Application of rapid on-site evaluation in computed tomography-guided percutaneous transthoracic needle biopsy of pulmonary nodules of ≤2.0 cm in diameter

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Received February 4, 2022; Accepted July 28, 2022

DOI: 10.3892/etm.2022.11599

Abstract. Histopathological findings are the gold standard for diagnosing lung nodules, and invasive diagnostic procedures such as percutaneous transthoracic needle biopsy (PTNB) are often inevitable for a confirmative diagnosis. However, the traditional biopsy method is inefficient for the diagnosis of small pulmonary nodules (diameter ≤2.0 cm). The present study aimed to investigate the application of rapid on-site evaluation (ROSE) in CT-guided PTNB of pulmonary nodules (≤2.0 cm in diameter). Data from patients undergoing PTNB in the Second Affiliated Hospital of Qiqihar Medical College between June 2018 and June 2021 were retrospectively analyzed. A total of 250 patients were included and divided into the ROSE (n=177) and the non-ROSE groups (n=73). The comparison of these two groups indicated significantly higher specimen adequacy [93.22% (165/177) vs. 71.23% (52/73)] and diagnostic accuracy [90.40% (160/177) vs. 68.49% (50/73)], as well as a significantly lower rate of secondary biopsies [5.08% (9/177) vs. 28.77% (21/73)], in the ROSE group. The coincidence rate between the diagnosis with ROSE and the final pathological results was 96.73%, indicating high consistency (κ=0.925). The results indicated that the application of ROSE in PTNB of pulmonary nodules with a diameter of ≤2.0 cm can ensure sufficient material sampling, improve the diagnostic accuracy and reduce the secondary biopsy rate, without increasing complications. ROSE can ensure high consistency with the results obtained from the pathological evaluation.

Introduction

Lung cancer is a common clinical malignant tumor, and its morbidity and mortality ranks first among malignant tumors (1). The cases of pulmonary are also increasing year by year (2). Histopathological specimens obtained by nodules lung biopsy are considered the gold standard for the diagnosis of lung cancer (3). The diagnostic accuracy of percutaneous transthoracic needle biopsy (PTNB) is ~91.1% (4). However, the accuracy of detecting pulmonary nodules with a diameter of ≤2.0 cm is usually low, owing to the difficulty in puncturing.

Rapid on-site evaluation (ROSE) can guide the operator in real time with regard to the direction of material sampling and ensure a rapid decision on the adequacy of the material obtained during the biopsy of certain lesions, which effectively improves the diagnostic success rate of the biopsy. ROSE is a safe method and has not been previously reported to exhibit serious surgical complications (5).

Agarwal et al (6) proposed that ROSE could reduce the inadequacy of biopsy specimens for bronchoscopy. Izumo et al (7) suggested that the number of punctures in endobronchial ultrasound with a guide sheath could be reduced and the accuracy of pathological results could be improved by the application of ROSE. However, the use of ROSE has not been previously examined for PTNB of pulmonary nodules with a diameter of ≤2.0 cm. Pulmonary nodules of >2.0 cm in diameter can be examined more accurately by needle biopsy, owing to the larger sizes of the lesions. When the size of the lesion is uncertain, the use of ROSE may reduce the accuracy of needle biopsy. The novelty of the present study was in the application of ROSE only for the biopsy of pulmonary nodules with a diameter of ≤2.0 cm, which increases its accuracy. The present study aimed to evaluate the specimen adequacy rate, diagnostic accuracy, secondary biopsy rate, complication rate and consistency with the final diagnosis of pulmonary nodules (≤2.0 cm in diameter) using ROSE in CT-guided PTNB. The effectiveness of ROSE was also analyzed.
Materials and methods

Study population. The present retrospective study was approved by the Ethics Committee of Qiqihar Medical College (Qiqihar, China; protocol no. 2021-193). The data used in the present study were anonymous; therefore, the present study was exempt from informed consent and was compliant with The Declaration of Helsinki. The medical records of patients undergoing PTNB in the Second Affiliated Hospital of Qiqihar Medical College, between June 2018 and June 2021, were retrospectively analyzed. Inclusion criteria included lesions >2.0 cm and fine needle aspiration biopsy. A total of 250 patients were included (age, 26-85 years; 152 males and 98 female); of these, 167 patients (age, 29-85 years; 101 males and 65 female) with malignant disease and 83 patients (age, 26-84 years; 51 males and 32 female) with benign disease. PTNB was selected for patients with lung lesions that were unsuitable for or did not provide sufficient specimens for transbronchial biopsy.

Procedure protocol and policies. All patients signed the preoperative informed consent for PTNB (the patients were informed regarding the purpose and possible risks of PTNB, as well as treatment options based on available technology). Each patient did not receive treatment with aspirin for >7 days, and their blood count and coagulation function met the guidelines of interventional radiology (8). DXW and YGW had >7 years of experience in CT-guided PTNB. The procedure was performed under the guidance of a 64-slice spiral CT (Aquilion; Canon Medical Systems Corporation) using an 18G semi-automatic biopsy needle with a matching 17G coaxial needle (TSK Surecut; TSK Laboratory). The patient position was determined by selecting the puncture route that was the shortest to the lesion and that could be used to avoid contact with the interlobar pleura, bullae and blood vessels. All biopsies were performed with a coaxial technique. Following insertion of the 17G coaxial needle into the edge of the lesion, the 18G semi-automatic biopsy needle was then used for biopsy.

Biopsy procedures. The following steps were performed: i) The skin puncture point, determined by a CT scan in an appropriate body position, was selected according to the puncture route set prior to the operation; ii) the lesion was punctured using a biopsy needle following local disinfection using Iodophor (20 ml) and local anesthesia (2% concentration of lidocaine hydrochloride injection; 5 ml); iii) when the biopsy needle reached the outside of the pleura, a CT scan was conducted to determine the forward direction and distance to the lesion (this step was not carried out in the ROSE group); iv) the CT scan was performed again when the biopsy needle reached the lesion, and the tissue specimens were obtained by sampling the tumor, following correction of the needle direction; and v) complications were immediately observed with the CT scan. Chest X-ray was performed at the 3- and 6-h follow-up periods to exclude the development of a pneumothorax.

ROSE technology. In the present study, on-site cytological evaluation for the ROSE group was conducted by pathologists who had >5 years of work experience. It has been previously reported that the accuracy of ROSE does not differ significantly when performed by a pulmonologist (with 1 month of cytopathology training) or a cytopathologist (9). An on-site assessment has also previously been performed by an experienced cytopathologist in the study by Anila et al (10). Therefore, the on-site cytological evaluation for the ROSE group in the present study was deemed to be unbiased. The specimens were smeared on the cytology slides to reduce the loss of tissue. The specimens were stored in 10% formalin fixative solution at room temperature for 12 h and sent to the Department of Pathology for histopathological diagnosis. The cytology slides were fixed at room temperature for 30-40s with a 95% ethanol solution on site, air-dried quickly and mounted immediately following rapid hematoxylin-eosin (H&E) staining (Room temperature, 90s.). The biopsy specimen was considered sufficient and the procedure was terminated if the cytoarchitecture was found to be consistent with the clinical findings. In case of inconsistency, a re-biopsy (change of the sampling site) was performed using the coaxial needle. The number of samplings per lesion was <5.

Pathological techniques. In the non-ROSE group, the identification of tissue adequacy was performed as follows: i) A sufficient specimen was a complete piece of tissue with a size of >10x1x1 mm³. Each lesion was sampled <5 times. Sufficient specimens were placed in 10% formalin at room temperature, 12 h and sent for histopathological diagnosis by two experienced pathologists; ii) fixed tissue was dehydrated and embedded in paraffin. The paraffin was cut into 3- to 5-μm thick slices, which were fixed to the slides. The slices were baked (50-60°C for 30-60 min) and sealed for conventional H&E staining at room temperature for 6-8 min). The sections were observed by two experienced histopathologists and final histopathological diagnosis (10) was made following consultation; and iii) immunohistochemical staining was performed if necessary. In brief, after the paraffin sections were dehydrated and rehydrated in absolute, 95, 90% ethanol, 80% ethanol, 70% ethanol and distilled water for 5 min each, the tissues were washed with phosphate-buffered saline (PBS) three times. According to the requirements of each antibody, the tissue antigen was retrieved by boiling in the pressure cooker (The reagent is composed of 5 l distilled water and 100 ml immunohistochemical antigen retrieval buffer, heated to 99°C for 3 min) and the tissues were again washed with PBS three times. Endogenous peroxidase was blocked using a 3% hydrogen peroxide methanol solution, and the tissues were again washed with PBS three times. Subsequently, 4% sheep serum (Beijing Nakasugi Company) was added for 30-50 min at room temperature to reduce non-specific staining. Sheep serum was removed, and different primary antibodies (cat. nos. TTF1/ZM-0250, NAPSIN/ZM-0473 or P40/ZM-0472, P63/ZM-0406, CD56/ZM-0057 and Syn/ZM-0246; Beijing Nakasugi Company) were added to samples at room temperature for 30-50 min. Following washing with PBS three times, the polymeric chelate (cat. no. UM-9002, Beijing Nakasugi Company, peroxidase) was added dropwise and incubated for 30 min (Room temperature). The samples were then
washed with PBS three times and diaminobenzidine color solution was added dropwise to each slice. The slices were washed with water, re-stained with hematoxylin (Room temperature, 5 min), dehydrated, transparentized and sealed. Histopathological diagnosis was performed with a light microscope (Olympus BX41).

**Measured variables.** The patient demographics, nodule size, nodule location (superior and middle lobes for upper lungs and inferior lobes for lower lungs), nodule-to-pleura distance and pleura-needle angle (acute angle between the needle and the tangent to the pleura) were recorded. The documented complications included hemoptysis, pneumothorax and chest tube placement. The final diagnosis was made according to the observations from H&E staining of paraffin-embedded histopathological sections and/or following ≥6 months of follow-up. The tumor histopathological findings (benign or malignant) (11) and secondary biopsy results were also recorded.

**Statistical analysis.** SPSS 18.0 software (SPSS, Inc.) was used for statistical analysis. The measured data are expressed as the mean ± standard deviation and the differences were compared using an unpaired t-test. The enumerated data are expressed as percentages, and the differences were compared using χ² test. P<0.05 was considered to indicate a statistically significant difference. The correlation of the ROSE results with the final diagnosis was analyzed using Cohen’s κ, with κ>0.75 indicating optimal consistency.

**Results**

**Comparison of patient demographics.** Between June 2018 and June 2021, 607 patients underwent PTNB. Of the 607 patients, a total of 309 patients with lesions >2.0 cm and 48 patients undergoing fine needle aspiration biopsy were excluded. Finally, 250 patients (26-85 years) were identified as study subjects, of whom 177 patients with ROSE (Fig. 1) were enrolled into the ROSE group and 73 patients without ROSE were included in the non-ROSE group (patients without ROSE were not available until April 2019). The demographic data and baseline values of the patients are presented in Table I. The differences between the two groups were not considered statistically significant (P>0.05).

### Table I. Demographics and baseline values of the two groups.

| Characteristic                        | ROSE            | Non-ROSE        | χ²- or t-value | P-value |
|---------------------------------------|-----------------|-----------------|----------------|---------|
| Age, years                          | 62.79±8.61      | 64.15±8.15      | 1.26           | 0.248   |
| Sex, male/female                     | 105 (59.32)/72  | 47 (64.38)/26   | 0.56           | 0.456   |
| Nodule size, cm                       | 1.72±0.84       | 1.63±0.69       | 1.61           | 0.109   |
| Nodule distribution, upper lung/lower lung | 115 (64.97)/62 | 45 (61.64)/28   | 0.25           | 0.618   |
| Distance from nodule to pleura, cm²   | 1.81±1.19       | 1.42±0.97       | 0.80           | 0.424   |
| Pleura-needle angle, 90°/<90°         | 71 (40.11)/106  | 32 (43.84)/41   | 0.30           | 0.587   |

Except where indicated, data are numbers of procedures, with percentages in brackets. *Data are expressed as the mean ± standard deviation; t-value; χ²-value. ROSE, rapid on-site evaluation.

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CT-guided percutaneous lung biopsy
June 2018–June 2021; n=607

Pulmonary nodules ≤2.0 cm PTNB; n=48

Comparison of histopathological diagnosis results. In the ROSE group, sufficient specimens (assessed by observation of paraffin sections) from 165/177 patients (93.22%) were obtained, whereas sufficient specimens from 153 patients were identified by on-site cytology. Moreover, in 128 patients, the first examination was sufficient. A total of 49 patients had insufficient specimens for the first time, and 25 patients obtained valid specimens after re-sampling. In addition, 101 patients presented with malignancies by on-site cytology, which was consistent with the paraffin section results (Fig. 2). A 71-year-old female patient underwent CT-guided PTNB for a 15-mm nodule in the left upper lobe (Fig. 2A). The initial diagnosis of ROSE was lung adenocarcinoma (Fig. 2B), and the histopathological diagnosis was lung adenocarcinoma (Fig. 2C). Immunohistochemistry was positive for TTF-1 (Fig. 2D), and the ROSE results in this case were consistent with histopathological findings of lung adenocarcinoma. Furthermore, 52 patients exhibited benign lesions as determined by on-site cytology, of whom 47 demonstrated absorption of pulmonary nodules to different degrees or no change following >6 months of follow-up; during the same period, 5 patients indicated an increase in the tissue content compared with the previous evaluation. Following surgical resection, the patients were finally diagnosed as lung cancer cases. Specifically, 24 patients were assessed to have insufficient specimens by on-site cytology, and 12 of them were found to possess sufficient materials for the determination of benign lesions using paraffin sections. The lesions were absorbed following treatment. An additional 12 patients with paraffin section specimens were classified as insufficient and...
underwent secondary biopsies (9 were malignant and 3 were benign).

In the non-ROSE group, 52/73 patients (71.23%) were classified as cases with sufficient material collection by H&E staining of paraffin sections, including 35 patients who were malignant cases and 17 patients who were benign cases. A total of 15 out of 52 patients indicated absorption of pulmonary nodules to different degrees or had no change, whereas 2 patients exhibited enlargement and were finally diagnosed as lung cancer cases following surgical resection and 6 months of follow-up. The specimens from 21 patients were characterized as insufficient (17 malignant and 4 benign specimens) and secondary biopsies were performed. No significant differences were noted in the histopathological types between the ROSE and the non-ROSE groups (Table II).

**Comparison of patient specimen adequacy rate, diagnostic accuracy rate, secondary biopsy rate and histopathological type.** The specimen adequacy rate [93.22% (165/177)] and diagnostic accuracy [90.40% (160/177)] of the ROSE group were significantly higher than those of the non-ROSE group [71.23% (52/73) and 68.49% (50/73), respectively; both P<0.05]. The rate of secondary biopsy [5.08% (9/177)] was significantly lower than that of the non-ROSE group [28.77% (21/73); P<0.05]. The comparison of the results between the two groups is shown in Table II.

**Comparison of patient complications and procedure time.** The comparison of complications and the procedure time between the two groups are listed in Table III. No significant difference was noted in the incidence of pneumothorax, thoracic tube placement, hemoptysis or the time of procedure (P>0.05). No case of air embolism or death was reported.

**Comparison of ROSE results with final diagnosis.** A comparison of the ROSE results with the final disease diagnosis is shown in Table IV. The sensitivity and specificity of ROSE diagnosis were 95.28 and 100.00%, respectively. The coincidence rate between the diagnosis of ROSE and the final pathological results was 96.73%, indicating high consistency (κ=0.925, P<0.001).

Figure 2. Representative results from a 71-year-old woman in the ROSE group. (A) CT-guided percutaneous transthoracic needle biopsy performed on a 15-mm diameter nodule (arrow) in the left upper lobe. (B) ROSE of atypical cells with an initial cytopathological diagnosis of lung adenocarcinoma (rapid H&E staining; magnification, x400). (C) Histopathological diagnosis of lung adenocarcinoma (routine H&E staining; magnification, x200). (D) Immunohistochemistry for thyroid transcription factor 1 (magnification, x200). ROSE, rapid on-site evaluation; H&E, hematoxylin-eosin.
**Discussion**

PTNB is one of the traditional methods used to obtain lung pathological specimens and exhibits high safety (9). However, the diagnosis of pulmonary nodules with a diameter of ≤2.0 cm is relatively difficult and prone to produce false-negative results. To improve the accuracy of PTNB in pulmonary nodules with a diameter of ≤2.0 cm, 250 patients undergoing biopsy of pulmonary nodules (≤2.0 cm) were evaluated. The results indicated that ROSE could significantly improve the diagnostic rate of PTNB. The incidence of pneumothorax and thoracic tube placement was higher in the ROSE group than in the non-ROSE group; however, only non-significant differences were noted.

No significant differences were noted in the demographic data or baseline values between the two groups. The specimen adequacy rate and diagnostic accuracy of the ROSE group were significantly higher than those of the non-ROSE group. Xu et al (12) demonstrated that the diagnostic accuracy of patients in the ROSE group was 85.7%, which is similar to the results of the present study. The secondary biopsy rate was reduced from 28.77 to 5.08% by the application of ROSE in the current study. In a recent meta-analysis of biopsies of pulmonary nodules with a diameter of ≤2.0 cm, Liu et al (13) indicated a higher rate of secondary biopsy, ranging from 14.4 to 31.2%. ROSE is used to assess specimen quality and provides the operator with real-time guidance. It can also significantly reduce the rate of secondary biopsy, notably in the biopsy of pulmonary nodules with a diameter of ≤2.0 cm.

The accuracy of CT-guided PTNB is associated not only with the proficiency of the operator, but also with the lesion size; notably, in the small nodules of the lower lungs, the operation may be complicated due to respiratory movement (14). Yarmus et al (15) suggested that ROSE does not decrease the rate of secondary biopsies. This suggestion is different from the conclusion of the current findings, possibly due to lack of differentiation between the pulmonary nodule sizes reported by Yarmus et al (15). It is suggested that the pulmonary nodules with a diameter of ≤2.0 cm benefit more from the use of ROSE in CT-guided PTNB. Based on the present study, ROSE can accurately assess whether the specimen is sufficient and improve the diagnostic accuracy of the method used. ROSE can also guide the location of the material during the puncture process and thereby enhance the technical skill of the medical practitioner.

Pneumothorax and hemoptysis are common complications of PTNB (16). The incidence of pneumothorax ranges from 5.3 to 37% (17,18). It has been reported that between 1.4 and 16.7% of these patients require chest tube placement (19,20). Hemoptysis is often self-limiting, with an incidence of 1-13% (21,22). These findings are in accordance with the

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**Table II. Comparison of specimen adequacy rate, diagnostic accuracy rate, secondary biopsy rate and histopathology between two groups of patients.**

| Characteristic                  | ROSE        | Non-ROSE   | χ²-value | P-value |
|--------------------------------|-------------|------------|----------|---------|
| Sufficient specimens           | 165.00 (93.22) | 52 (71.23) | 21.81    | <0.001  |
| Accurate diagnosis             | 160.00 (90.40) | 50 (68.49) | 7.18     | 0.007   |
| Secondary biopsy               | 9 (5.08)    | 21 (28.77) | 27.45    | <0.001  |
| Malignant diagnosis            | 115 (64.97) | 52 (71.23) | 0.914    | 0.339   |

Data are numbers of procedures, with percentages in brackets. ROSE, rapid on-site evaluation.

**Table III. Comparison of complications and procedure times between the two groups of patients.**

| Characteristic                  | ROSE        | Non-ROSE   | χ² or t-value | P-value |
|--------------------------------|-------------|------------|---------------|---------|
| Pneumothorax                   | 28 (15.82)  | 11 (15.07) | 0.02          | 0.882   |
| Thoracic catheterization       | 9 (5.08)    | 3 (4.11)   | 0.11          | 0.743   |
| Hemoptysis                     | 12 (6.78)   | 6 (8.22)   | 0.16          | 0.689   |
| Time of procedure, minb        | 20.06±3.37  | 19.34±3.22 | 1.54         | 0.124   |

Except where indicated, data are numbers of procedures, with percentages in brackets. χ²-value; data are expressed as the mean ± standard deviation; t-value. ROSE, rapid on-site evaluation.

**Table IV. Comparison of ROSE results with final diagnosis.**

| ROSE results | Final diagnostic results | Malignant | Benign | Total |
|--------------|--------------------------|-----------|-------|-------|
| Malignant    |                          | 101       | 0     | 101   |
| Benign       |                          | 5         | 47    | 52    |
| Total        |                          | 106       | 47    | 153   |

Sensitivity, 95.28% (101/106). Specificity=100 (47/47)%. ROSE, rapid on-site evaluation.
present study suggestion that the incidence rates of the complications were not significantly different between the two groups. This evidence suggests that the incidence of common complications of PTNB will not be increased by the use of ROSE. Moreover, no serious complications, such as air embolism and death, occurred in the current study. Furthermore, ROSE did not prolong the time of the puncture procedure and was safe and reliable.

In addition, the sensitivity and specificity of ROSE for the diagnosis of pulmonary nodules with a diameter of ≤2 cm were 95.28 and 100.00%, respectively. These results are highly consistent with the final pathological findings and aid the confirmation of the initial diagnosis of emergency cases. Similarly, Anila et al (10) indicated that the sensitivity and specificity of ROSE in 50 patients with pulmonary nodules were 92.00 and 100.00%, respectively.

The present study contains certain limitations. Firstly, this is not a multicenter study and it therefore may include certain biases. Secondly, this is not a prospective study and may be subjected to certain confounding factors (for example: incomplete data). Finally, the sample size used was fairly small. Although ROSE was used, 5 malignant tumors were missed. The use of ROSE can reduce the incidence of missed diagnoses; however, it requires regular patient follow-up. It is suggested that the use of ROSE should be implemented only in the puncture of small nodules of the lung. The use of puncture in other organs could also be beneficial, which will be investigated in future studies.

In conclusion, the current study indicated that when CT-guided PTNB was performed for pulmonary nodules with a diameter of ≤2.0 cm, ROSE not only ensured the adequacy of sampling, but also improved the diagnostic accuracy and significantly reduced the secondary biopsy rate. ROSE ensures high consistency between diagnosis and the final pathological results, without increasing the incidence of PTNB complications.

Acknowledgements

Not applicable.

Competing interests

The authors declare that they have no competing interests.

Funding

This study received funding from the Qiqihar Science and Technology Plan Joint Guidance Project (grant no. LHYD-202045).

Availability of data and materials

The datasets used and/or analyzed during the current study are available from the corresponding author on reasonable request.

Authors' contributions

DW was responsible for the conception and design of the present study. QZ and YW participated in study design and data collection. DW, WD, GD, QW, YH, YD and BL analyzed and interpreted the data. DW drafted the manuscript. QZ and DW critically revised the manuscript for intellectual content. All authors have read and approved the final manuscript. QZ and YW confirm the authenticity of all the raw data.

Ethics approval and consent to participate

The Ethics Committee of Qiqihar Medical College (Qiqihar, China; protocol no. 2021-193) provided ethical approval for the present study. As it is a retrospective study, the ethics committee has exempted the patients' right of informed consent, but the patient information should remain anonymized.

Patient consent for publication

Not applicable.

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