Fast cytological evaluation of lymphatic nodes obtained during transcervical extended mediastinal lymphadenectomy

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‡

Abstract

OBJECTIVES: Evaluation of the diagnostic efficiency of the intraoperative cytological examination of lymphatic nodes obtained during transcervical extended mediastinal lymphadenectomy (TEMLA).

METHODS: All mediastinal nodes obtained during consecutive TEMLA operations in patients with confirmed lung cancer were examined. Cytological imprints from cross sections of nodes were performed, fixed in 96 proof alcohol and stained with Haematoxylin–Eosin. The cytological slides were evaluated by light microscopy intraoperatively, and a standard paraffin histological examination of the same nodes was done afterwards for confirmation of the final diagnosis.

RESULTS: Intraoperative cytological studies were performed in 63 patients (17 women and 46 men; overall in 453 mediastinal nodal stations) from 1 April 2009 to 28 February 2011. The mean number of nodes/procedure was 27.8. The mean time of performance of the examination was 37 min, including 7 min for smears, 13 min for staining and 17 min for microscopic examination (overall 37 min). The cytological study discovered neoplasmatic cells in 12 of 63 patients, nodal stations in 22 of 453 and nodes in 44 of 1724. According to the analysis of the 63 patients, the imprint cytology technique had a sensitivity of 92.3%, specificity of 100%, accuracy of 98.4%, positive predictive value of 100% and negative predictive value of 98.0%, as was confirmed by the final histopathological examination.

CONCLUSIONS: (i) Cytological imprints examination was characterized by a very high specificity and sensitivity, is technically simpler and faster and allows for the examination of several dozens of lymphatic nodes during a single TEMLA procedure within an acceptable time, and after the exclusion of N2 nodes enables the simultaneous performance of a radical lung resection. (ii) The presented technique was the alternative for the traditional histopathological examination of the material frozen in cryostat.

Keywords: Imprint cytology • Transcervical extended mediastinal lymphadenectomy • Mediastinal lymph nodes • Intraoperative evaluation

INTRODUCTION

The diagnostic work-up of patients with non-small-cell lung cancer (NSCLC) includes the discovery and staging of the disease. According to the TNM classification, the proper choice of the treatment and the prognosis are based upon the pathological type and the stage of NSCLC. Staging of the nodal (N) factor is critically important. There are several staging techniques of the N factor, including the imaging modalities with computer tomography (CT), positron emission tomography (PET) combined with CT (PET/CT), endoscopic techniques combined with ultrasonography, like endobronchial ultrasound (EBUS) with transbronchial needle aspiration and endosonographic ultrasound (EUS) with fine needle aspiration [1–6]. Surgical staging techniques include standard cervical mediastinoscopy, anterior mediastinotomy, videothoracoscopy (VATS) and two new procedures—video-assisted mediastinoscopic lymphadenectomy (VAML) and transcervical extended mediastinal lymphadenectomy (TEMLA) [7–11]. The last technique enables the removal of the largest number of lymph nodes, which is essential for accurate N staging. The mean number of nodes removed by TEMLA was reported to be 37.9. In our institution, TEMLA has been routinely performed for the staging of NSCLC since 2004. The subsequent resection in patients with negative results of the TEMLA is usually performed after 14–20 days. Recently, we have started to perform the combined procedure including TEMLA with the intraoperative examination of all resected nodes with an immediate VATS lobectomy in patients suitable for such minimally
invasive procedures. The use of the standard frozen section analysis for the study of a large number of nodes removed during TEMLA is too time-consuming. The alternative to this technique is an imprint cytology technique, which has been previously reported in the diagnostics of lung pathology and for the study of the lymph nodes biopsies removed by mediastinoscopy [12–17]. This study examined retrospectively the diagnostic value of the intraoperative examination of the lymph nodes removed during TEMLA with the imprint cytology technique and its usefulness for combined TEMLA–VATS lobectomy procedures.

**MATERIALS AND METHODS**

There were 61 patients (17 women and 46 men) with NSCLC and two patients with malignant mesothelioma undergoing invasive staging with the use of TEMLA from 1 April 2009 to 28 February 2011. The technique of the TEMLA was described in detail elsewhere [9]. In brief, it includes a 5- to 8-cm collar incision in the neck, elevation of the sternal manubrium with a special retractor, bilateral visualization of the laryngeal recurrent and vagus nerves and dissection of all mediastinal nodal stations except for the pulmonary ligaments nodes (station 9). The non-fixed nodes were sent to the Pathology Department.

The slides were examined by two cytologists and one consultant pathologist. The intraoperative cytological studies were performed when the first author (M.J.) and one of the other authors (A.O., M.L. or M.S.) were on duty at the Pathology Department. No other criteria were used to select patients for the intraoperative imprint cytology study. During the same period, the whole number of 169 TEMLA procedures and 251 pulmonary resections for NSCLC were performed.

The nodes were counted and sectioned along the long axis. Cytological smears were performed from both surfaces of the sectioned nodes. The smears were performed from each node; usually there were 2–8 smears for each nodal station. Immediate fixing of the nodes in 96% alcohol was critically important to avoid excessive drying of the material. The slides were stained with Haematoxylin–Eosin (H&E). The slides were examined by light microscopy. In each case, the cytological study was confirmed by the subsequent final histological examination, which was the gold standard at our institution. The methodology of the histopathological examination included fixing of the whole nodes in 10% formalin. On the second day, the fixed nodes were prepared in the tissue processor and then embedded in the paraffin blocks. Finally, the standard histological slides were prepared and stained with the standard H&E technique. All histological studies were performed by one pathologist (J.P.) who was always blinded to the results of the cytological examinations.

**Statistical analysis**

The sensitivity, specificity, accuracy, positive predictive value (PPV) and negative predictive value (NPV) [including 95% confidence interval (CI)] were calculated using the standard definitions and GraphPad InStat 3.05 software (GraphPad Software, San Diego, CA, USA) was used.

**RESULTS**

There were 63 patients, of whom 61 had NSCLC. Primary staging was performed in 43 patients and re-staging after neoadjuvant therapy in 20 patients (chemotherapy in 14 patients and chemoradiotherapy in six patients). The patients who underwent neoadjuvant therapy were initially staged at our institution with CT and PET/CT (selective patients) and EBUS/EUS and with CT and PET/CT (selective patients) if they were referred from the other institutions. There were 453 nodal stations (1724 nodes) examined. The mean number of nodes/procedure was 27.8, with a mean number of 30 nodes/procedure for patients undergoing primary staging and 23.5 nodes/procedure for re-staging. The mean total time for preparing of the slides and the cytological examination was 37 min (24–51 min), including preparation of the smears taking 7 min (4–10 min), staining and covering of the slides–13 min (10–15 min) and cytological examination–17 min (10–30 min). In 12 of 63 patients, malignant cells were found. The positive results were obtained in 22 of 453 nodal stations and in 44 of 1724 nodes. In one patient with malignant mesothelioma, the cytological examination revealed the malignant cells but it was insufficient to diagnose the metastases of the malignant mesothelioma. The diagnosis could be made only after further histological study with immunohistochemistry performed. In one node discovered in the cytological examination, the final histological study did not confirm the presence of the malignant cells (false-positive result) (Table 1). Histological types of tumours included squamous cell carcinoma in two patients, adenocarcinoma in three patients, malignant mesothelioma in two patients and NSCLC (a more precise diagnosis could not be made) in five patients. According to the analysis of the 63 patients, the imprint cytology technique had a 92.3% sensitivity, 100% specificity, 98.4% accuracy, 100% PPV and 98.5% NPV (Table 2). According to the analysis of the 453 nodes, the imprint cytology technique had a 95.5% sensitivity, 100% specificity, 99.8% accuracy, 100% PPV and 98.8% NPV (Table 3).
DISCUSSION

Intraoperative analysis for the removal of lymph nodes during mediastinoscopy has been practiced by many institutions worldwide. Gephardt and Rice [18] used the frozen section analysis technique [18]. The authors performed 122 mediastinoscopies, biopsying the total number of 620 nodes (mean number of five nodes/procedure). The results were acceptable with a 92.2% sensitivity, 100% specificity, 100% PPV and 99.3% NPV. The author concluded that the frozen section analysis technique might be used for the intraoperative analysis of the limited number of nodes. The imprint cytology analysis was first used by Ghandur-Mnaymneh and Paz in 1985 [16]. They examined 300 nodes comparing the imprint cytology technique with the frozen section analysis. The accuracy of the imprint cytology and of the frozen section was 99 and 98.7%, respectively. However, the imprint cytology was regarded the preferable technique because it was simple and less time-consuming.

Tamiolakis et al. [17] reported that the time necessary for the intraoperative imprint cytology and frozen section analysis of the pulmonary and the mediastinal lesions was 1 min for the imprint cytology and 10 min for the frozen section one biopsy.

The reported sensitivity of the imprint cytology for evaluation of the mediastinal nodes has been in the range of 90–100%. Okuba et al. [14] reported the analysis of 520 nodal stations in 157 patients (mean 3.3 stations/procedure) with a 95.7% sensitivity, 99.4% specificity 99.4% PPV and 99.3% NPV. Clarke et al. [13] examined 121 mediastinal nodes removed during 38 operations (mean 3.3 nodes/procedure) and reported a 96.6 sensitivity, 100% specificity, 98.9% PPV, 100% PPV and 99.2% accuracy. Orki et al. [12] examined 1050 mediastinal lymph nodes removed during 255 procedures including mediastinoscopies, mediastinotomies or VATS (mean 4.1 nodes/procedure). The authors reported a 93.1% sensitivity, 99.5% specificity, 95.6% PPV, 99.1% NPV and 98.8% accuracy. All the authors agreed that the imprint cytology technique can be used for the standard examination of the mediastinal lymph nodes due to its simplicity, accuracy and reliability and, most importantly—being less time-consuming than the frozen section analysis.

In our study, the number of nodes undergoing the intraoperative examination was much higher in comparison with the other reports—27.8 nodes/procedure. The interesting finding was that the number of the nodes in patients undergoing primary staging was higher than that in restaged patients (30.0 vs 23.5). Such a finding has not been reported yet and it deserves further investigation in the future. The other important finding of our study was the proof of the high effectiveness of imprint cytology in patients who underwent neoadjuvant therapy.

Our results in regard to the diagnostic yield were comparable to those reported by the other authors. Additionally, we proved that it was possible to examine such a big number of nodes in the relatively short time (mean 37 min/procedure). We have not measured the mean time of the frozen section analysis of one node in our institution but it takes at least 10 min, so the total time for the intraoperative examination of 27.8 nodes would be much beyond any reasonable limits. The technical problems of the cytological analysis include the difficulties of the preparation of the smear in the case of very small nodes, in the case of dried and damaged nodes and fibrotic or necrotic nodes. In the case of silicosis, the histiocytes might mimic the neoplastic cells in the cytological study [4]. In these cases, the final test is a standard histological examination.

For the intraoperative examination, the most important issue is the lack of the malignant cells. The next important issue is the subtyping of NSCLC. In most of our patients, however, it was possible to report the subtype of NSCLC, with exception of two patients with squamous cell carcinoma and adenocarcinoma in whom the diagnosis of NSCLC was reported on the cytological examination.

In one patient with malignant mesothelioma, the cytological examination was insufficient to diagnose the metastases of the malignant mesothelioma. The diagnosis could be made only after the further histological study with immunohistochemistry performed.

The single false-positive result was due to the presence of two benign histiocytes mimicking the neoplastic cells (Fig. 1). The final diagnosis excluding the presence of metastasis was made with the histological study with immunohistochemistry. The other reason for false positive cytological results is a contamination of the smear by the neoplastic cells from the other nodal station during the preparation of the smears. The reasons for false negative results include the very small micrometastasis. In such patients, the final diagnosis can be made with immunohistochemistry or polymerase chain reaction, which cannot be done intraoperatively [19, 20].

The imprint cytology technique enabled us to introduce a combined procedure of TEMLA for final staging of the mediastinum with subsequent pulmonary resection performed with VATS in the case of a negative result of TEMLA. In our previous

Table 3: Results of imprint cytology according to the 453 nodal stations

<table>
<thead>
<tr>
<th>Variable</th>
<th>Value (%)</th>
<th>95% CI</th>
</tr>
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<tbody>
<tr>
<td>Sensitivity</td>
<td>95.5</td>
<td>0.772–0.999</td>
</tr>
<tr>
<td>Specificity</td>
<td>100</td>
<td>0.992–1.000</td>
</tr>
<tr>
<td>Accuracy</td>
<td>99</td>
<td>0.985–1.000</td>
</tr>
<tr>
<td>PPV</td>
<td>100</td>
<td>0.839–1.000</td>
</tr>
<tr>
<td>NPV</td>
<td>99.8</td>
<td>0.987–1.000</td>
</tr>
</tbody>
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Figure 1: A false-positive result of the cytological smear of the lymph node (lack of metastasis on the subsequent histological examination).

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reports, we showed that TEMLA was an extremely accurate staging procedure significantly better than mediastinoscopy, EBUS, EUS and combined EBUS/EUS [21, 22]. In the patients with the metastatic nodes discovered intraoperatively VATS lobectomy was not performed and the patients were referred for the neoadjuvant treatment. In our opinion, the presented strategy facilitates an optimal staging due to the very high diagnostic accuracy of TEMLA. The combination of two minimally invasive procedures—TEMLA and VATS lobectomy—was acceptable to our patients, and the time for the whole procedure including TEMLA, imprint cytology analysis and VATS lobectomy was in the range of 3.5–4.5 h. In our opinion, such combined procedures might be used in our normal practice.

The efficient and fast performance of imprint cytology necessitated the cooperation of two cytologists and one consultant pathologist; on the other hand, this is a relatively simple technique, without the need for sophisticated expensive technology.

CONCLUSIONS

(1) Cytological imprints examination is characterized by very high specificity and sensitivity, is technically simpler and faster and allows for the examination of several dozens of lymphatic nodes during a single TEMLA procedure within an acceptable time, and after the exclusion of N2 nodes, enables the immediate performance of a radical lung resection.

(2) The presented technique is the alternative to the traditional histopathological examination of the material frozen in cryostat.

Conflict of interest: none declared.

REFERENCES


APPENDIX. CONFERENCE DISCUSSION

Dr P. Dartevelle (LePlessis-Robinson, France): I would like to ask only one question. When you have N2 disease, what do you do? Recently I asked my co-workers to review all patients who had a lobectomy or a pneumonectomy with an N2 disease over 10 years. Half were clinical, limited but clinical N2 disease. The other half were N2 disease discovered at surgery. I was very surprised by the results which demonstrate a 5-year survival rate of 47% in this series of approximately 300 patients. Is it possible to contraindicate these patients for surgery? I think no. What is your answer?

Dr Zielinski: Our policy is to send such patients who had N2 nodes discovered during TEMLA to induction chemotherapy without pulmonary resection with the intention that they will come back after induction. I know that the topic is controversial and I know your policy well. We still believe that the patients who had N2 nodes had micro-metastasis spread elsewhere. The N2 nodes are just a marker for dissemination. That’s why we send these patients to induction therapy. But I also know that the patients with very limited N2 involvement have much better results than the rest of N2 patients. Maybe this strategy you described is justified. I think the future will tell us which strategy is better.

Dr A. Dokhan (Cairo, Egypt): You have 13 patients positive out of 63. What about the rest? What about the comparison between these 50 and the paraffin sections? And you said 100% specificity, and accuracy, almost the same. Will you kindly comment? Do you understand my question?

Dr Zielinski: Yes. There was only one false result. There were no false negatives, but in one patient the result was false positive, the one I showed. The
results are close to 100% in this small group, which I would like to underline once again. This is the initial study which probably should be confirmed in the larger groups. What was your first question?

Dr Dokhan: You said that only 13 out of 63 were positive cytologically. Is that right?

Dr Zielinski: Yes, 13 patients out of 63 patients.

Dr Dokhan: And what about the others?

Dr Zielinski: They were negative.

Dr Dokhan: And the patients proved to be lung cancer before surgery?

Dr Zielinski: Yes.

Dr Dokhan: What about these 50?

Dr Zielinski: They underwent pulmonary resection.