapid staining with Hematoxylin–Eosin; (d) Stained tissue prints slides.

The stained slides (Figure 1d) were examined by the cytologist in light microscopy using a 40× objective. The total duration of the procedure is three to six minutes. The results were expressed as benign or malignant.

When possible, also a histological description was made for benign slides [tuberculosis (TB), sarcoidosis] and for malignant slides (adenocarcinoma, squamous cell carcinoma, or simply non-small cell carcinoma, small cell carcinoma, lymphoma).

The classical histology technique was performed in the Laboratory of Pathology of the Hospital, using the typical method of fixation, embedding in paraffin, slicing with a microtome, staining with HE. The slides were examined in light microscopy with successive objectives (10×, 20×, 40×), all by the same pathologist. The results were expressed as benign or malignant, as well as by a complete histological description of the examined tissue, depicting the histological type of carcinoma or of benign lesions.

The database included the site of the excision (lymph node, lung nodule or mass, mediastinal mass, pleura), the tissue print result and the pathology result. Tissue print examination was compared to the pathology results in order to assess the sensibility, specificity, positive predictive value and negative predictive value of tissue prints examination.

## Results

The study included 311 tissue fragments, obtained by surgery from 290 patients operated between March 2010 and February 2012. In 23 patients, two or three fragments were obtained from different sites (e.g., lung and lymph node).

From the 311 tissue fragments, 143 were lymph nodes, 116 lung nodules or lung tumor fragments, 36 were pleural

fragments, and 16 fragments of mediastinal masses.

Classical histology examination showed 208 malignant lesions and 103 benign. The tissue prints showed 227 malignant and 84 benign.

### Malignant fragments

From the 208 malignant fragments, 93 were obtained from lymph nodes, 78 were lung nodules or tumors, 26 were pleural fragments, and 11 were mediastinal masses.

Among the 208 fragments proven malignant by pathology, the tissue prints showed: 190 (91.34%) malignant and 18 (8.65%) benign, which are false negative tissue prints.

Table 1 depicts the distribution of malignant fragments according to the site of excision, as well as the presence of malignancy as judged by tissue prints for each site.

**Table 1 – Distribution of malignant fragments according to excision site and the corresponding tissue print result**

Tissue type	Malignant histology (208 fragments)				
	Lymph node (93)	Lung nodule (78)	Pleura (26)	Mediastinal mass	
Tissue print					
	<i>Malignant</i>	80 (86.02%)	73 (93.58%)	26 (100%)	11 (100%)
	<i>Benign</i>	13 (13.97%)	5 (6.41%)	–	–

Tissue prints seem to be most accurate for pleural and mediastinal fragments, with a 100% match, and for lung tissue, with a 93.58% match, while the prints from lymph nodes show 13.97% false negative results.

Histological types defined by classical pathology examination in the 208 malignant fragments showed adenocarcinoma in 98 fragments, squamous cell carcinoma in 32, small cell carcinoma in 15, large cell carcinoma in two cases, Hodgkin lymphoma in 10 fragments, eight non-Hodgkin lymphoma, and six mesothelioma. Also, other rare histological types were identified, one case

each: anaplastic carcinoma, adeno-squamous carcinoma, mucoepidermoid, undifferentiated, two fragments of atypical carcinoid, two Askin's tumors. Other less defined malignancies were encountered in 29 fragments. These were confirmed as malignant but needed extra-immunohistochemical tests to define a specific histological diagnosis.

As tissue printing not only judged the slides as malignant or benign, but also described when possible a histological type, we checked the correspondence between these results and the actual histological type (Table 2).

**Table 2 – Histological correspondence between tissue print and histology slide (malignant fragments)**

Investigation	Histological type						
	ADK (98)	SCC (32)	SCLC (15)	LCLC (2)	Hodgkin (10)	Non-Hodgkin (8)	Mesothelioma (6)
<i>Pathology examination</i>	ADK (26)	ADK (6)	SCLC (7)	ADK (2)	Hodgkin (7)	Lymphoma (4)	ADK/NSCLC (4)
<i>Tissue print examination</i>	ADK/NSCLC (53)	ADK/NSCLC (15)	NSCLC (5)	–	Lymphoma (3)	Hodgkin (1)	Mesothelioma (1)
	NSCLC (4)	SQ/NSCLC (3)	–	–	–	NSCLC (1)	–
	Other malignancies (9)	NSCLC (6)	–	–	–	–	–
	Benign (6)	Benign (2)	Benign (3)	–	–	Benign (2)	Benign (1)

ADK: Adenocarcinoma; SCC: Squamous cell carcinoma; SCLC: Small cell lung carcinoma; LCLC: Large cell lung carcinoma; NSCLC: Non-small cell lung carcinoma; SQ: squamous carcinoma. Values are expressed as number of cases.

For adenocarcinomas (ADK), tissue prints identified the histological type certainly in 26 (26.53%) cases and suggested ADK or non-small cell carcinoma (NSCLC) in other 53 (54.08%) cases. For squamous cell carcinoma, there were far fewer matches, most of the prints suggesting just the NSCLC. Small cell carcinoma was recognized on prints only in half of the cases, the others being considered NSCLC or benign. A better match is seen in lymphomas, with 15 confirmations of lymphoma from the 18 cases.

Figures 2 and 3 show tissue prints (a) compared to paraffin slides (b) for a case of Hodgkin's lymphoma and a case of adenocarcinoma, respectively.

### Benign fragments

The 103 benign fragments were obtained from: lymph

nodes (50), lung nodules or masses (38), pleura (10) and mediastinal masses (5).

Among the fragments proven benign by classical pathological examination, the tissue printing showed: only 66 (64.07%) benign and 37 (35.92%) malignant, which are false positive.

The distribution of benign fragments according to the site of excision and to the corresponding tissue printing is summarized in Table 3.

One can note that, while for the lymph nodes the tissue print results correspond in 76% of cases with the pathology, the tissue prints from lung nodules show malignancy in only 57.89% of cases, while most pleural fragments are misjudged as malignant.

The pathological description of the benign fragments

was: sarcoidosis in 22 fragments, tuberculosis in 14, reactive lymph nodes in 13, hamartoma in three, typical carcinoid in two, one fragment each: eosinophilic pneumonia, bronchiolitis obliterans organizing pneumonia (BOOP), fibroma, teratoma, thymoma, while 43 fragments were described just as benign.

Among the 22 sarcoidosis fragments, tissue prints described: 11 as sarcoidosis, 19 as benign and three as malignant. The tuberculosis fragments were described by tissue prints as: one tuberculosis, 10 benign, and four malignant.

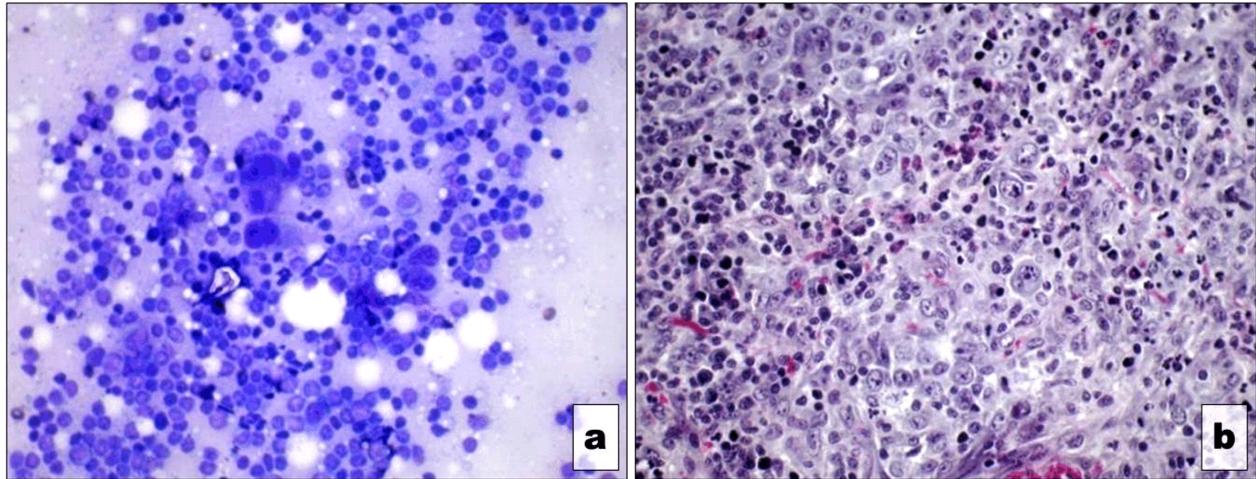


Figure 2 – Distribution of malignant fragments according to excision site and the corresponding tissue print result.

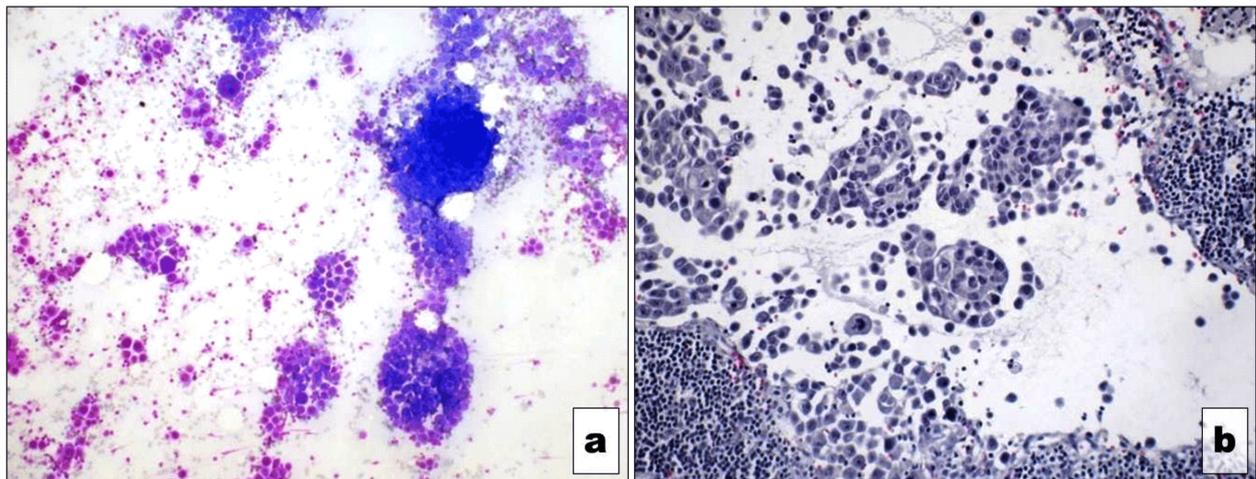


Figure 3 – Histological correspondence between tissue print and histology slide (malignant fragments).

Table 3 – Distribution of benign fragments according to the excision site and the corresponding tissue print result

Tissue type		Lymph node (50)	Lung nodule (38)	Pleura (10)	Mediastinal mass (5)	Total (103)
Tissue print	Benign	38 (76%)	22 (57.89%)	3	3	66
	Malignant	12 (24%)	16 (42.11%)	7	2	37

**Sensibility and specificity**

Table 4 allows us to calculate the sensibility, specificity, positive and negative predictive values of tissue prints for identifying malignancy in the examined fragments.

Table 4 – Sensibility, specificity, positive predictive value and negative predictive value of tissue prints

Tissue type	Malignancy (pathological confirmation)		Total
	Positive	Negative	
Tissue prints	Positive	190	227
	Negative	18	84
	Total	208	311

There are 18 (8.65%) false negative prints (prints that fail to diagnose malignancy) and 37 (35.92%) false positive

prints (prints that suggest cancer when there is none). The sensibility of the method, the capability of the tissue prints to confirm a positive diagnosis, is 190/208: 0.91, and the specificity, the capability of a negative test to exclude the disease, is 66/103: 0.64.

Positive predictive value (the proportion of positive test results that are true positives, correct diagnosis made by tissue prints), is 190/227: 0.83.

Negative predictive value (the proportion of subjects with a negative test result who are correctly diagnosed) is 66/84: 0.78.

In Table 5, we separated the results of the tissue prints according to the site of fragment excision, to see if this influences the sensibility and specificity.

For the pleura and mediastinal masses, the sensibility

is very high, but the specificity is very low, suggesting that tissue prints are susceptible to indicate too many false malignancies and should probably not be used for rapid diagnosis, at least for pleural fragments. The sensibility

of tissue prints for lung tissue fragments is quite high, but with low specificity, while for the lymph nodes sensibility is good, with less false positive results than for lung tissue.

**Table 5 – Sensibility and specificity of tissue prints according to the excised tissue**

Malignant (210 fragments)			Benign (103 fragments)		
Tissue type	Malignant print	Benign print	Tissue type	Malignant print	Benign print
Lymph node (93)	80 (0.86)	13	Lymph node (50)	12	38 (0.76)
Lung tissue (78)	73 (0.93)	5	Lung tissue (38)	16	22 (0.57)
Pleura (26)	26 (1.00)	0	Pleura (10)	7	3 (0.30)
Mediastinal mass (11)	11 (1.00)	0	Mediastinal mass (5)	2	3 (0.60)

## Discussion

The tissue printing method is attractive due to several features: it is cheap, it is rapid, it is low tech, all these making it suitable for many hospital facilities. However, its capacity to help taking a decision in the operation room needs to be assessed.

This study included fragments excised during thoracic surgery from patients operated with different indications, cancers or benign diagnosis, which were not taken into account, the golden standard being considered the pathological examination of the fragments. The patients were not selected as cancer patients, but also other indications were included, this resulted in a population with about one-third benign lesions.

The overall sensibility of the method is high (0.91), suggesting the capability of tissue prints to identify malignancy in most true malignant cases. This seems to be better for lung nodules or masses (0.93) than for lymph nodes (0.86).

The overall specificity of tissue printing is low, of 0.64, showing a low capability of the method to exclude malignancy in the benign cases. If the method is used for taking decisions during surgery, the high percentage of false positive might induce un-needed extensive resections in benign cases. The specificity is higher for lymph nodes (0.76), suggesting the method can be useful to assess the extension of malignancy to regional lymph nodes.

For pleural fragments, almost all tissue prints suggested malignancy, with a very low specificity (0.3), so probably the method is not useful to assess malignancy of the pleura.

The low specificity encountered in our study might be influenced by the fact that fragments from many benign indications were included, building up one third of all fragments examined. If the method would be applied in a selected population (*e.g.*, only in patients diagnosed with cancer before surgery), it is possible that the specificity would be higher.

Comparing our results with the published data regarding the frozen section method applied on fragments obtained by open lung surgery or mediastinoscopy, we can see similar sensibility (0.94) but higher specificity, ranging between 0.90 and 1 [7–13].

Regarding the capability of tissue prints to identify the histological type of the lesions, tissue prints are most accurate for lymphomas, and are capable to identify most adenocarcinomas as such or at least as non-small cell carcinomas, which is useful information during surgery.

The tissue prints are less performant in identifying small-cell carcinomas, which are misinterpreted in half of the cases.

## Conclusions

The method of tissue prints is attractive as a rapid method for identifying malignancy during surgery because it is fast, easy to perform and low tech and also cheap. The method has a high sensibility for cancer, but still a low specificity in the studied group. A better selection of the cases might improve the results, by applying the method only in patients operated for lung cancer, in order to confirm the lymph node or local extension, and not for benign or uncertain cases.

## Conflict of interests

The authors declare that they have no conflict of interests.

## Author contribution

The authors had equal contribution to the article achievement.

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