Diagnostic impact of safety protocols for processing peritoneal washing specimens during the global pandemic of coronavirus disease 2019: A comparative study from 195 cytological samples

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Abstract
Background: The global pandemic of the coronavirus disease 2019 represents a major concern for health services worldwide, and has also induced major changes in cytopathology practice.

Aim: We aimed to verify the diagnostic performance of cytological evaluation under a new safety protocol during the pandemic compared to the standard pre-pandemic procedure. We also aimed to assess how cytological diagnoses and sampling were impacted during the pandemic period compared to the pandemic-free period in 2019.

Materials and methods: Cytological samples of peritoneal washings taken during the first 10 months of the pandemic emergency in Italy (March 11, 2020 to January 11, 2021) were compared to samples from the preceding 10-month time frame (May 11, 2019 to March 10, 2020).

Results: One hundred ninety-five specimens were analysed in the present study. We observed no noticeable differences in cytological diagnoses during the pandemic period compared to the pre-pandemic period. The case numbers by diagnostic category for the pre-pandemic vs pandemic periods, respectively, were as follows: non-diagnostic, 0 vs 0 cases; negative for malignancy, 86 vs 52 cases; atypia of uncertain significance, 7 vs 1 cases; suspicious for malignancy, 0 vs 2 cases; malignant, 42 vs 4 cases.

Conclusion: While a consistent reduction in the number of cytological examinations has been observed during the COVID-19 period, our institutional safety protocol for processing cytological samples did not affect the diagnostic reliability of peritoneal washing cytology.
1 | INTRODUCTION

Intraperitoneal spread of neoplastic cells is a well-established phenomenon occurring in several advanced-stage epithelial neoplasms, which may result in serosal involvement with or without concomitant effusion. Therefore, peritoneal washings (PWs) in patients with abdomino-pelvic neoplasms represent an important additional value that can be easily processed for cytological examination.

Neoplastic cells in PWs indicate intraperitoneal diffusion of the neoplasm beyond the original tumour site; this phenomenon often correlates with a worst prognosis and aggressive biological behaviour.

The prognostic significance of PW cytology is well documented in gynaecological, gastric, pancreatic, and oesophageal tumours. Recently, the worldwide cytopathologists’ community introduced a 5-tier International System for Reporting Serous Fluid Cytology (TIS) using specific diagnostic categories based on well-defined criteria and risk of malignancy (ROM); unsatisfactory (ND), benign (NFM), atypia of uncertain significance (AUS), suspicious for malignancy (SFM), and malignant (MAL). In this regard, a recent paper on the cytology of serous fluids demonstrated the good diagnostic performance of the TIS system in terms of specificity and predictive value, confirming its useful role in the cytological diagnosis of effusions.

The global spread of the coronavirus disease 2019 (COVID-19) has induced major changes in public health services, also affecting the practice of cytopathology. In fact, according to the World Health Organization guidelines, all cytological samples must be processed and handled as they carry an infectious potential. There are only two large institutional experiences reported for cytopathology practice during the pandemic period thus far. Both studies revealed a significant reduction in the number of cytological procedures performed; at the same time, given the prioritisation of oncological procedures, an increase in malignant diagnoses was recorded.

Recent international surveys have investigated major changes related to biosafety procedures affecting cytopathology services worldwide following the spread of the coronavirus disease. The global pandemic has brought about a significant reduction in the number of cytological procedures performed; however, an increase in malignant diagnoses rate has been recorded.

In Italy, a novel method for the decontamination of cytological materials processed by liquid-based cytology (LBC) has been proposed and utilised to protect laboratory personnel from infectious risk. In the present study we aimed to verify the diagnostic performance of cytological evaluation with the new safety protocol for processing cytological samples compared to standard practice. Moreover, we also assessed how cytological diagnoses and sampling were impacted during the pandemic period compared to the pandemic-free period in 2019.

2 | MATERIALS AND METHODS

Data regarding PWs were collected during the 10 months before and after March 11, 2020, the watershed between the standard and the new protocol.

From March 11, 2020, all cytological samples processed in our institution have been considered as potentially infectious. Serous fluids from PWs are processed by specialised technicians wearing protective equipment inside a dedicated biosafe hood. Glass slides are then placed into a 70% alcohol fixative solution and 99% ethanol is added to the solution for decontamination purposes. Cytological materials are prepared by the ThinPrep method of LBC. Specifically, all LBC specimens processed in our laboratory adhere to the following strict protocol to reduce the risk of contamination (see also Table 1):

1. Collect the sample in a 70% ethyl alcohol solution.
2. Centrifuge at 600 g for 10 min or 1200 g for 5 min.
3. Decant the supernatant fluid and resuspend the cell pellet.
4. Utilise CytoLyt solution (30 ml).
5. Centrifuge at 600 g for 10 min.
6. Decant the supernatant fluid.
7. Resuspend the pellet.
8. Examine the pellet and if necessary, repeat from step 5.
9. Enrich the PreservCyt solution vial with an appropriate amount of the specimen.
10. PreservCyt for 15 min.
11. Process the material with a ThinPrep 2000 or 5000 processor.

Cytological specimens before March 11, 2020 were processed with our institutional standard liquid-based cytology protocol. Namely, cytological samples were fixed in methanol-based buffered preservative solution and processed using ThinPrep (Hologic Inc; see Table 1 for details).

For both the pandemic and pandemic-free data, the demographic details and the patient’s history were recorded. PW samples were classified into five categories, according to the well-defined criteria and risk of malignancy (ROM) for serous fluids proposed in 2019 as the TIS: ND, NFM, AUS, SFM, and MAL.
**TABLE 1** Slide preparation methods in pre-pandemic and pandemic period

| Pre-pandemic period slide preparation | Pandemic period slide preparation |
|--------------------------------------|----------------------------------|
| 1. Fix the collected sample in the haemolytic and methanol-based, buffered, preservative solution of CytoLyt™ after rinsing the needle in this solution | 1. Collect the sample in a 70% ethyl alcohol solution |
| 2. Cells are spun at 524 g | 2. Centrifuge sample at 600 g |
| 3. Sediment is transferred to the PreservCyt™ solution | 3. Pour off the supernatant fluid and resuspend the cell pellet |
| 4. Run on a ThinPrep 2000 processor or a ThinPrep 5000 processor. | 4. Add 30 ml of CytoLyt solution to reduce biological contamination |
| 5. Fix the resulting slide in 95% ethanol | 5. Centrifuge at 600 g for 10 min |
| 6. Stain slide with Papanicolaou | 6. Pour off the supernatant fluid |
| 7. Store the remaining material in the PreservCyt solution for further investigation | 7. Resuspend the cell pellet |
| | 8. Evaluate the cell pellet; if 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 specimen to stand in PreservCyt for 15 min. |
| | 11. Run on a ThinPrep 2000 processor or a ThinPrep 5000 processor. |

**TABLE 2** Demographic details of patients in the pre-COVID-19 and COVID-19 periods

|                  | Pre-COVID-19 | COVID-19 |
|------------------|--------------|----------|
| PW (number)      | 135          | 60       |
| Female (%)       | 75 (56%)     | 42 (70%) |
| Male (%)         | 60 (44%)     | 18 (30%) |
| Mean age (years) | 64           | 55       |
| Age range (years)| 19–90        | 41–98    |
| Age distribution |              |          |
| <20 years        | 1 (1%)       | 0 (0%)   |
| 21–40 years      | 4 (3%)       | 0 (0%)   |
| >41 years        | 130 (96%)    | 60 (100%)|

Abbreviation: PW, peritoneal washings.

All five categories determined under the pandemic protocol and the pre-pandemic standard procedure.

Additionally, 48 and 13 PW patients from the pre-pandemic and pandemic periods, respectively, had corresponding peritoneal biopsies.

Continuous data are reported as counts and percentages. Categorical data are reported as counts and percentages. Variations between the two groups in the percentages of all five cytological categories, and the concordance with the peritoneal biopsies, were evaluated using Pearson’s chi-squared or Fisher’s exact test. P-values lower than 0.05 were considered statistically significant.

**3 | RESULTS**

One hundred ninety-five PW specimens were analysed in the present study. In detail, during the 10-month period under the sanitary emergency (March 11, 2020 to January 11, 2021) there was a 56% reduction in PWs performed (n = 60) compared to the 10-month pre-COVID-19 period (May 11, 2019 to March 10, 2020; n = 135). An average age of 63 years (range 19–91 years) was recorded for patients in the pre-COVID-19 period, compared to 54 years (range 41–98 years) during the COVID-19 emergency (Table 2). Concerning the pre-COVID-19 vs COVID-19 rates of benign (40% vs 32%), as opposed to malignant conditions (60% vs 57%) justifying the need to perform PW cytology, we did not observe significant differences. Gynaecological tumours represented half of the neoplastic conditions for both groups (30% vs 29%). Data are summarised in Figure 1. For the breakdown by TIS diagnostic category, no ND samples were observed in either period, while there were 86 (64%) cases of NFM in the pre-COVID-19 and 52 (87%) in the COVID-19 periods (P = 0.060), and 7 (5%) cases of AUS for pre-COVID-19 and 1 (2%) for COVID-19 (P = 0.263). Similarly, there were 0 diagnoses of SFM in the pre-pandemic period and 3 (3%) in the pandemic period (P = 0.009), and 42 (31%) cases diagnosed as MAL for pre-COVID-19 and 4 (7%) for COVID-19 (P = 0.001; see Figure 2, Table 3). Clinical conditions resulting in PW cytological exams during the pandemic period included 41 cases of ascites, 24 of which were due to chronic liver disease, inflammation and heart failure, along with 17 cases associated with malignancy, of which 9 were related to a clinical-instrumental suspicion of carcinomatosis.

During the COVID-19 period there was a 73% reduction of peritoneal biopsies performed compared to the pre-pandemic period (13 against 48). In the pre-COVID-19 period, 97% of MAL serous fluids had biopsy-proven peritoneal involvement (n = 32). Two AUS PWs were unsolved also on biopsies because of inadequate biotic sampling; both cases showed low cellularity and artefactual changes that altered the morphology of malignant cells, invalidating also the immunohistochemical results. The primary tumour was a gastric poorly cohesive carcinoma in one case, and an unknown primary site in the other case. Over the course of the COVID-19 period, 100% of MAL PWs had biopsy-proven peritoneal involvement (n = 4). However, 8 out of 52 NFM PWs underwent peritoneal biopsy, and 3 (38%) had positive results on histology. By comparing the two groups, statistically significant differences were not observed. Examples on cyto-histological comparison between peritoneal washing specimens and corresponding histological samples are illustrated in Figure 3.
Finally, regarding the COVID-19 status of the laboratory staff, none of the staff contracted COVID-19 during processing the fluids due to the precautionary steps taken.

4 | DISCUSSION

The COVID-19 emergency has drastically changed laboratory organisation and cytopathology practice. The potential presence of the coronavirus in cytological samples requires strict biosafety protocols, according to the recent World Health Organization laboratory biosafety guidelines.

Liquid-based cytology still appears to be a safer technical alternative, considering that according to this procedure the cytological specimen is directly collected in the fixative, where it is possible to inactivate the virus, and is processed in a closed system. Moreover, the adoption of TIS diagnostic criteria for reporting serous fluid cytology represents an important step for...
standardising cytological procedures, laboratory handling, and reporting terminology.\textsuperscript{8}

The results from our study, based on 195 PW specimens analysed according to TIS terminology, indicate that significant differences in diagnostic cytological categories during the pandemic and pre-pandemic period were not observed. However, a relative decrease of 24% (31% vs 7%) was recorded in the MAL category. The cases in the benign NFM category increased by 23% (64% vs 87%), however this was not statistically significant (P-value of 0.079).

This difference observed between malignant and benign diagnoses can be explained by (1) a reduction in the number of cytological procedures performed during the pandemic period; (2) the fact that our hospital is considered a tertiary referral centre mainly focused in oncological patients. However, we have also observed an intriguing result: Despite the recommended prioritisation of oncological patients during the COVID-19 pandemic, the number of malignant cases was not superior to the pre-lockdown period. This result seems to be quite different from other studies,\textsuperscript{11,12} which have not only reported a significant reduction in the number of the cytological procedures but at the same time also described an increase in malignant diagnoses. We should note specifically that all analysed samples came from either (1) patients with suspicion for neoplastic/malignant disease; or (2) patients affected by malignant neoplasms, already treated in our hospital. In these latter cases the PW would represent a staging procedure, a prognostic tool, or also a

Figure 3 Cytological/histological comparisons between peritoneal washing specimens and corresponding histological samples. (A) Peritoneal washing sample from a male patient presenting with peritoneal carcinomatosis, demonstrating clusters of atypical cells with increased nucleocytoplasmic ratio and prominent nucleoli (ThinPrep, Papanicolaou, 20\texttimes). (B) The corresponding histological sample confirmed the presence of a moderately differentiated mucinous carcinoma originating from the left colon (H&E, 10\texttimes). (C) Peritoneal washing sample from a female patient presenting with peritoneal carcinomatosis suspicious for ovarian origin. Highly atypical and pleomorphic tumour cells growing in solid sheets and papillary clusters visible in cytological examination (ThinPrep, Papanicolaou, 40\texttimes). (D) The corresponding peritoneal biopsy sample confirmed the presence of malignant papillary aggregates constituted by pleomorphic tumour cells with increased mitotic activity, consistent with ovarian origin (H&E, 20\texttimes). (E) Peritoneal washing sample of a male patient with known history of adenocarcinoma of the gallbladder, presenting with peritoneal carcinomatosis. Focal but highly suggestive aggregates of adenocarcinoma cells with high nucleocytoplasmic ratio and evidence of cytoplasmic vacuoli visible on cytological examination (ThinPrep, Papanicolaou, 20\texttimes). (F) Histological sample confirming the presence of peritoneal dissemination of malignant cells, which formed solid and glandular aggregates (H&E, 10\texttimes) [Colour figure can be viewed at wileyonlinelibrary.com]
component of follow-up programmes in the patient clinical management. In this perspective, despite the decrease of overall malignancy rate, the prioritisation of neoplastic patients has been ensured.

Furthermore, an important finding that emerged from our study is represented by the diagnostic performance of our institutional ethanol-based safety protocol for processing specimens. The latter protocol has caused only very limited changes mainly concerning cellular morphology and nuclear details. By this modified technique, we have observed a slight increase of the fibrin amount in the background, probably due to the rapid fixation of the haemorrhagic material in a large volume of ethanol. Moreover, a decrease in cellularity with smaller and more scattered cells in comparison with the pre-pandemic method of preparation was also noted.

However, based on our routine experience in the pre-COVID period with the standard methanol-based protocol, we did not observe significant differences in cytoarchitectural features as well as diagnostic categories. These results are in line with a previous study reporting the cytological experience with this novel ethanol-based protocol. In fact, ND, AUS, and SFM diagnoses were respectively 0, 7, and 0 during the COVID-19 period, and 0, 1, and 2 in 2019, before the pandemic spread.

Finally, during the COVID-19 period, similar to the reduction in cytological procedures, there was also a 73% reduction of peritoneal biopsies performed as compared to the pre-COVID-19 period. Moreover, for both periods, using different adopted procedures, we can confirm good correlation between MAL cytological diagnoses and biopsy-proven peritoneal involvement on histology, in this way highlighting the role of TIS in confirming the definitive diagnosis of malignancy.

5 CONCLUSION

We reported our institutional experience in the processing and evaluation of cytological specimens during the COVID-19 period, in which a consistent reduction in the number of performed procedures has been observed. Despite high-risk oncological patients having been prioritised, the overall malignancy rate did not increase.

Moreover, given the risks associated with the methanol-based procedure, our results showed that the new described laboratory adaptations to the COVID-19 emergency provided safe procedures for sanitary operation, without compromising diagnostic conclusions and making our experience useful also for other pathological labs dealing with the pandemic. Finally, the implementation of TIS in diagnostic reports has provided good results in terms of diagnostic performance and adequate clinical decision-making.

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CONFLICT OF INTEREST

No conflict of interests are declared.

AUTHOR CONTRIBUTIONS

Conceptualisation: A.S., F.I., G.F.Z. Methodology: P.S., D.A., M.G.M. Software: P.S., D.A., S.S. Validation: G.F.Z., A.S., G.S. Formal analysis: A.S., G.A., F.C. Investigation: A.S., G.A., F.I. Resources: G.S., S.S., N.D.A. Data curation: G.F.Z., E.D.R., D.A. Writing—original draft preparation: A.S., G.A. Writing—review and editing: A.S., M.G.M., G.A., F.I. Supervision: G.F.Z., A.S., E.D.R., N.D.A., G.S. All authors have read and agreed to the published version of the manuscript.

DATA AVAILABILITY STATEMENT

The data that support the findings of this study are available from the corresponding author upon reasonable request.

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