Description of a New Biosafe Procedure for Cytological Specimens From Patients With COVID-19 Processed by Liquid-Based Preparations

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BACKGROUND: Coronavirus disease 2019 is caused by severe acute respiratory syndrome coronavirus 2 (SARS-CoV-2) and represents the causative agent of a potentially fatal disease. The spread of the infection and the severe clinical disease have led to the widespread adoption of social distancing measures. Special attention and efforts to protect or reduce transmission have been applied at all social levels, including health care operators. Hence, this reports focuses on the description of a new protocol for the safe management of cytological samples processed by liquid-based cytology (LBC) with an evaluation of the changes in terms of morphology and immunoreactivity. **METHODS:** From March 11 to April 25, 2020, 414 cytological cases suspicious for SARS-CoV-2 were processed with a new virus-inactivating method suggested by Hologic, Inc, for all LBC specimens. **RESULTS:** The samples showed an increased amount of fibrin in the background. A slight decrease in cellular size was also observed in comparison with the standard method of preparation. Nonetheless, the nuclear details of the neoplastic cells were well identified, and the immunoreactivity of the majority of those cells was maintained. The cell blocks did not show significant differences in morphology, immunoreactivity, or nucleic acid stability. **CONCLUSIONS:** Despite some minor changes in the morphology of the cells, the results of this study highlight that the adoption of the new protocol for the biosafety of LBC-processed samples in pathology laboratories is important for minimizing the risk for personnel, trainees, and cytopathologists without impairing the diagnostic efficacy of the technique. ***Cancer Cytopathol* 2020;128:905-909**.

KEY WORDS: coronavirus disease 2019 (COVID-19); cytology; diagnosis; liquid-based cytology.

INTRODUCTION

Coronaviruses are enveloped viruses with a positive-sense single-stranded RNA genome. They infect birds and mammals and cause a variety of lethal diseases, and they can also infect humans and cause disease to varying degrees ranging from upper respiratory tract infections resembling the common cold to lower respiratory tract infections such as bronchitis, pneumonia, and even severe acute respiratory syndrome (Hubei Province, China, 2019).[1-6]

In late December 2019, several health facilities reported clusters of patients with pneumonia of unknown cause that were epidemiologically linked to a seafood and wet animal wholesale market in Wuhan, Hubei Province, China.[7]

The causative agent of this unidentified pneumonia has been confirmed to be a novel coronavirus by sequencing and etiological investigations by several independent laboratories in China.[8] The new coronavirus,

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originally called 2019 novel coronavirus (2019-nCoV) and officially renamed severe acute respiratory syndrome coronavirus 2 (SARS-CoV-2) by the International Committee on Taxonomy of Viruses, and the disease it causes, namely coronavirus disease 2019 (COVID-19), have quickly become of tremendous concern worldwide. There have been significant outbreaks in many regions of China as well as global expansion to Asia, Europe, North America, South America, Africa, and Oceania. Person-to-person transmission occurs mostly through contact and respiratory transmission (droplets) but also by the fecal-oral route.[9] For this reason, there is an international push to contain the virus and prevent its spread. The response to the COVID-19 pandemic can be regarded at all social levels (eg, social community, hospital, laboratory, and individual levels).

Because it is possible that infected samples may be submitted to pathology and cytopathology laboratories for diagnostic purposes, it is important for us to take adequate precautions to protect ourselves and our staff. The World Health Organization recommends that all specimens collected for laboratory investigations be regarded as potentially infectious. Health care workers who collect, handle, or transport any clinical specimens should adhere rigorously to the standard precautionary measures and biosafety practices.

The role of the cytology laboratory for a patient with known SARS-CoV-2 is limited, although it may involve the examination of samples from the oropharyngeal and respiratory tract, which is likely to host a significant amount of viruses. Because the laboratory personnel might be exposed to contamination during the preparation and handling of fresh specimens from such patients, a new procedure for the sterilization of material to be processed by liquid-based cytology (LBC) has been applied.[10] This study is focused on a description of this new procedure and on an evaluation of the changes in terms of morphology and cell immunoreactivity that this technique produces in cellular material.

MATERIALS AND METHODS

From March 11 to April 25, 2020, 414 cytological cases considered to be possibly infected by SARS-CoV-2 were sent to the Cytopathology Laboratory of the Agostino Gemelli University Hospital of Rome (IRCCS).

The cytological material was processed in a dedicated high-level biosafe hood by specialized technicians wearing adequate personal protective equipment (eg, mask, face or eyes protection, disposable medical gloves, a disposable water-repellent gown or coveralls with sleeves fully covering the forearms, and shoe covers or dedicated shoes). To each vial is added an amount of 95% alcohol ethanol for at least the same amount of its volume to the material; this is considered the safest way of handling cytological samples infected by SARS-CoV-2. The following is the modified method adopted at the study institution for all LBC specimens processed under the protocol suggested by Hologic, Inc (Marlborough, Massachusetts):

1. Collect the sample in a 70% ethyl alcohol solution.
2. Centrifuge it at 600g for 10 minutes or at 1200g for 5 minutes.
3. Pour off the supernatant fluid and resuspend the cell pellet.
4. Add 30 mL of CytoLyt solution to reduce biological contamination.
5. Centrifuge at 600g for 10 minutes.
6. Pour off the supernatant fluid.
7. Resuspend the cell pellet.
8. Evaluate the cell pellet; if it is necessary, repeat from step 5.
9. Add an appropriate amount of the specimen (depending on the size of the cell pellet) to the PreservCyt solution vial.
10. Allow it to stand in PreservCyt for 15 minutes.
11. Run on a ThinPrep 2000 processor or a ThinPrep 5000 processor.

Immunohistochemistry was performed on either LBC slides or formalin-fixed, paraffin-embedded cell blocks obtained from stored ThinPrep material. All molecular testing was performed on cell block material only. All patients consented to their procedure. We received institutional (Catholic University of the Sacred Heart) ethical approval for this study.

Molecular Analysis

A mutational analysis of epidermal growth factor receptor (EGFR) was performed with the Therascreen EGFR Rotor-Gene Q (RGQ) polymerase chain reaction kit (Qiagen) in the RGQ 5plex high resolution melt analyzer instrument according to the manufacturer's protocol (sensitivity < 1%). The mutation nomenclature used in this work follows the guidelines indicated by the Human Genome Variation Society.[11]

TABLE 1. Summary of Cytological Samples and Distribution of Molecular and Immunohistochemical Analyses

Cytological Sample	Number of Cases	Molecular Analysis, Number	Immunohistochemical Analysis, Number
Thyroid	61	0	15
Urine	90	0	6
liquor in cerebrospinal fluid	45	0	0
Lung/mediastinal FNA	20	3	8
Bronchoalveolar washings	57	0	0
Pleural effusions	91	0	5
Peritoneal effusions	32	0	3
Pericardial effusions	18	0	2

Abbreviation: FNA, fine-needle aspiration.

TABLE 2. Comparison of the Morphological Features of the New Method and the Standard Method

Feature	New Method	Standard Method
Cellular size	Slightly smaller	Normal
Nucleoli	Present	Present
Cytoplasm	No change	Normal
Background	Fibrin, mucus	Clear
Cellularity	Decrease	Normal

RESULTS

The material, processed according to the modified method, consisted of 414 specimens. In all, 61 thyroid specimens, 90 urine specimens, 45 cerebrospinal fluid specimens, 20 lung aspiration specimens, 57 bronchoalveolar washings, 91 pleural effusions, 32 peritoneal effusions, and 18 pericardial effusions were evaluated (Table 1). The series included 186 men and 228 women, and the median patient age was 60 years (range, 20-91 years). The morphological features of the modified and standard methods were compared (Table 2). All cytological samples, particularly the fine-needle biopsies, showed an increased amount of fibrin in the background. A decrease in cellularity in comparison with the standard method of preparation was also noted (Figs. 1-5). In all cytological samples, we observed that the cells were smaller and more scattered in comparison with samples processed with the original technique. Therefore, the distinction between normal, reactive, and atypical cells was slightly more difficult in the samples treated with the modified preparation in comparison with the standard method. Nonetheless, the nuclear details of the neoplastic cells were generally well identified, and the immunoreactivity of the majority of the cells was maintained. The cell blocks taken from the material processed by LBC did not show significant differences in morphology, immunoreactivity, or nucleic acid stability in comparison with the standard LBC method. Molecular test data were available for 3 lung fine-needle aspiration specimens. Approximately 10 ng of genomic DNA was isolated from the samples, quantified, and amplified by polymerase chain reaction (Sanger sequencing). A molecular analysis for EGFR (exons 19 and 21) was ordered for all specimens. Mutations were identified in 2 of the 3 cases. We found 2 EGFR-mutated non–small cell lung

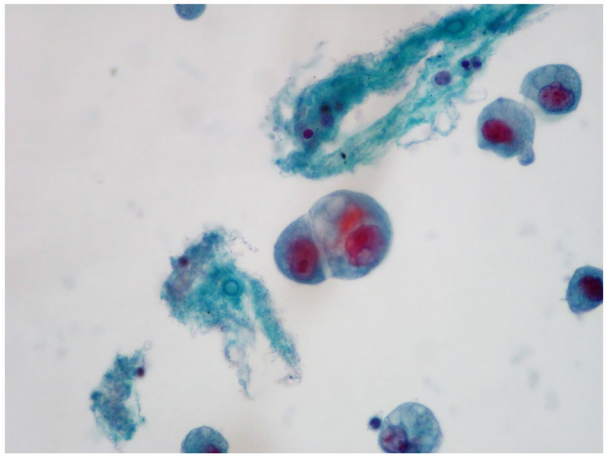

FIGURE 1. Urothelial cells suspicious for high grade urothelial carcinoma (SHGUC) (ThinPrep, Papanicolaou, ×500).

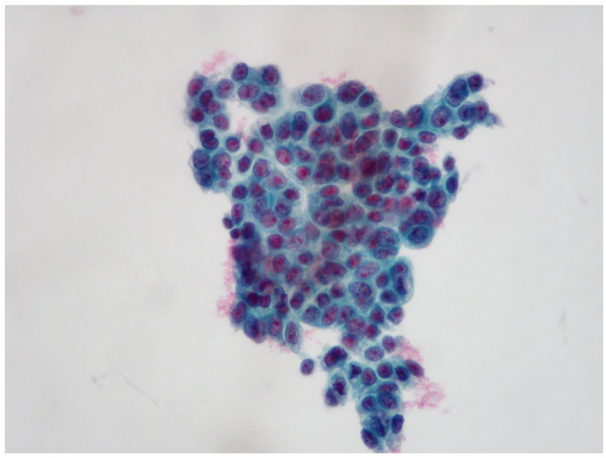

FIGURE 2. Cluster of neoplastic cells from a lung adenocarcinoma (ThinPrep, Papanicolaou, ×500).

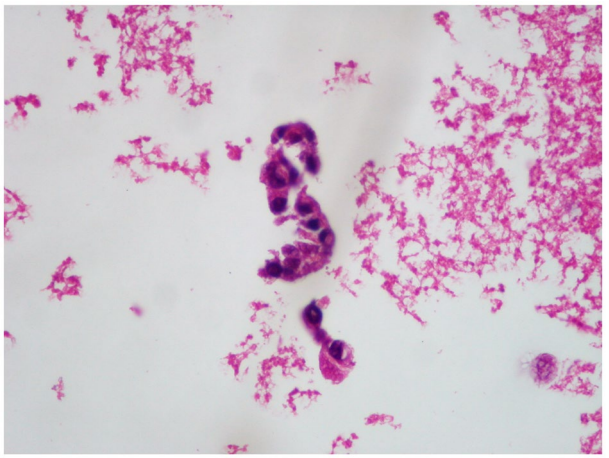

FIGURE 3. Same cells identified in the cell block taken from the sample shown in Figure 2.

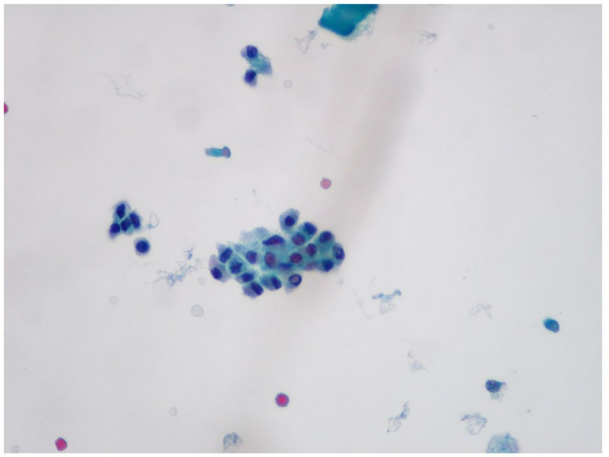

FIGURE 4. Clusters of neoplastic follicular cells of a papillary thyroid carcinoma (ThinPrep, Papanicolaou, ×400).

cancers (1 case with short in-frame deletions of exon 19 and 1 case with a single-nucleotide substitution in exon 21 characterized by the missense mutation p.L858R)

DISCUSSION

Given the extraordinarily fast spread of the disease and the pace of change in the information and procedures concerning how to deal with the various aspects of fighting this infection, one can give only general suggestions for a cytology laboratory's response.[12]

In this study, we report a series of cytological samples processed with a modified protocol that ensures effective biosafety in handling the samples for the staff exposed to the viral load. The use of this protocol

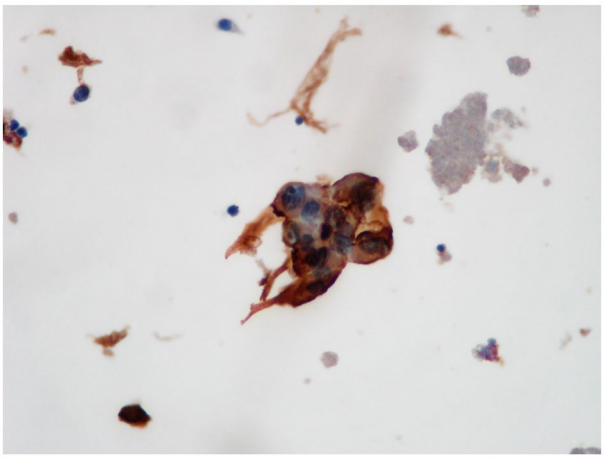

FIGURE 5. Group of neoplastic cells from a nodal metastasis of oropharyngeal carcinoma showing strong positivity for pancytokeratins (Avidin Biotin Complex, ThinPrep, ×500).

suggests that the morphological details and quality of the cellular component can be preserved to achieve the diagnostic efficacy of the original method. Our results show that this modified technique might increase the amount of fibrin in the background, especially for fine-needle aspiration biopsies; this is probably related to the sudden fixation of the hemorrhagic material in a large volume of ethanol. When we analyzed the efficacy of the cytological diagnosis, only minimal differences from the standard procedure, mostly concerning some nuclear details, were noted. In fact, the degrees of nuclear hyperchromasia and nuclear atypia are more difficult to assess only if the cells are less preserved or show artifactual changes.

Despite the difficulty in diagnosing atypical cells due to these overlapping cytomorphological features, the results of our study show that the morphological details, combined with the use of immunohistochemical techniques (whose quality is not affected by the procedure), can lead to a definitive diagnosis of malignancy in the large majority of cases.

As recently reported in the literature,[12-14] during the COVID-19 pandemic, the adoption of strict protocols and guidelines is important for establishing and maintaining a safe work environment. Because the pandemic will probably last for months from this point, the adoption of protocols for the biosafety of the laboratory and the staff will enable the processing of cytological material until the end of the danger and can be useful for future critical situations. Although the modification of the original

protocol results in limited changes in the morphology of cells, the benefits in terms of laboratory biosafety during this COVID-19 pandemic have to be considered significantly more important.

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CONFLICT OF INTEREST DISCLOSURES

The authors made no disclosures.

AUTHOR CONTRIBUTIONS

Patrizia Straccia: Conceptualization, data curation, formal analysis, investigation, methodology, project administration, resources, software, supervision, validation, visualization, writing–original draft, and writing–review and editing. **Esther Diana Rossi:** Data curation, formal analysis, investigation, methodology, resources, validation, visualization, writing–original draft, and writing–review and editing. **Maurizio Martini:** Data curation, investigation, and resources. **Antonino Mulè:** Data curation, investigation, and resources. **Federica Cianfrini:** Data curation, investigation, and resources. **Mariangela Curatolo:** Data curation, investigation, and resources. **Alessandra Cancellieri:** Data curation, investigation, and resources. **Chiara Brunelli:** Data curation, investigation, and resources. **Gian Franco Zannoni:** Data curation, investigation, and resources. **Guido Fadda:** Data curation, formal analysis, investigation, methodology, resources, validation, and writing–review and editing.

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