Rapid On-Site Evaluation (ROSE) in Diagnostic Interventional Pulmonology

Volume 1: Infectious Diseases

Jing Feng
Xin Li
Pei Li
Qiang Li
Yi Shi
Editors

Springer

Rapid On-Site Evaluation (ROSE) in Diagnostic Interventional Pulmonology

Jing Feng • Xin Li • Pei Li
Qiang Li • Yi Shi
Editors

Rapid On-Site Evaluation (ROSE) in Diagnostic Interventional Pulmonology

Volume 1: Infectious Diseases

Springer

Editors
Jing Feng
Division of Pulmonary and Critical Care Medicine, Department of Interventional Pulmonology
Tianjin Medical University General Hospital
Tianjin
China

Pei Li
Department of Respiratory Medicine
Taikang Xianlin Gulou Hospital
Nanjing University School of Medicine
Nanjing, Jiangsu
China

Yi Shi
Department of Respiratory and Critical Care Medicine
Jinling Hospital of Nanjing University
Nanjin, Jiangsu
China

Xin Li
Department of Pathology
Tianjin Haihe Hospital, Tianjin Institute of Respiratory Diseases
Tianjin
China

Qiang Li
Department of Respiratory and Critical Care Medicine
Shanghai East Hospital
Tongji University School of Medicine
Shanghai
China

ISBN 978-981-13-3455-9 ISBN 978-981-13-3456-6 (eBook)
https://doi.org/10.1007/978-981-13-3456-6

Library of Congress Control Number: 2019930759

This Springer imprint is published by the registered company Springer Nature Singapore Pte Ltd.
The registered company address is: 152 Beach Road, #21-01/04 Gateway East, Singapore 189721, Singapore

Contents

1 Rapid On-Site Evaluation (ROSE) in Diagnostic Interventional Pulmonology: Introduction and Detailed Methods

Jing Feng, Qiang Li, Yi Shi, and Ke Wang

In recent years, the use of diagnostic interventional pulmonology has been booming due to the increased prevalence of lung cancer, more drug-resistant pathogen infections of lower respiratory tract, and urgent request for diagnosis of baffling and critical respiratory diseases. The efficiency of interventional diagnostics has become one of the most important references for rating pulmonology or cancer center, which promote the clinical application of numerous advanced technologies and facilities. As a "real-time accompany technique" for diagnostic interventional pulmonology, rapid on-site evaluation (ROSE) has also been paid an unprecedented attention and develops promptly.

J. Feng (✉)
Division of Pulmonary and Critical Care Medicine, Department of Interventional Pulmonology, Tianjin Medical University General Hospital, Tianjin, China

Q. Li
Department of Respiratory and Critical Care Medicine, Shanghai East Hospital, Tongji University School of Medicine, Shanghai, China

Y. Shi
Department of Respiratory and Critical Care Medicine, Jinling Hospital of Nanjing University Nanjin, Jiangsu, China

K. Wang
Department of Respiratory Medicine, The 1st Affiliated Hospital of Guangxi Medical University, Nanning, China

1.1 Definition and Work Content of Diagnostic Interventional Pulmonology ROSE

The diagnostic interventional pulmonology ROSE is a real-time cytological examination technique which accompanies sequential sampling. The process of ROSE is as follows: A small part of every tissue specimen sampled from target lesion is smeared on a slide without losing tissue material significantly. Then the cytological slide is stained as soon as possible. Finally, the stained slide is interpreted immediately under specialized microscope integrating with all the available clinical information. The cytological content to be interpreted includes cellular morphology, differential cell counts, constituent ratio, cellular array, mutual relation, cytological background, and analysis of exotic substance.

As a carrier of cells, ROSE slide plays the following roles: evaluation of adequate sampling, real-time guidance for interventional methods and modalities, approaching a preliminary diagnosis or narrowing differential diagnosis spectrum, optimizing processing scheme for target lesion specimen, analyzing patients' disease status, and prognosis in combination with all available clinical and cytological information. It is still controversial about whether ROSE can increase the rate of successful diagnosis in diagnostic interventional pulmonology.

J. Feng et al. (eds.), *Rapid On-Site Evaluation (ROSE) in Diagnostic Interventional Pulmonology*,
https://doi.org/10.1007/978-981-13-3456-6_1

1.2 Historic Evolution and Prospect Forecast of ROSE Clinical Practice

"Modern" ROSE was first applied in interventional pulmonology in 1981. Pak HY et al. [1] used a quick staining and rapid interpretation technique in the procedure of percutaneous transthoracic fine needle aspiration biopsy under fluoroscopic guidance. Staining could be accomplished within 5 min, and interpretation was available less than 15 min after sampling target lesions. Successful diagnosis was achieved in 36 of 37 patients (97%). An improved rate of successful diagnosis following the "bedside" rapid stain was then proposed, which reflected the advantage of being able to determine the adequacy of specimens before releasing patients from the procedure room.

The use of "modern" ROSE in interventional operation with flexible bronchoscopy began from 1990. Davenport RD [2] suggested that ROSE might significantly improve the diagnostic yield of transbronchial aspirates.

Around 2005, "minimally invasive internal medicine" techniques with favorable sensitivity and specificity, including transbronchial needle aspiration (TBNA), began to spread widely. These techniques were not only applied to the diagnosis of lung/mediastinal malignancies but also benign diseases such as sarcoidosis, tuberculosis, etc.

If operators are satisfied with the specimen got through these procedures, it is not necessary to perform more invasive surgeries such as mediastinoscopy, video-assisted thoracoscopic surgery (VATS), and open lung biopsy. Meanwhile, interventional pulmonologists have to answer questions regarding the following: Whether the target specimen is obtained and sufficient? How to deal with target specimen appropriately? Can a preliminary diagnosis be achieved or a wide differential diagnosis spectrum be narrowed? Can patients' disease status and prognosis be analyzed comprehensively in combination with all available clinical and cytological information? Obviously, this "real-time feedback" ROSE information is invaluable.

During interventional procedures, if target specimen is satisfactory, the procedure stops where it should stop, which can not only save time and medical resources but also reduce pain, trauma, and complications. On the contrary, the procedure should be continued, and interventional methods and modalities may have to be changed appropriately. If a preliminary diagnosis is made, differential diagnosis spectrum is narrowed, or disease status is integrated, an important reference may be provided for clinicians to establish a thorough diagnostic protocol and treatment regimen. And it can also help to select processing scheme for target lesion specimen including oncological examinations such as immunohistochemistry, polymerase chain reaction (PCR), chromosome fluorescence in situ hybridization (FISH), electron microscopy, and microbiological examinations such as special staining, grinded tissue culture, etc. And it can also assist in the selection of further means of procedures. In a case for which ROSE in TBNA has provided a relatively definite diagnosis of malignant tumor and obtained satisfactory specimens for follow-up oncology-related examination, the transbronchial lung biopsy (TBLB) with higher risks of complications is then not necessary [3]. The entire interventional diagnostic operation is thus considered optimized. Therefore, ROSE has been widely accepted and utilized during this period [4] and is matured in about 2010 [5, 6].

Since 2010, high-tech equipment represented by virtual bronchoscopy navigation (VBN), ultrathin bronchoscopy, endobronchial ultrasound (EBUS), electromagnetic navigation (EMN) bronchoscopy, etc. was widely used in interventional pulmonary diagnosis and treatment [7, 8]. Due to the high cost of such technical equipment, relatively complicated manipulating process, and expensive consumable items, an extremely high success rate of intervention diagnosis is required; with the addition of the urgent needs of microbial etiology in critical respiratory disease, ROSE has almost become a "standard configuration" in interventional lung disease diagnosis and treatment center.

In 1997, the birth of clone sheep shocked the world, and it showed that single somatic cell could contain almost all the life information. Recently, rapid development and extensive application of molecular diagnostics has brought cytological technology to rejuvenate. At present, the ability to diagnose of cytology is almost compa-

rable to that of histology [9] and is distinctly advantageous in many aspects [10, 11]. ROSE glass slide, as a cell carrier, can not only be used to make cytological interpretation but also can be a "treasure trove" for preserving and studying cells. All cell-based molecular biology and gene technology can be carried out using the ROSE glass slides, including PCR, FISH, immunocytochemistry, second-generation gene sequencing, etc. [11, 12]. The development of biotechnology is at a tremendous pace; especially the progress of molecular biology and genetic technology is beyond imagination. In this scenario, the future of ROSE is anticipated.

1.3 Basic Working Conditions and Equipment Requirements of ROSE

1.3.1 ROSE Cytological Microscope

The main equipment of ROSE is a dedicated cytological microscope, and the ocular lens are usually ×10 (10 times), while the wide-field objective lens are ×10 (10 times) and ×40 (40 times). "Oil-free" × 100 objective lens (100 times) is recommended, which is not only necessary for observing characteristics of microorganisms but also an easy access to get high-quality graphic information.

1.3.2 Graphic Imaging and Photographic System

It should be equipped with high-resolution graphic imaging and photographic system for report making, data summary, case review, academic exchange, clinical education, etc. A high-resolution camera with autofocus function is recommended to integrate on a microscope as its graphic system.

1.3.3 ROSE for Infectious Diseases

In principle, the preparing of infection-related ROSE slides should be carried out in Class II biosafety cabinet. The slides and staining liquor should be specially treated after interpretation. After all, the operators must get biosafety-related training and have the required qualifications.

1.3.4 Location Requirements

ROSE must be positioned at the procedure room, providing primary cytological interpretation and exchanging real-time impression. The advanced interventional pulmonary center may be equipped with a professional ROSE room, which should connect to the procedure site or can show microscope graphic information directly to operators in real time through electronic systems.

1.3.5 Preparation for ROSE

Sterile cytological slides with cell adhesion, absorbent paper, powder-free latex gloves, and disposable 2.5/5 mL syringe needles should be prepared before procedure, and a full set of Diff-Quik (DQ) staining liquor can be poured into sealed glass dyeing cylinders for convenience.

1.3.6 Conservation of Stained Slides

Stained slides and dyeing liquor for infectious diseases should be treated after use following Class II biosafety protocols. It is recommended to place stained cytological slides in a cool and dry place directly for long-term preservation and not to use neutral gum for slide sealing to avoid missing cytological information.

1.4 The Detailed Work Process for ROSE

ROSE is to proceed with the three steps of preparing, staining, and interpreting continuously. As ROSE needs to "guide interventional pulmonary procedures" real time, in clinical practice, preparing, staining, and interpreting of ROSE slides should be accomplished in succession promptly.

1.4.1 The Preparation of Cytological Slides for ROSE

1.4.1.1 Imprinting (Rolling)

It is the most commonly used method, suitable for transbronchial lung biopsy (TBLB), conventional transbronchial needle aspiration (TBNA) with tissue-incising needles (such as Wang's MW-319 needle), mucosa biopsy under direct bronchoscopic vision, medical thoracoscopy biopsy under direct scopic vision, and percutaneous tissue-incising needle lung biopsy.

After target site sampled, the tissue pellets are picked up with a disposable 2.5/5 mL syringe needle from biopsy forceps cup or percutaneous tissue-incising needle groove or are pushed out from tissue-incising needle (such as Wang's MW-319 needle). Then the specimens are smeared roundly on the one-third dyeing side of cytological slide, which should have a strong cell adhesion, with a diameter of about 1 cm and a proper thickness without losing materials for histopathological exam as its premise. After that, the tissue pellets are processed conventionally step by step including pathologic or microbiologic exams, and the target specimen flow direction is optimized according to the results of ROSE interpretation, thus adjusting further process means.

1.4.1.2 Brushing

It is applicable to specimens brushed with ordinary cell brush, protected specimen brush, or ultrafine cell brush, as well as semiliquid specimens including sputum, viscous body fluid, etc. After target site is drawn, the brush tip is pushed out, and the specimens are smeared on the one-third dyeing side of cytological slide, which should have a strong cell adhesion, forming a rectangle of about 1×2 cm. Slides in other processes such as regular slides sent to pathology department and microbiology laboratory should be still prepared according to the conventional methods.

1.4.1.3 Spraying

It is applicable for fine needle aspiration (FNA) and conventional transbronchial needle aspiration (TBNA) with cytological needle such as SW-121, SW-122, SW-521, and SW-522 type of Wang's needle and so on. After target site sampled, the needle tip is pressed against the one-third dyeing side of cytological slide, which should have a strong cell adhesion. As air pressurizing at needle tail, the specimens are smeared roundly with a diameter of about 1 cm and a proper thickness without losing materials for histopathological exam as its premise. Slides in other processes such as regular slides sent to pathology department and microbiology laboratory should be still prepared according to conventional methods.

1.4.1.4 Leaving

It is appropriate to endobronchial ultrasonography (EBUS)-induced transbronchial needle aspiration (TBNA), so called EBUS-TBNA. After target site sampled, the needle tip is press against the one-third dyeing side of cytological slide, which should have a strong cell adhesion, and the tissue paste is pushed out with the inner needle. After most of the tissue specimens are taken away with filter paper hold by pointed tweezers, the cytological material will be left on the slide to become a ROSE film. Then the tissue paste sent to pathology department and microbiology laboratory should be still prepared according to conventional methods. Or, ROSE cytological slides in EBUS-TBNA can also be prepared using the aforementioned "Spraying" method.

1.4.2 Rapid Staining of ROSE Cytological Slides (Staining)

The World Health Organization (WHO) recommends the use of Diff-Quik (DQ) staining liquor to rapidly stain ROSE cytological slides. DQ staining has been modified from Romanowsky stain technology, which has the similar interpreting results to Wright's staining. DQ staining liquor contains acid dye (eosin) and alkaline dye (methylene blue). DQ staining's rationale is the constituents to be dyed have different affinities to staining liquor and show different colors for identifying the morphological characteristics. It consumes very short time (only about 30–70 s) for cytological slides to be stained after the target

site is sampled. Thus, the interpreting process of ROSE forms a "real-time" feedback to interventional procedure because of time-saving preparing and staining.

It is recommended to use "dip" staining rather than "drop" staining to improve quality and efficiency. DQ A solution, DQ B solution, phosphate-buffered saline (PBS), and water are poured respectively in glass vials with lids. Individual ROSE slide is dipped in DQ A solution for 10–30 s and transferred to PBS vial washing DQ A solution. Then the slide is soaked in DQ B solution for 20–40 s and washed in water tank. Finally, residual liquid is removed from slide with bibulous paper. Glass vials holding DQ A solution, DQ B solution, and PBS should be sealed after use because these solutions are volatilizable.

1.4.3 To Interpret ROSE Cytological Slides Promptly and Comprehensively

The stained ROSE slide should be delivered immediately to the assistant and interpreted real timely with specialized cytological microscope. Cytological interpreting impression is indispensable part of the information needed for analyzing disease status comprehensively. In practice, ROSE interpretation should be based on all the available knowledge and clinical information, which should include the following:

1. Multidisciplinary knowledge about respiratory diseases, interventional pulmonology, pathology, clinical microbiology, infectious diseases, oncology, etc.
2. Detailed medical history and physical examination.
3. All the diagnosis and treatment process and development of the disease.
4. Imaging manifestations, especially comparison of imaging data before and after treatment.
5. Laboratory tests, comparison of laboratory data before and after treatment.
6. Manifestations of endoscopic vision and physical properties of the specimens obtained during the interventional procedure.
7. "Real-time" ROSE impression of the cytological interpretation after target site is confirmed and sampled precisely.

1.5 ROSE Implications for Disease/Disease Status

In general, ROSE is significative and putative in the diagnosis and differential diagnosis of lung disease/disease status as listed below:

1. Most common types of solid malignancies and histological typing of tumor.
2. Tuberculosis and its different development stages.
3. Sarcoidosis.
4. Mycoplasma pneumonia.
5. Viral pneumonia.
6. Some kinds of mycotic pneumonia (such as aspergillus, cryptococcus, or candida).
7. Organizing pneumonia or organizing status (i.e., organization) or fibrosis.
8. Pyogenic infection.
9. Necrotic infection or necrotic changes (necrosis).
10. Some kinds of allergic diseases or allergic changes.
11. Some kinds of rheumatic diseases, immune diseases (such as certain types of vasculitis), or immune changes.
12. Others, such as post-chemotherapy immune reconstitution or related changes after lung transplantation.

1.6 Consensus and Controversy in Clinical Practice of ROSE

1.6.1 ROSE and Histopathology/Laboratory Medicine Are Mutual Complementation, Rather than Mutual Repulsion

ROSE is a carrier of cytological information, and it is independent and interrelated among cytology, histopathology, and ecsomatics. ROSE will not compromise the status of histopathology or ecso-

matics in clinical diagnosis. On the contrary, high-quality specimens can be obtained and delivered to the department of pathology and laboratory with the help of ROSE. Thus, the target specimen quality can be controlled, and the use of specimens can be optimized, when it will provide focus of attention to the auxiliary departments without delay. Similarly, the evaluation of ROSE cytological significance should not depend absolutely on whether the histopathology/laboratory examination has "positive results" or not. Cytological interpretation of ROSE is based on its own analysis index. ROSE impressions should be considered as a key component of diagnosis basis and integrated with all the available clinical information. It is not appropriate to excessively limit the flow of specimens according to ROSE impressions unless ROSE diagnosis is definite or the specimen amount is insufficient and further sampling is impossible. It is recommended to add nonstandard specimen inspection process not designed originally, such as special pathogen staining on tissue sections, according to ROSE results, thereby increasing the diagnostic efficiency.

1.6.2 Obtaining Target Lesion Is the Basis of ROSE Interpretation

The interpreting and comprehensive analyzing of ROSE should not be carried out until the specimen is obtained precisely from target lesions. Otherwise, the ROSE interpretation is worthless or even misleading clinical decision. If target lesion is not obtained, interventional modes and modalities should be modified to attempt repeatedly with the help of ROSE.

1.6.3 ROSE Is Not Exactly "Observe the Pathogenic Microorganism Itself"

In some kinds of mycotic pneumonia pathogens (such as aspergillus, cryptococcus, or candida), ROSE can interpret the pathogen directly according to microbial morphology. In case of other infectious diseases like tuberculosis, interpreting ROSE should be based more on cytological background integrated with available clinical information. ROSE is not only a "real-time" state analysis of illnesses but also an auxiliary beforehand anticipation for progression of disease.

1.6.4 It Is Still Controversial Whether ROSE Can Increase the Yield Rate of Diagnostic Interventional Pulmonology

In the 1980s, emergence of ROSE was aimed at improving the yield rate of diagnostic interventional pulmonology [1, 2]. As a carrier of cytological information, ROSE clinical value is continuously explored, and it is utilized further with the development of biotechnology. In recent years, there have been researches to question ROSE's "original intention" in improving diagnostic yield rate.

The controversy is mainly reflected in TBNA for lymph nodes, regardless of conventional lymph node TBNA or EBUS-lymph node TBNA. In cases of conventional lymph node TBNA, some researchers argue that ROSE can improve the positive rate [13, 14], while others thought that it cannot [5]. Similar arguments have been put forward for EBUS-lymph node TBNA. Some argue ROSE's positive value [15–17], especially for lymph node malignancy genotyping [18] or benign diseases [19], when others deem there is no difference [20–22]. However, from the perspective of reducing complications and improving the "exact diagnosis" efficiency, using ROSE is not only recommended in conventional lymph node TBNA [5] but also suggested in EBUS-lymph node TBNA [3], which was demonstrated by a large multicenter study.

In other diagnostic interventional procedures in applications of "high-tech equipment" except of lymph node TBNA, such as pulmonary peripheral lesion TBNA [23], positioning biopsy with peripheral lung radial EBUS (R-EBUS) [24, 25], peripheral lung precise bronchoscopic brushings [26], and positioning biopsy with peripheral pul-

monary EMN [27–29], ROSE is proven to increase the positive rate of diagnostic interventional procedures in majority of current studies. More prospective randomized controlled studies are warranted for further conclusions.

1.6.5 Applying ROSE May Benefit More to the Following Interventional Procedures

1. Procedures applying "high-tech equipment" such as EMN and R-EBUS.
2. Target lesions difficult to sample, such as lesions that cannot directly be viewed through endoscope, very small target lesions, or lesions difficult to access.
3. Procedures with high risk of complications, to minimize the sampled material and stop where it should stop.
4. Short of sampled material may optimize the use of specimens with the help of ROSE preliminary impression.
5. Diagnosis and treatment that should be completed at the same time, such as EMN positioning thermal ablation for pulmonary peripheral nodules.
6. Urgent target lesion assessment for critical respiratory diseases requires timely differential diagnosis and treatment plan.
7. To narrow the spectrum of differential diagnosis or analyze patients' disease status and prognosis in combination with all available clinical and cytological information.
8. "Exact diagnosis" or "immediate diagnosis" must be made in a single procedure or the obvious existence of psychological and objective pressure.
9. Operation demo, academic exchange, technical training, or clinical teaching.

1.6.6 Who Will Interpret ROSE Slides?

ROSE should be completed under the predominance of a clinical (interventional) physician, and so is a comprehensive evaluating process rather than just a histopathology/laboratory process. Staffs involved in ROSE interpretation should be cytopathologists, cytopathological technicians, laboratorians and trained clinical/interventional physicians, nurses, common technicians, interns, etc. [30]. If ROSE report is needed for medical records or charges, it can be issued by a qualified cytopathology physician or laboratorian.

References

1. Pak HY, Yokota S, Teplitz RL, Shaw SL, Werner JL. Rapid staining techniques employed in fine needle aspirations of the lung. Acta Cytol. 1981;25(2):178–84.
2. Davenport RD. Rapid on-site evaluation of transbronchial aspirates. Chest. 1990;98(1):59–61.
3. Eapen GA, Shah AM, Lei X, Jimenez CA, Morice RC, Yarmus L, Filner J, Ray C, Michaud G, Greenhill SR, Sarkiss M, Casal R, Rice D, Ost DE, American College of Chest Physicians Quality Improvement Registry, Education. Complications, consequences, and practice patterns of endobronchial ultrasound-guided transbronchial needle aspiration: results of the AQuIRE registry. Chest. 2013;143(4):1044–53.
4. Gasparini S. It is time for this 'ROSE' to flower. Respiration. 2005;72(2):129–31.
5. Trisolini R, Cancellieri A, Tinelli C, Paioli D, Scudeller L, Casadei GP, Parri SF, Livi V, Bondi A, Boaron M, Patelli M. Rapid on-site evaluation of transbronchial aspirates in the diagnosis of hilar and mediastinal adenopathy: a randomized trial. Chest. 2011;139(2):395–401. https://doi.org/10.1378/chest.10-1521.
6. Diacon AH, Koegelenberg CF, Schubert P, Brundyn K, Louw M, Wright CA, Bolliger CT. Rapid on-site evaluation of transbronchial aspirates: randomised comparison of two methods. Eur Respir J. 2010;35(6):1216–20. https://doi.org/10.1183/09031936.00050809.
7. Asano F, Eberhardt R, Herth FJ. Virtual bronchoscopic navigation for peripheral pulmonary lesions. Respiration. 2014;88(5):430–40. https://doi.org/10.1159/000367900.
8. Zaric B, Stojsic V, Sarcev T, Stojanovic G, Carapic V, Perin B, Zarogoulidis P, Darwiche K, Tsakiridis K, Karapantzos I, Kesisis G, Kougioumtzi I, Katsikogiannis N, Machairiotis N, Stylianaki A, Foroulis CN, Zarogoulidis K. Advanced bronchoscopic techniques in diagnosis and staging of lung cancer. J Thorac Dis. 2013;5(Suppl 4):S359–70. https://doi.org/10.3978/j.issn.2072-1439.2013.05.15.
9. Travis WD, Brambilla E, Noguchi M, Nicholson AG, Geisinger K, Yatabe Y, Ishikawa Y, Wistuba I, Flieder DB, Franklin W, Gazdar A, Hasleton PS, Henderson DW, Kerr KM, Petersen I, Roggli V, Thunnissen E, Tsao M. Diagnosis of lung cancer in small biopsies and cytology: implications of the 2011 International Association for the Study of

Lung Cancer/American Thoracic Society/European Respiratory Society classification. Arch Pathol Lab Med. 2013;137(5):668–84. https://doi.org/10.5858/arpa.2012-0263-RA.
10. Fischer AH, Cibas ES, Howell LP, Kurian EM, Laucirica R, Moriarty AT, Renshaw AA, Zakowski MF, Young NA. Role of cytology in the management of non-small-cell lung cancer. J Clin Oncol. 2011;29(24):3331–2.; author reply 3332–3. https://doi.org/10.1200/JCO.2011.35.2534.
11. Chandra S, Chandra H, Sindhwani G. Role of rapid on-site evaluation with cyto-histopathological correlation in diagnosis of lung lesion. J Cytol. 2014;31(4):189–93. https://doi.org/10.4103/0970-9371.151128.
12. Rekhtman N, Brandt SM, Sigel CS, Friedlander MA, Riely GJ, Travis WD, Zakowski MF, Moreira AL. Suitability of thoracic cytology for new therapeutic paradigms in non-small cell lung carcinoma: high accuracy of tumor subtyping and feasibility of EGFR and KRAS molecular testing. J Thorac Oncol. 2011;6(3):451–8. https://doi.org/10.1097/JTO.0b013e31820517a3.
13. Bruno P, Ricci A, Esposito MC, Scozzi D, Tabbì L, Sposato B, Falasca C, Giarnieri E, Giovagnoli MR, Mariotta S. Efficacy and cost effectiveness of rapid on site examination (ROSE) in management of patients with mediastinal lymphadenopathies. Eur Rev Med Pharmacol Sci. 2013;17(11):1517–22.
14. Madan K, Dhungana A, Mohan A, Hadda V, Jain D, Arava S, Pandey RM, Khilnani GC, Guleria R. Conventional transbronchial needle aspiration versus endobronchial ultrasound-guided transbronchial needle aspiration, with or without rapid on-site evaluation, for the diagnosis of sarcoidosis: a randomized controlled trial. J Bronchology Interv Pulmonol. 2017;24(1):48–58.
15. Nakajima T, Yasufuku K, Saegusa F, Fujiwara T, Sakairi Y, Hiroshima K, Nakatani Y, Yoshino I. Rapid on-site cytologic evaluation during endobronchial ultrasound-guided transbronchial needle aspiration for nodal staging in patients with lung cancer. Ann Thorac Surg. 2013;95(5):1695–9. https://doi.org/10.1016/j.athoracsur.2012.09.074.
16. Cardoso AV, Neves I, Magalhães A, Sucena M, Barroca H, Fernandes G. The value of rapid on-site evaluation during EBUS-TBNA. Rev Port Pneumol (2006). 2015;21(5):253–8. https://doi.org/10.1016/j.rppnen.2015.02.003.
17. Guo H, Liu S, Guo J, Li B, Li W, Lu Z, Sun J, Zhang B, Yu J. Rapid on-site evaluation during endobronchial ultrasound-guided transbronchial needle aspiration for the diagnosis of hilar and mediastinal lymphadenopathy in patients with lung cancer. Cancer Lett. 2016;371(2):182–6. https://doi.org/10.1016/j.canlet.2015.11.038.
18. Trisolini R, Cancellieri A, Tinelli C, de Biase D, Valentini I, Casadei G, Paioli D, Ferrari F, Gordini G, Patelli M, Tallini G. Randomized trial of endobronchial ultrasound-guided transbronchial needle aspiration with and without rapid on-site evaluation for lung cancer genotyping. Chest. 2015;148(6):1430–7. https://doi.org/10.1378/chest.15-0583.
19. Plit ML, Havryk AP, Hodgson A, James D, Field A, Carbone S, Glanville AR, Bashirzadeh F, Chay AM, Hundloe J, Pearson R, Fielding D. Rapid cytological analysis of endobronchial ultrasound-guided aspirates in sarcoidosis. Eur Respir J. 2013;42(5):1302–8. https://doi.org/10.1183/09031936.00128312.
20. Joseph M, Jones T, Lutterbie Y, Maygarden SJ, Feins RH, Haithcock BE, Veeramachaneni NK. Rapid on-site pathologic evaluation does not increase the efficacy of endobronchial ultrasonographic biopsy for mediastinal staging. Ann Thorac Surg. 2013;96(2):403–10. https://doi.org/10.1016/j.athoracsur.2013.04.003.
21. Griffin AC, Schwartz LE, Baloch ZW. Utility of on-site evaluation of endobronchial ultrasound-guided transbronchial needle aspiration specimens. Cytojournal. 2011;8:20. https://doi.org/10.4103/1742-6413.90081.
22. Oki M, Saka H, Kitagawa C, Kogure Y, Murata N, Adachi T, Ando M. Rapid on-site cytologic evaluation during endobronchial ultrasound-guided transbronchial needle aspiration for diagnosing lung cancer: a randomized study. Respiration. 2013;85(6):486–92. https://doi.org/10.1159/000346987.
23. Mondoni M, Sotgiu G, Bonifazi M, Dore S, Parazzini EM, Carlucci P, Gasparini S, Centanni S. Transbronchial needle aspiration in peripheral pulmonary lesions: a systematic review and meta-analysis. Eur Respir J. 2016;48(1):196–204. https://doi.org/10.1183/13993003.00051-2016.
24. Chen CH, Cheng WC, Wu BR, Chen CY, Chen WC, Hsia TC, Liao WC, Tu CY, Shih CM, Hsu WH, Wang KP. Improved diagnostic yield of bronchoscopy in peripheral pulmonary lesions: combination of radial probe endobronchial ultrasound and rapid on-site evaluation. J Thorac Dis. 2015;7(Suppl 4):S418–25. https://doi.org/10.3978/j.issn.2072-1439.2015.12.13.
25. Zaric B, Eberhardt R, Herth F, Stojsic V, Carapic V, Popovic ZP, Perin B. Linear and radial endobronchial ultrasound in diagnosis and staging of lung cancer. Expert Rev Med Devices. 2013;10(5):685–95. https://doi.org/10.1586/17434440.2013.827512.
26. Steinfort DP, Leong TL, Laska IF, Beaty A, Tsui A, Irving LB. Diagnostic utility and accuracy of rapid on-site evaluation of bronchoscopic brushings. Eur Respir J. 2015;45(6):1653–60. https://doi.org/10.1183/09031936.00111314.
27. Loo FL, Halligan AM, Port JL, Hoda RS. The emerging technique of electromagnetic navigation bronchoscopy-guided fine-needle aspiration of peripheral lung lesions: promising results in 50 lesions. Cancer Cytopathol. 2014;122(3):191–9. https://doi.org/10.1002/cncy.21373.
28. Balbo PE, Bodini BD, Patrucco F, Della Corte F, Zanaboni S, Bagnati P, Andorno S, Magnani C. Electromagnetic navigation bronchoscopy and rapid on site evaluation added to fluoroscopy-guided

assisted bronchoscopy and rapid on site evaluation: improved yield in pulmonary nodules. Minerva Chir. 2013;68(6):579–85.

29. Karnak D, Ciledağ A, Ceyhan K, Atasoy C, Akyar S, Kayacan O. Rapid on-site evaluation and low registration error enhance the success of electromagnetic navigation bronchoscopy. Ann Thorac Med. 2013;8(1):28–32. https://doi.org/10.4103/1817-1737.105716.
30. Bonifazi M, Sediari M, Ferretti M, Poidomani G, Tramacere I, Mei F, Zuccatosta L, Gasparini S. The role of the pulmonologist in rapid on-site cytologic evaluation of transbronchial needle aspiration: a prospective study. Chest. 2014;145(1):60–5. https://doi.org/10.1378/chest.13-0756.

2 Anatomic Distribution and Morphology of Common Tracheal/Bronchial/Pulmonary Cells

Jing Feng, Pei Li, Xin Li, and Hongmei Zhou

2.1 Native Tracheal/Bronchial/Pulmonary Cells

2.1.1 Cell Components of Proximal Airways

Ciliary columnar epithelial cells (ciliated cells); goblet cells; brush cells; basal cells; neuroendocrine cells.

2.1.2 Cell Components of Terminal Airways (Bronchioli)

These include ciliary columnar epithelial cells (ciliated cells) and glabrous cells.

Glabrous cells: Glabrous cells are mainly Clara cells; others are bronchiolar neuroendocrine cells (labeled as K, very few) and various types of bronchiolo-epithelial cells (e.g., occasional distal airway basal cells); these cells can be collectively referred to as distal/terminal bronchiolar epithelial cells, or distal airway epithelial cells, or Feng's cells, labeled as F.

2.1.3 Cell Components of Gas Exchange Areas

Type II pneumocytes; Type I pneumocytes.

2.1.4 Other Native Cell Components

Fibroblasts and fibrocytes; glandular cells; myocytes; basement (membrane) cells of blood vessel (difficult to differentiate from airway basal cells); others.

2.2 Common Nonnative Tracheal/Bronchial/Pulmonary Cells

Erythrocytes; neutrophils; eosinophils; basophils; lymphocytes; monocytes; macrophages; histiocytes; epithelioid cells; multinuclear giant cells; others.

Morphology of Common Tracheal/Bronchial/Pulmonary Cells (DQ Staining).

J. Feng (✉)
Division of Pulmonary and Critical Care Medicine, Department of Interventional Pulmonology, Tianjin Medical University General Hospital, Tianjin, China

P. Li
Department of Respiratory Medicine, Taikang Xianlin Gulou Hospital, Nanjing University School of Medicine, Nanjing, China

X. Li
Department of Pathology, Tianjin Haihe Hospital, Tianjin Institute of Respiratory Diseases, Tianjin, China

H. Zhou
Department of Respiratory and Critical Care Medicine, Affiliated Zhongshan Hospital of Guangdong Medical University, Guangzhou, China

J. Feng et al. (eds.), *Rapid On-Site Evaluation (ROSE) in Diagnostic Interventional Pulmonology*,
https://doi.org/10.1007/978-981-13-3456-6_2

2.3 Native Tracheal/Bronchial/ Pulmonary Cell Morphology

2.3.1 Cell Morphology of Proximal Airways

Ciliary columnar epithelial cells (ciliated cells): The cells are roughly cylindrical, with the nucleus located in the middle. The end of the cell body is gradually tapered, the other end has a flat terminal bar, and the terminal bar is attached by pink-stained cilia.

Goblet cells: The cells are polar, the long axis of the nucleus is perpendicular to the long axis of the cell, the nucleus is located at one side which is narrow relatively, and the top of the other side expands, mostly empty vacuoles in cytoplasm, like a stemmed goblet.

Brush cells: Cells are roughly cylindrical and the nucleus is located in the middle. The cell body at one end is tapered, the other end has a flat terminal bar, and the terminal bar is lined with well-arranged microvilli. Another type of brush cells are hairless small fusiform (i.e., tapering at both ends) epithelial cells embedded in pseudostratified ciliated columnar epithelial structure.

Basal cells (reserve cells): The cells are small in size; the nucleus is similar in size to erythrocytes, conical or cuboidal in shape, and progressively smaller in nuclear-to-plasma ratio from deep to superficial; and cytoplasm is gradually increasing and eosinophilic, but nucleoplasm ratio is larger as a whole. These cells form a structure, appearing as a sheet. They are multidirectional stem cells and can differentiate and supplement other types of epithelial cells.

Neuroendocrine cells: The cells are rare and cylindrical or cubic, cytoplasm is abundant, and the overall nuclear-to-plasma ratio is small. Thick neurosecretory granules can be seen in the cytoplasm.

2.3.2 Cell Morphology of Terminal Airways (Bronchioli)

Clara cells: The nucleus size is about 1.2–1.5 times larger than that of erythrocyte; nucleus size of these cells is further increased under disease conditions, but the overall nuclear-to-plasma ratio and morphology still suggest nonmalignant cells. The nuclear chromatin is delicate, overall lightly stained, and occasionally deeply stained under disease conditions.

The chromatin is slightly thick; the cell membrane is thin, incomplete, or even invisible, and there is a clear cytoplasm, but not much, which is grayish blue or gray in color; the nucleus located in the cytoplasm without cell membrane is an important feature of the cell, and there is no polarity; there are also Clara cells without cytoplasm, and they must be differentiated from reactive lymphocytes.

2.3.3 Cell Morphology of Gas Exchange Areas

Type II pneumocytes: Nuclear-to-plasma ratio is relatively small; nuclear is circular or quasi-circular; cytoplasmic staining is deeper compared with lung macrophages and histiocytes; and vacuoles are in the cytoplasm, but have no phagocytosis like macrophages.

Type I pneumocytes: These cells can be seen only in the destruction of large amounts of lung tissue; the nucleus is oval-shaped with thin cytomembrane.

2.3.4 Other Native Cell Morphology

Fibroblasts and fibrocytes: Fibroblasts are large and round, abundant in cytoplasm, darkly stained, cyanophilic, with larger nuclei, and often more than 2 times the diameter of erythrocytes; nuclear membrane is thick and cyanophilic and also has deep staining; the nuclei and the whole size of fibrocytes are smaller than those of the fibroblasts. Fibrocytes are elongated or spindle-shaped, often appearing in an aggregated manner and arranged in series.

Glandular cells: These cells are often sheet structure arranged, with abundant cytoplasm and vacuoles, lightly stained, and neutrophile; nuclear-to-plasma ratio is relatively small; and most of the eosinophilic nuclei are eccentric.

2.4 Common Nonnative Tracheal/Bronchial/ Pulmonary Cell Morphology

Erythrocytes: Erythrocytes have a diameter of 6–9 μm and average 7.2 μm and are light red or gray in DQ staining and often used as a cell size ruler.

Neutrophils: Neutrophils have a diameter of 10–12 μm; cytoplasm in the DQ staining is colorless, the nuclei are darkly stained like curved rods (horseshoes) or lobulated, and the lobulated nuclei have usually 2–5 leaves, which are connected by filaments.

In the transbronchial lung biopsy (TBLB), neutrophils are in a very low magnitude; when there are no obvious infection and bleeding, it is extremely difficult to see neutrophils; generally, when obvious distribution of neutrophils is seen, you can confirm that the relevant infection exists; when the density of neutrophils is relatively high, it can be confirmed that the relevant infection exists and is severe; it should be noted that the density of neutrophil distribution in the mucus/secretory substance is relatively high, so it must be considered comprehensively when making the interpretation.

In the activated phase, neutrophils are mainly composed of rod-shaped nuclei and two-lobule nuclei. The cytomembrane is relatively intact, and the cytoplasm is plump, showing "poisoning changes"; in the necrosis phase, the neutrophils are mainly composed of 3–5 nuclei and have often no membrane and no cytoplasm, showing neutrophil "debris and fragmentation."

In most cases of bacterial infections, "neutrophil phagocytosis of bacteria" is visible, which is of further significance for the interpretation of infections; according to cytology pertinent theory, neutrophils seldom phagocytize "colonized bacteria" but tend to phagocytize "pathogenic bacteria." Neutrophils are found in bacterial and fungal infections, that is, suppurative infections, partial rheumatism, and destructive damages to some lung lesions.

Eosinophils: Eosinophils have a diameter of 13–15 μm, their nuclear shape is similar to neutrophil, and they can have 2–3 leaves, generally two leaves like glasses, and are dark purple stained; cytoplasm may have splintery eosinophilic particles; eosinophilic cytoplasm is pale red; eosinophils are easy to crumble, and particles can be distributed around the cells; when a large number of eosinophils disintegrate, diamond-shaped "Charcot-Leyden" crystals can be formed; and eosinophils are seen in tuberculosis, parasitic diseases, tumors, allergies, etc.

Basophils: They are 10–14 μm in diameter and round, and their cytoplasm contains coarse, different-sized, unevenly distributed, and blue-violet-dyed basophilic granules; granules cover the nuclei, so nuclei of the basophils are often unclear, though their shape looks like the nuclei of the neutrophils with 2–3 leaves, usually 2 leaves; and increased basophils can also be seen in allergic diseases.

Lymphocytes: Lymphocytes are classified into three types, large (11–18 μm), medium (7–11 μm), and small (4–7 μm), according to diameter; medium and small lymphocytes can be seen in TBLB; large lymphocytes can be seen at the time of transbronchial needle aspiration (TBNA); and in TBLB, the nuclear-to-plasma ratio of lymphocytes is more and the cytoplasm is less. In the mature and stable lymphocytes, nuclei are of round type, with more chromatin and deeper staining, and the cytoplasm is blue.

The nuclei of reactive lymphocytes, often appearing in TBLB, are larger; the chromatin is even and loose, and the staining is more light than that in mature and stable lymphocytes; the cytoplasm is very little or no cytoplasm. In TBNA, the large lymphocytes are round, and the cytoplasm is more and light blue; nuclei types are round and can have notch; nuclear chromatin is concentrated, and nucleoli remnants are visible. The small lymphocytes are circular or quasi-circular and pale blue and have little or no cytoplasm and no particles; the nuclei are round, with visible notches and pits; nuclear chromatin clumps, purple, and no nucleoli.

Plasmocytes: These cells, also known as effector B lymphocytes, are derived from the B lymphocytes by the stimulation of CD4+ lymphocytes, so they are consistent with the morphology of some B lymphocytes; plasmocyte diameter is 10–20 μm; nucleus is located on one side, and

double nuclei are seen occasionally; the chromatin is thick and dense, and it is dyed into uneven lilac; there is often a half-moon lightly stained area near the nucleus, and there may be vacuoles in the cytoplasm.

Plentiful of lymphocytes are likely to represent the acute phase of the lesion, seen in various types of inflammatory reactions, viral infections, tuberculosis (especially obvious), some rheumatism, some allergic reactions, and immune responses such as graft versus host. When plasmocytes appear, it is suggested that a chronic phase begins (but does not negate the acute phase).

2.4.1 Mononuclear Cell-Derived Non-epithelial Cells

2.4.1.1 Monocyte-Macrophage

Monocytes have a diameter of 12–20 μm and are round or irregular in shape; occasionally, pseudopodia are visible; nucleus morphology is irregular and can be kidney-shaped, horseshoe-shaped, and lobulated, often accompanied by notches, pittings, and obvious twisting and folding. The nuclear chromatin is more delicate, loose and silky, or cord-like; generally, there is no nucleolus; cytoplasm is more and grayish blue or pink in color; fine purple-red particles are seen in the cytoplasm; once the monocytes migrate into the lungs, they become pulmonary macrophages; therefore, the lung is dominated by its macrophage subtype, and typical monocytes are rare.

Pulmonary macrophages differentiate from monocytes and are widely distributed in the stroma, with more in airways and alveolar septa below the bronchioles; some migrate to the alveoli, so are called alveolar macrophages; macrophages are 9–40 μm in diameter, their nuclei are circular or quasi-circular, and they are rich in cytoplasm and characterized by phagocytosis or being foamy. Early-phase lung macrophages are relatively small, with less cytoplasm and phagocytosis.

Histiocytes: These cells are differentiated from monocytes or transformed from alveolar macrophages (also monocyte origin) after phagocytosis for pathogens (such as tubercle bacillus); cells are of different sizes, generally more than 7 μm, and round, oval, or irregular in shape; cytoplasm is abundant and lightly stained, with thin or even incomplete cell membrane, and can undergo "cytoplasm-shedding" to form naked nuclei; nuclei contain fine vacuoles and show irregular round, oval, long, or kidney shapes, often sidely located; sometimes nucleoli are visible.

Epithelioid cells: Their morphology is similar to epithelial cells, so it is called. Epithelioid cells are the main cellular components of granuloma; they can be directly differentiated from monocytes or from histiocytes or pulmonary macrophages (all of which are monocyte in origin) after phagocytosis and digestion of pathogens (such as the tubercle bacilli containing waxy membrane); they are fusiform or polygonal, rich in cytoplasm, and lightly stained and have thin or even incomplete cell membrane; a considerable part of epithelioid cells can be "cytoplasm-shedding" to form naked nuclei; nuclei have fine vacuoles and are kidney-shaped, crescent-shaped, shoe-like, narrow rod-shaped, or cucumber-shaped, with both ends blunt round.

Lots of histiocytes suggest a chronic phase and the appearance of hyperplasia and reparation (but not negate acute phase).

It can be considered that monocyte-macrophage, histiocyte, and epithelioid cell are different stages of the differentiation and evolution of the same monocyte cell line; during this evolution, the cells gradually become irregular; the cytoplasm gradually increases; the cytomembrane gradually becomes thin and gradually undergoes "cytoplasm-shedding" to form naked nuclei; the nuclei gradually change from a round shape into an irregular shape and then become a kidney shape, a long shape, and a cucumber shape, which are growing longer and longer. These cells can array circularly together with lymphocytes to become multinucleated giant cells or become granuloma directly with more of these cells.

The multinucleated giant cells: More than three or even up to hundreds of epithelioid cells protrude out of cytoplasm, and then the cell bodies approach each other. Finally, the fusion of cytoplasmic protrusion causes the epithelioid cells to align circularly with lymphocytes and fuse together to form the multinucleated giant

cells, so the giant cells are abundant in cytoplasm; the nuclei of epithelioid cells scatter in the cytoplasm of giant cells; multinucleated giant cells in tuberculosis are also called Langhans giant cells.

Mastocytes: Basophils are called mastocytes when they are in connective tissue or in mucosal epithelium. So their structure and functions are similar to those of basophils. Like blood basophils, they have basophilic granules. In DQ staining, they are characterized by the presence of toluidine blue-positive rose-red particles in the cytoplasm.

2.5 The Labeling of Cell Types and Cell States in this Book

In this book, we use two letters, in which the initial one is capitalized, of the cell name as the abbreviation of the cell type for legend, and use the italic form of the two letters, also in which the initial one is capitalized, as the abbreviation of the cell state for legend.

- Ciliated cell, labeled as Ci.
- Glabrous cells: Glabrous cells are mainly Clara cells; others are bronchiolar neuroendocrine cells (labeled as K, very few) and various types of bronchiolo-epithelial cells (e.g., occasional distal airway basal cells); these cells can be collectively referred to as distal/terminal bronchiolar epithelial cells, or distal airway epithelial cells, or Feng's cells, labeled as F.
- Goblet cell, labeled as Go.
- Brush cell (including the glabrous spindle epithelial cell), labeled as Br.
- Basal cell (also known as reserve cell), labeled as Ba.
- Neuroendocrine cell (also known as small granulosa cell), labeled as K.
- Type II alveolar epithelial cells (Type II pneumocytes), labeled as Al2.
- Type I alveolar epithelial cells (Type I pneumocytes), labeled as Al1.
- Fibrocyte, labeled as Fi.
- Fibroblast, labeled as Fb.
- Glandular cell, labeled as Gl.
- Myocyte, labeled as My.
- Erythrocyte, labeled as Er.
- Neutrophil, labeled as Ne.
- Eosinophil, labeled as Eo.
- Basophil, labeled as Bp.
- Lymphocyte, labeled as Ly.
- Plasmocyte, labeled as Pl.
- Mononuclear-macrophage system.
- Migratory macrophage, or monocyte, collectively known as mononuclear cell, labeled as Mo.
- Macrophage, labeled as Ma.
- Histiocyte, labeled as Hi.
- Epithelioid cell, labeled as Ei.
- Multinucleated giant cell, labeled as Gi.
- Mastocyte, labeled as Mc.
- Mucus, labeled as Mu.
- Mesothelial cell, labeled as Me.
- Gomphosis, labeled as Gp.
- Keratinization, labeled as Ke.
- Cytoplasm, labeled as Cp.
- Cell state (italic).
- Hyperplasia, labeled as *Hy*.
- Necrosis, labeled as *Nr*.
- Aggregate, labeled as Ag.
- Giant cell reaction (cytomegalic), labeled as Gc (Cm).
- Some external objects.
- Hypha, labeled as Hp.
- Septum, labeled as Se.
- Spore, labeled as Sp.
- Bifurcate, labeled as Bi.
- Inclusion body, labeled as Ib.
- Amorphous material, labeled as Am.
- Tumor cell, labeled as Tc.
- All the others not mentioned here need to be specially marked.

3 Clustering (Categorizing) Analysis in ROSE Interpretation of Common Nonneoplastic Disease States of Lung/Mediastinum

Jing Feng, Pei Chen, Wei Chen, and Yao Li

For nonneoplastic diseases, ROSE is often the "cytologic version" of histopathology, that is, the manifestation of the cell shedding of corresponding tissue content, so the interpreter should have a deep understanding of the histopathology of corresponding diseases.

It should be noted that ROSE clustering (categorizing) analysis is the most common method we use in ROSE interpretation. This method is used throughout this book.

In general, nonneoplastic disease states of the lung/mediastinum can be classified into the following categories in ROSE interpretation (the detailed classification (clusters) of clustering analysis). Some of these categories can be classified as mild, moderate, and severe according to the specific situations.

J. Feng (✉)
Division of Pulmonary and Critical Care Medicine, Department of Interventional Pulmonology, Tianjin Medical University General Hospital, Tianjin, China

P. Chen
Department of Respiratory Medicine, Xijing Hospital, Air Force Military Medical University, Xi'an, China

W. Chen
Department of Respiratory Medicine, The Affiliated Huaian No.1 People's Hospital of Nanjing Medical University, Huai'an, China

Y. Li
Department of Respiratory Medicine, Shanghai Ninth People's Hospital, Shanghai Jiaotong University School of Medicine, Shanghai, China

3.1 Poorly Prepared ROSE Slides May Result in Meaningless Interpretations

At this point, the interpreter can be prompted to suggest that a bad slide preparation is got, so as to make corresponding corrections in subsequent process.

3.2 "Inflammatory Changes"

The "inflammatory changes" are lacking in specificity, and there are differences in the degree.

The cells of tissue specimen drawn from target anatomic site/lesion (especially airway epithelial cells) are found to have hyperplasia, degeneration, necrosis, and denaturation; occasionally, a small number of inflammatory cells, such as scattered neutrophils, reactive lymphocytes or plasma cells, and excessive alveolar macrophages, are seen.

3.3 Approximately Normal/Mild Non-specific Inflammatory Response

Scattered clear macrophages/clear macrophages are abundant, with mild "inflammatory changes."

J. Feng et al. (eds.), *Rapid On-Site Evaluation (ROSE) in Diagnostic Interventional Pulmonology*,
https://doi.org/10.1007/978-981-13-3456-6_3

3.4 Suppurative Infections (with or without Visible Pathogens)

A variety of inflammatory cells mainly neutrophils, including lots of reactive lymphocytes and macrophages, are seen. Necrosis is obvious. Epithelial cell proliferation, degeneration, necrosis, and denaturation are visible.

3.5 May Conform to Viral Infections; May Conform to Mycoplasma Infections

In viral pneumonia, a variety of inflammatory cells mainly reactive lymphocytes, including scattered neutrophils and macrophages, and varying degrees of "inflammatory changes" are presented; type II pneumocyte hyperplasia is obvious. It may have "cytomegaly and karyomegaly," viral inclusions, "ciliocytophthoria," and some other features.

In mycoplasma pneumonia, a variety of inflammatory cells mainly mononuclear cells (early migratory macrophages), including scattered neutrophils, are presented. "Inflammatory changes" are obvious.

3.6 Granulomatous Inflammation

In inflammation phase, the key feature of "epithelioid cell subpopulation among reactive lymphocytes" is presented; namely, histocytes and/or epithelioid cells are among lots of reactive lymphocytes.

In proliferation phase, many inflammatory cells mainly composed of histocytes and/or epithelioid cells are found; multinucleated giant cells are visible.

3.7 May Conform to Organization

Organization is found secondary to infection or immune reasons. Abundant foamy macrophages aggregate, and scattered reactive lymphocytes and fibroblasts, with or without basophilic necrosis, can be seen.

3.8 May Conform to Fibrosis (Fibroblast Dominant/ Fibrocyte Dominant)

There are abundant fibroblasts and some of them have evolved to fibrocytes.

3.9 Lymphocyte-Based Immune Inflammatory Response

Abundant reactive lymphocytes are presented, and there are varying degrees of "inflammatory changes."

3.10 Eosinophil-Based Immune Inflammatory Response

Abundant eosinophils are presented, and there are varying degrees of "inflammatory changes."

3.11 Proliferative/Reparative Inflammatory Response

Histocytes are dominating, and occasionally multinucleated giant cells and atypical granulomas are seen, with or without different numbers of reactive lymphocytes and plasma cells; varying degrees of "inflammatory changes" may be seen.

3.12 There Are Visible Pathogens, Characteristic Manifestations, or Foreign Objects

It may have hyphae, spores, cysts, bacteria, parasites, and other visible pathogens in the background, and some pathogens may be associated with eosinophils.

3.13 Necrotic "Inflammatory Changes"

Necrosis is evident. Most cells break up and disintegrate in the mucus background, and it is difficult to classify and count these cells.

3.14 Inconclusive Interpretation May Be Reached or the Interpretation Is Inconsistent with Clinical Information

At this point, the interpreter may be prompted to suggest that an invalid specimen is got or suggest that the clinical value of this interventional procedure is insufficient, and further clinical evidence should be sought.

4 Interpretation of ROSE Characteristics of Common Infectious Disease States of Lung/Mediastinum

Jing Feng, Meijun Song, Yue Gu, Yingyan Zhou, and Shifeng Peng

It should be noted that ROSE clustering (categorizing) analysis is the most common method we use in ROSE interpretation. This method is used throughout this book.

Clustering (categorizing) analysis of ROSE characteristics in common infectious disease states of lung/mediastinum [the detailed classification (clusters) of clustering analysis].

4.1 Tuberculosis (Five Subtypes)

The key ROSE features of this disease:

1. "Inflammatory changes"

The "inflammatory changes" are lacking in specificity, and there are differences in the degree.

The cells of tissue specimen drawn from target anatomic site/lesion (especially airway epithelial cells) are found to have hyperplasia, degeneration, necrosis, and denaturation; occasionally, a small number of inflammatory cells, such as scattered neutrophils, reactive lymphocytes or plasma cells, and excessive alveolar macrophages, are seen.

2. Granulomatous inflammation

In inflammation phase, the key feature of "epithelioid cell subpopulation among reactive lymphocytes" is presented; namely, histocytes and/or epithelioid cells are among lots of reactive lymphocytes.

In proliferation phase, many inflammatory cells mainly composed of histocytes and/or epithelioid cells are found; multinucleated giant cells are visible.

3. Lymphocyte-based immune inflammatory response

Abundant reactive lymphocytes are presented, and there are varying degrees of "inflammatory changes."

J. Feng (✉)
Division of Pulmonary and Critical Care Medicine, Department of Interventional Pulmonology, Tianjin Medical University General Hospital, Tianjin, China

M. Song
Department of Emergency Medicine, Ningbo No.2 Hospital, Ningbo, China

Y. Gu
Department of Respiratory and Critical Care, First Hospital, Jilin University, Jilin, China

Y. Zhou
Department of Respiratory Medicine, Ningbo Medical Center Li Huili Hospital, Ningbo, China

S. Peng
Department of Respiratory Medicine, Chengdu Second People's Hospital, Chengdu, China

J. Feng et al. (eds.), *Rapid On-Site Evaluation (ROSE) in Diagnostic Interventional Pulmonology*,
https://doi.org/10.1007/978-981-13-3456-6_4

4. Necrotic "inflammatory changes"
Necrosis is evident. Most cells break up and disintegrate in the mucus background, and it is difficult to classify and count these cells.

4.1.1 Pulmonary Tuberculosis (Nine Cases)

4.1.2 Endobronchial Tuberculosis (Three Cases)

4.1.3 Miliary Pulmonary Tuberculosis (Two Cases)

4.1.4 Mediastinal Tuberculous Lymphadenitis (Two Cases)

4.1.5 Tuberculous Pleurisy (Two Cases)

4.2 Bacterial Pneumonia (Six Cases)

The key ROSE features of this disease:

1. "Inflammatory changes"
The "inflammatory changes" are lacking in specificity, and there are differences in the degree.

The cells of tissue specimen drawn from target anatomic site/lesion (especially airway epithelial cells) are found to have hyperplasia, degeneration, necrosis, and denaturation; occasionally, a small number of inflammatory cells, such as scattered neutrophils, reactive lymphocytes or plasma cells, and excessive alveolar macrophages, are seen.

2. Suppurative infections (with or without visible pathogens)
A variety of inflammatory cells mainly neutrophils, including lots of reactive lymphocytes and macrophages, are seen. Necrosis is obvious. Epithelial cell proliferation, degeneration, necrosis, and denaturation are visible.

3. There are visible pathogens, characteristic manifestations, or foreign objects
It may have hyphae, spores, cysts, bacteria, parasites, and other visible pathogens in the background, and some pathogens may be associated with eosinophils.

4. Necrotic "inflammatory changes"
Necrosis is evident. Most cells break up and disintegrate in the mucus background, and it is difficult to classify and count these cells.

4.3 Viral Pneumonia (Two Cases)

The key ROSE features of this disease:

1. "Inflammatory changes"
The "inflammatory changes" are lacking in specificity, and there are differences in the degree.

The cells of tissue specimen drawn from target anatomic site/lesion (especially airway epithelial cells) are found to have hyperplasia, degeneration, necrosis, and denaturation; occasionally, a small number of inflammatory cells, such as scattered neutrophils, reactive lymphocytes or plasma cells, and excessive alveolar macrophages, are seen.

2. May conform to viral infections
In viral pneumonia, a variety of inflammatory cells mainly reactive lymphocytes, including scattered neutrophils and macrophages, and varying degrees of "inflammatory changes" are presented; type II pneumocyte hyperplasia is obvious. It may have "cytomegaly and karyomegaly," viral inclusions, "ciliocytophthoria," and some other features.

3. Lymphocyte-based immune inflammatory response
Abundant reactive lymphocytes are presented, and there are varying degrees of "inflammatory changes."

4.4 Cytomegalovirus Pneumonia (Two Cases)

The key ROSE features of this disease:

1. "Inflammatory changes"
The "inflammatory changes" are lacking in specificity, and there are differences in the degree.

The cells of tissue specimen drawn from target anatomic site/lesion (especially airway epithelial cells) are found to have hyperplasia, degeneration, necrosis, and denaturation; occasionally, a small number of inflammatory cells, such as scattered neutrophils, reactive lymphocytes or plasma cells, and excessive alveolar macrophages, are seen.

2. May conform to viral infections
In viral pneumonia, a variety of inflammatory cells mainly reactive lymphocytes, including scattered neutrophils and macrophages, and varying degrees of "inflammatory changes" are presented; type II pneumocyte hyperplasia is obvious. It may have "cytomegaly and karyomegaly," viral inclusions, "ciliocytophthoria," and some other features.

3. Lymphocyte-based immune inflammatory response
Abundant reactive lymphocytes are presented, and there are varying degrees of "inflammatory changes."

4.5 Mycoplasma Pneumonia (Three Cases)

The key ROSE features of this disease:

1. "Inflammatory changes"
The "inflammatory changes" are lacking in specificity, and there are differences in the degree.

The cells of tissue specimen drawn from target anatomic site/lesion (especially airway epithelial cells) are found to have hyperplasia, degeneration, necrosis, and denaturation; occasionally, a small number of inflammatory cells, such as scattered neutrophils, reactive lymphocytes or plasma cells, and excessive alveolar macrophages, are seen.

2. May conform to mycoplasma infections
In mycoplasma pneumonia, a variety of inflammatory cells mainly mononuclear cells (early migratory macrophages), including scattered neutrophils, are presented. "Inflammatory changes" are obvious.

3. Necrotic "inflammatory changes"
Necrosis is evident. Most cells break up and disintegrate in the mucus background, and it is difficult to classify and count these cells.

4.6 Aspergillosis (Six Cases)

The key ROSE features of this disease:

1. "Inflammatory changes"
The "inflammatory changes" are lacking in specificity, and there are differences in the degree.

The cells of tissue specimen drawn from target anatomic site/lesion (especially airway epithelial cells) are found to have hyperplasia, degeneration, necrosis, and denaturation; occasionally, a small number of inflammatory cells, such as scattered neutrophils, reactive lymphocytes or plasma cells, and excessive alveolar macrophages, are seen.

2. Suppurative infections (with or without visible pathogens)
A variety of inflammatory cells mainly neutrophils, including lots of reactive lymphocytes and macrophages, are seen. Necrosis is obvious. Epithelial cell proliferation, degeneration, necrosis, and denaturation are visible.

3. Eosinophil-based immune inflammatory response
Abundant eosinophils are presented, and there are varying degrees of "inflammatory changes."