

ORIGINAL ARTICLE

Interventional cytopathology in the COVID-19 era

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Abstract

Introduction: The dramatic spread of COVID-19 has raised many questions about cytological procedures performed in and out of the laboratories all over the world.

Methods: We report a heterogeneous series of fine needle aspirations performed during the period of phase 1 of the lockdown for the COVID-19 pandemic to describe our experience and measures taken during this period.

Results: A total of 48 fine needle aspirations (ultrasound, computed tomography and endoscopic ultrasound guided) were processed and reported.

Conclusions: Pre-existing procedures have been modified to allow healthcare professionals to work safely ensuring patients the necessary assistance with samples suitable for cellularity, fixation and staining for an accurate cytological diagnosis.

KEYWORDS

COVID-19, fine needle aspiration, pandemic, rapid on-site evaluation, safety devices

1 | INTRODUCTION

The outbreak of the COVID-19 pandemic, caused by a new coronavirus (SARS-CoV-2) capable of developing severe acute respiratory syndrome, has strained the whole world. The high contagiousness of the virus has put the various governments and health care systems in considerable distress. Hospitals have undergone a superhuman *tour de force* in many countries. New guidelines and biosecurity measures from disease control and prevention centres have been introduced. In each hospital, elective procedures were deferred while essential clinical services had to be enhanced to reduce the turnout and to allow social distancing.

In this global situation, cytology laboratories and their staff, cytopathologists and cytotechnicians, have faced a new challenge. It is not the first time that cytology laboratories, routinely working with not-fixed material, are in contact with potentially infected samples. However, the dramatic spread of this new virus and the scarce knowledge about the course of its infection have posed many questions about cytological procedures performed into and out of the laboratories.¹ Some questions have not found a definite answer yet, such as: can we continue rapid on-site evaluation (ROSE)? How can the interventional cytopathologists carry out their activities in

complete safety? How should the samples be handled? How can multidisciplinary tumour boards be maintained?

After the initial disorientation and the inevitable management problems, several strategies have been implemented in hospitals to continue ensuring health assistance, taking into account the guidelines by World Health Organization, European Center for Disease Prevention and Control and, in Italy, the Italian National Institute of Health- Istituto Superiore di Sanità.

SARS-CoV-2 appears to be mainly transmitted at the beginning of the incubation period, when affected patients lack symptoms or exhibit non-specific symptoms.^{2,3} Consequently, each patient should be considered potentially infected. The interventional cytopathologists could be unaware that they exposed to the virus while performing fine needle aspiration (FNA) through respiratory droplets, touching contaminated objects or having close unprotected contact with patients. In this view, the Pathology Service of the Vanvitelli University has established some measures to try to contain the infection within its laboratory and to allow cytopathologists to work safely. Among the activities carried out by cytopathologists in our Pathology Service, ROSE and the interventional cytology with execution of FNA in clinic outpatients represent two of our strengths. We report a heterogeneous series of FNA performed during the

period of phase 1 of the lockdown COVID19-pandemic, in order to describe our activity and the relative precautionary measures taken during this period.

2 | MATERIALS AND METHODS

Thirty ultrasound (US)-guided FNAs (nine of lymph node, 12 of breast, three of thyroid, four of salivary gland, two of soft tissue), 15 computed tomography (CT)-guided FNAs (14 of lungs, one in retroperitoneal region) and three endoscopic US (EUS)-guided FNAs (all of pancreas) were performed in our department during the lockdown for COVID 19-pandemic, from 9 March to 4 May 2020. ROSE was performed in 41 cases (23 US-FNAs, 15 CT-FNAs, three EUS-FNAs). Direct smears were obtained in all cases. Cell-block (CB) material was collected in 41 cases (14 lungs, three pancreas, eight lymph node, nine breast, four salivary gland, two soft tissue, one retroperitoneal region) and suspended in formalin. An additional pass was performed in four lymph node FNAs and the material was suspended in 5 mL of PBS for flow cytometry (FC) evaluation. The following combined fluorescein-labelled antibodies were used: CD10, CD19, CD23, FMC7, CD5, CD3, CD2, CD7, CD4, CD8, and κ and λ light chains (Becton Dickinson). In addition, predictive markers were evaluated when necessary, including PD-L1 (seven cases) and ALK (five cases) by immunocytochemistry, ROS1 (five cases) and RET (five cases) by fluorescence in situ hybridisation; BRAF and EGFR (five cases) by next generation sequencing. Immunocytochemical evaluation of oestrogen and progesterone receptors, Ki-67 and HER2, was performed in seven cases.

We implemented some specific measures to limit the possibility of infection, according to the different clinical settings and in collaboration with other specialists involved in the procedures. The number of daily procedures was reduced by 30%-50% to allow the equipment and surfaces shared with the patient to be properly cleaned and disinfected. Furthermore, a limited number of people were admitted to the procedure room. Patients' temperature was measured and each patient underwent point-of-care serological diagnostic tests before starting each procedure. The procedure rooms were differently ventilated (from 60 L/s to at least 160 L/s per patient) depending on whether the ROSE took place in radiology service (CT-guided FNA) or in endoscopy service (EUS-FNA).⁴ Adequate safety devices (personal protective equipment [PPE]) were worn. Standard medical masks or EU FFP2 were considered mandatory for US, CT and EUS-guided procedures. Eye protection, water-resistant long-sleeve gown covers and shoe-covers and were also used for each type of procedure. PPE and waste were properly disposed of.⁵ The operators washed their hands before and after contact with each patient. Fixation time of DiffQuik® staining during ROSE was extended to 60-90 s, while the times of the following staining passes (reagent B and C) were not modified. As for the alcohol-fixed slides subsequently stained by Papanicolaou stain, we used 95% alcohol solutions with fixation times prolonged at least to 30 min.

TABLE 1 Fine needle aspiration cytology diagnoses

Lungs	
Adenocarcinoma	4
Squamous cell carcinoma	3
SCLC	1
NSCLC, NOS	4
Metastasis	1
Negative	1
Total	14
Lymph nodes	
Metastasis	6
Lymphoma	2
BRH	2
Total	10
Breast	
Benign	3
Suspicious	2
Malignant	7
Total	12
Salivary glands	
Warthin tumour	2
Adenoid cystic carcinoma	1
Sialolithiasis	1
Total	4
Pancreas	
Ductal adenocarcinoma	2
Serous cystadenoma	1
Total	3
Soft tissues	
Nodular fasciitis	1
Inadequate	1
Total	2
Thyroid	
Goitre	3
Total	3

Abbreviations: BRH, benign reactive hyperplasia; NSCLC, NOS, non-small cell lung cancer, not otherwise specified; SCLC, small cell lung carcinoma.

3 | RESULTS

A total of 48 FNA were processed and reported in our cytopathology laboratory during the first phase of the COVID-19 pandemic. Samples were adequate and representative in 47 cases. The only inadequate case was a US-guided soft tissue FNA. For all adequate cases, a final cytological diagnosis was rendered (Table 1), in some cases with the aid of ancillary techniques. Predictive markers were assessed on cytological material in nine cases of pulmonary carcinoma, as the patients were declared inoperable by our multidisciplinary lung group. In seven cases of breast carcinoma, the

immunophenotype (including oestrogen and progesterone receptors, HER2 and Ki-67) of the neoplasm was assessed in order to programme a potential neoadjuvant chemotherapy.

4 | DISCUSSION

Current knowledge about detection of viable viruses in clinical samples is limited. Viable SARS-CoV-2 has been mainly isolated from upper respiratory tract and bronchoalveolar lavage fluid samples.⁶⁻⁷ However, SARS-CoV-2 RNA has been detected in other types of samples such as faeces, blood, tears and conjunctival secretions, as well as anal swabs.⁸⁻¹¹

In their commentary, Chen and Chi categorized the cytopathology samples into 3 groups, according to the data published in international literature.^{6,8-12}: high-risk, intermediate-risk, and low-risk for COVID-19 infection.⁴ The high-risk specimens include upper and lower respiratory tract samples, nasopharyngeal and oropharyngeal swabs, sputum, and all types of bronchoscope sampling, blood and bloody samples, faeces and anal swabs, tear drops and conjunctival discharges. Intermediate-risk specimens are pleural and pericardial effusions, and urine. The low-risk specimens include ascites and peritoneal washing, uterine cervical and vaginal smears, cerebrospinal fluid, synovial fluid, and semen.⁴ The high-risk and intermediate-risk samples must be processed in a Class II biosafety cabinet, whereas the processing for low-risk samples can be done according to good microbiological practices and procedures.¹³⁻¹⁴ Formalin and alcoholic solutions with alcohol concentrations above 70%-95% are considered effective to inactivate COVID-19. The effectiveness of other fixatives with lower alcohol concentrations, which are generally used in cytology laboratories, is not established.¹⁵ Since the outbreak of the COVID-19 pandemic, we have limited the number of liquid-based cytology (LBC) processed samples, because the fixatives required by this method contain low alcohol levels (Hologic Inc.).^{15,16} However, if the use of LBC is necessary, a pre-fixation of the sample in a 70% alcohol solution is recommended according to the modified protocol described by Rossi et al,¹⁶ which fully meets technical and safety requirements. A pre-fixation in 70% alcohol solution was used for cytospin preparations. In this circumstance, an adhesive substance was added to the glass slides to increase the cellular adherence. Regarding the preparation of samples during the ROSE, the air-dried smears are fixed in a methanol-based solution (MGG Quick Stain Reagent A, Bio Optica) and the fixation times vary from 20 to 40 s depending on the thickness of the smear and the quantity of blood present in the sample. This activity is certainly high risk for the cytopathologists especially in case of EBUS-TBNA and CT-guided lung FNA. In fact, Pambuccian recently declared that ROSE should be performed during the pandemic only if absolutely necessary to ensure the success of the biopsy procedure and with a high protection for the medical team.¹⁵ We have extended the fixation times for DiffQuik stain to 60-90 s in order to maximize the time of exposition of the virus to alcohol.¹⁷

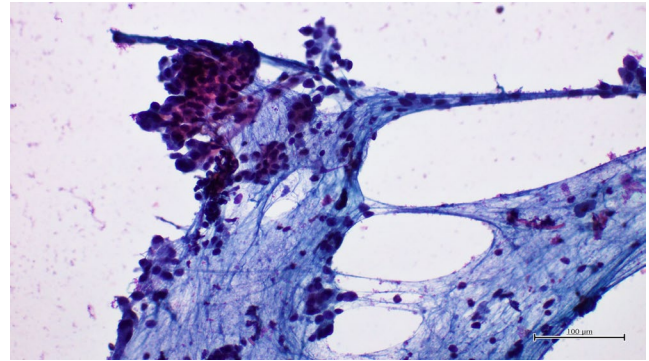


FIGURE 1 Lung adenocarcinoma lymph node metastasis: isolated and aggregated epithelial cells with distorted, hyperchromatic nucleus in a proteinaceous background with debris. (Papanicolaou stain, original magnification 20×)

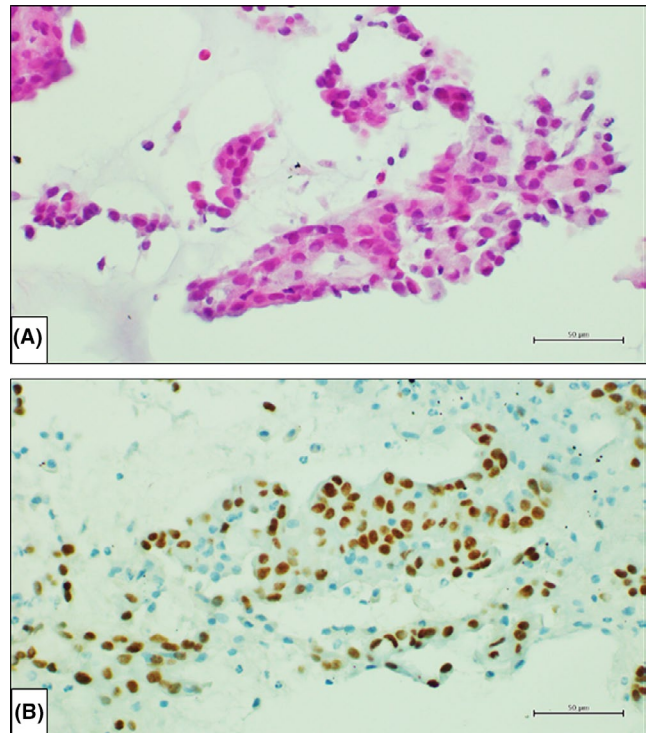


FIGURE 2 (A) Cell-block section haematoxylin-eosin stained: fragments with glandular-like architecture (original magnification 20×). (B) TTF1 immunocytochemical positivity (TTF1 immunostain, original magnification 20×)

Concerning the alcohol-fixed slides subsequently stained by Papanicolaou stain, we are using 95% alcohol solutions with fixation times prolonged to 30 min at least; although this method lowers the yield of the cytological sample, it allows to manage the smears safely. The main artefact is represented by cell distortion due to shrinkage (Figure 1) and the proteinaceous background is dirtier due the presence of debris, but cytological evaluation has never been severely affected in our experience. Importantly, the

extended fixation time in DiffQuik reagent A did not determine any artifacts. Regarding the samples suspended in formalin to set up a CB, as formalin inactivates the virus, the only precaution has consisted in the extension of the fixation time to at least 24 h (Figure 2). The pre-analytical phase for FNA samples is extremely important. The management of the cytological material and the use of different technical supports (eg, vials, additional smears, cytospin slides, LBC slides, FTA-cards, resins) for one ancillary technique rather than another one will also have to take into account the good microbiological practices and procedures. The preparation of cell suspensions in PBS or physiological solution for FC evaluation is performed in order to prevent environmental contamination.¹⁸ All the steps (aliquoting of sample, use of the centrifuge and vortex) for the preparation are carried out under the chemical hood. Our laboratory has chemical hoods with full suction and total expulsion to the outside after HEPA filtration. Furthermore, a post-fixation in formalin is provided, making the preparation of the method reasonably safe. In general, the use of the under-hood set up is intended for all samples that require the use of vortex or cytocentrifuge. All technical personnel dedicated to these procedures have been properly trained and use the appropriate PPE. Obviously, the use of PPE during all these activities is mandatory. After the procedures, the samples are transported to the pathology laboratory inside a bio-box in a secondary container (trans-bag). In our laboratory, the technical procedures have been adapted to this new situation.

For other clinical activities where cytopathologists are involved, such as multidisciplinary tumour boards, clinicians, surgeons, radiologists and pathologists can share clinical data and imaging of patients using web-based video conferencing tools to discuss management of cases.

Finally, the COVID-19 pandemic has put hospitals and pathology laboratories in front of situation unprecedented since the last century. Therefore, many of the pre-existing procedures have been modified and will have to be modified further to allow healthcare professionals to work safely. Certainly, the close collaboration of the different specialists and the continued use of biosecurity measures will ensure the best health care to each patient.

CONFLICT OF INTEREST

The authors have no conflict of interest to report.

AUTHOR CONTRIBUTIONS

Ronchi A.: contributed to the conceptual interpretation of the data; contributed to the statistical analysis and the writing of the manuscript. Pagliuca F.: performed the analysis of the records in the database and provided a language revision of the manuscript; contributed to the conceptual interpretation of the data. Zito Marino F.: contributed to the statistical analysis and the preparation of figures and tables and contributed to the conceptual interpretation of the data. Montella M.: contributed to the conceptual interpretation of the data; performed the analysis of the records in the database and contributed to the writing of the manuscript. Franco R.: contributed

to the conceptual interpretation of the data and editing the manuscript. Cozzolino I.: revised the cytological slides; contributed to the conceptual interpretation of the data and the writing of the manuscript.

DATA AVAILABILITY STATEMENT

Research data are not shared.

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