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COVID-19 AND BREAST FINE NEEDLE ASPIRATION CYTOLOGY METHOD: WHAT SHOULD WE CHANGE?

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Dear Professor Sheaff,

On behalf of my co-Authors, I'm submitting the revised version of the manuscript trying to meet the Editor's and Reviewers' comments hoping it can be suitable for publication.

As English language needed to be improved, a mother tongue revised the manuscript.

Reviewer 1: we thank the Reviewer and followed the suggestion to increase the number of references adding 7 more refs.

As suggested, we have added some figures explaining the methods and two cytological pictures; and we have added a couple of tables with the clinico-radiological and cytopathological characteristics of patients evaluated during the two periods.

Finally, we thank the reviewer for the suggestion and we have to synthetized the result by a graph.

Reviewer 2:

We thanks the Reviewer for the suggestion and the manuscript has been revised by a mother tongue in order to improve English language, as well as we have added more tables.

Looking forward to hearing news from You,

Best regards,

Mauro G. Mastropasqua

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COVID-19 AND BREAST FINE NEEDLE ASPIRATION CYTOLOGY METHOD: WHAT SHOULD WE CHANGE?

Safety of fine needle aspiration cytology during COVID-19

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ABSTRACT

Objective: Air-dried slide preparation for fine needle aspiration cytology procedures, is currently considered unsafe because the risk of aerosols of infectious material of Coronavirus 19. This study compares the safety and accuracy of two different protocols, with and without air-dried slides.

Methods: Starting from March 3rd, 2020, we stopped using air-dried slides during breast fine needle aspiration procedures. We selected cases collected during two periods: two months before and two months after the March 3rd. In both groups, the number of procedures was recorded together with the distribution of the diagnostic categories and the concordance between cytological and histological results on surgical specimens for lesions suggestive of malignancy, using the chi-squared test.

Results: Considering the 100 procedures performed during the pre-COVID-19 period, 55% were negative (C2), 3% were non diagnostic (C1) and 40% were positive (C4 or C5). Considering the 75 procedures obtained during the COVID-19 period, 44% were negative (C2), 2.7% were non diagnostic (C1) and 52% were positive (C4 or C5). Despite the use of a new protocol during the COVID-19 period, we observed concordance between cytological and histological results for lesions suggestive of malignancy and there was no statistically significant difference concerning the distribution of the diagnostic categories in the two groups.

Conclusions: Taking into account the slightly lower amount of procedures being analysed during the COVID-19 period, the introduction of a new protocol not including air-dried slides is safe and reliable.

Keywords: COVID 19, Breast, fine needle aspiration, cytology, surgery.

Data availability: Data sharing is not applicable to this article as no new data were created or analysed in this study.

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Permission to reproduce material from other sources: There is no material from other sources.

Clinical trial registration: There is no registration.

INTRODUCTION

Fine Needle Aspiration Cytology (FNAC) has been an essential step in the evaluation of breast lesions since 1968, when Franzen and Zajicek published the first series of cytology results based on 2111 patients, obtaining excellent results [1].

Indeed, FNAC has been confirmed as an excellent method for the diagnosis of both palpable and non-palpable lesions, under Ultra-Sound guidance, with satisfactory levels of sensitivity and specificity [2,3].

In comparison to other breast diagnostic procedures, FNAC has many advantages: it can be performed in the outward clinic, most of the equipment needed for a high-quality product is low-priced and readily available, results are generally available in a few hours with a low rate of complications and good patient tolerability [4,5].

In most breast oncology centres, standard FNAC slide production consists of preparing a thin smear of aspirated material, avoiding crush artefacts. The slides are rapidly air-dried and quickly fixed in 95% ethanol [6]. However, since the outbreak and dramatic spread in the North of Italy of Coronavirus Disease 2019 (COVID-19) [7,8] the preparation of air-dried slides has become a risky procedure due to the aerosols of potentially infectious material. Specimen processing should follow biosafety level 2 guidelines with personal protective equipment (PPE) at all times [9-11].

Further, the risk of infection of mucous membranes of the nose, eyes or mouth from potential COVID-19 contamination of dry surfaces is also a complication [12,13].

In contrast, alcohol fixed smears significantly reduce coronavirus infectivity [14,15] and so are preferred over air-dried ones.

In accordance with this, our Breast Unit at European Institute of Oncology (Milan, Lombardy, the epicentre of the pandemic area), decided to change the FNAC protocol avoiding air-dried slides, in

favour of an alcohol-based method. Samples were ethanol fixed to avoid the production of potentially infectious aerosols during the expulsion of material onto slides for air drying.

However, in order to ensure diagnostic accuracy of the procedure, we aimed to verify the efficacy of this new protocol compared to the standard one.

METHODS

We have retrospectively analysed all breast ultrasound guided FNACs for suspicious lesions performed between January 2020 and May 2020 in our Breast unit.

The 3rd of March was the cut-off between the old and the new protocols.

Selected cases were from routine screening and follow-up programs or symptomatic patients, provided they presented visible lesions on ultrasonography, either palpable or not palpable.

Lesions were morphologically classified according to the Breast Imaging Reporting and Data System (BI-RADS) [16] (Table 1).

Cytological characterization of samples was recorded and classified into five categories (C1-C5) according to European Guidelines for Quality Assurance in Breast Cancer Screening [17] (Table 2).

Surgery was performed when high-risk or malignant lesions (C4 and C5) were detected and the final pathological diagnosis was recorded.

All FNAC procedures were ultrasound-guided and performed by two operators using PPE, specifically FFP2 or FFP3 masks, protective glasses and gloves.

All the operators used the aspiration technique with a 23 Gauge needle connected to a 20 ml syringe with extension tubing (Figure 1).

Following identification of the lesion by ultrasonography and alcohol disinfection of the skin, the needle was inserted into the suspected lesion. The syringe plunger was pulled back by the second operator, creating negative pressure, and cells were collected into the cutting edge of the needle. The needle was then withdrawn from the lesion, and its content expelled onto previously labelled slides. This is the first risky moment that may lead to aerosol/droplet formation.

Smears were prepared by gently spreading the aspirated material, avoiding crush artefacts, using a second slide. The standard procedure prior to COVID-19 involved fixing one slide in 95% alcohol (Figure 2) while the other one was air-dried (Figure 3). The rest of the specimen was put into a ready-to-use "ThinPrep CytoLyt"® solution for subsequent automated processing with ThinPrep 5000 Processor® (according to manufacturer's instruction, Hologic Corporation, Marlborough, MA, USA) (Figure 4). The entire procedure was repeated twice. The slide containers, labelled with the patient data, were sent to the cytology laboratory for analysis along with a complete Cytopathology Requisition Form, including all the pertinent patient history.

During the COVID-19 period, air-dried slides were no longer used due to the high risk of infectious aerosol formation while performing all the cyto-preparatory steps.

For C4 and C5 lesions, we evaluated the accuracy and reliability of the protocols used in the pre-COVID-19 and during COVID-19 periods using subsequent diagnosis on surgical specimens as a reference point.

We also compared the number of cytological procedures performed two months before and two months after the cut-off point (March 3rd). In particular, we compared the percentage of inadequate (C1) results obtained by the COVID-19 protocol, with the percentage of inadequate results obtained with the pre-COVID-19 standard procedure.

Continuous data are reported as median and ranges. Categorical data are reported as counts and percentages.

The Pearson's chi-squared ($\chi 2$) test assessed the concordance between cytological and histological results on surgical specimens in case of lesions suggestive of malignancy. P values less than 0.05 were considered statistically significant.

RESULTS

During the pre-COVID-19 period (January 2nd-March 2nd), 100 FNAC breast procedures were performed compared to 75 procedures made during the COVID-19 period (March 3rd-May 3rd).

Patients' average age was 50 years for both groups (range 25-80 years and 35-65 years).

For the pre-COVID-19 period, 55 out of 100 cases (55%) were negative (C2) on FNAC, 3/100 (3%) were non diagnostic (C1) and 2/100 (2%) were probably benign (C3), subsequently confirmed histologically. The remaining 40/100 (40%) were positive (C4 or C5), in detail: 30/100 were C5 and 10/100 were C4. All these patients underwent surgery, and only 1 (previously diagnosed as C4) resulted negative on histology (Graph).

For the COVID-19 period, 33 out of 75 cases (44%) were negative (C2) on FNAC, 2/75 (2.7%) were non diagnostic (C1) and 1/75 (1.4%) was probably benign (C3), subsequently confirmed histologically. The remaining 39/75 (52%) patients were positive (C4 or C5), in detail: 32/75 were C5 and 7/75 were C4. All these patients underwent surgery, and only 1 (initially diagnosed as C4) resulted negative on histology (Graph).

By comparing the two groups, we observed no statistically significant differences in terms of distribution of the diagnostic categories nor agreement between cytological and histological diagnoses of potentially malignant lesions (Table 3).

DISCUSSION

FNAC is an essential tool for breast cancer diagnosis: its availability, rapidity, cost-effectiveness and low associated risks are the main strengths of this procedure.

Generally speaking, air-dried slides offer optimal definition of cytoplasmic and nuclear features in terms of quality, clear evidence of nuclear shape and dimension, providing useful details for a definite diagnosis of malignancy [18]. This depends on the distention of cells on smears unopposed by the immediate alcohol fixation. In many challenging cases, comparing the morphological features provided by air-dried and alcohol-fixed specimens could be of paramount importance in establishing the diagnosis (Figure 5).

However, the extraordinary COVID-19 emergency forced us to rethink the organization and practices of FNAC considering that, according to the WHO, the preparation of air-dried slides could be potentially dangerous due to the risk of operator contamination with infectious material [10]. The aim of our work was to show the preliminary results of FNAC performed using an exclusively alcohol-based protocol in comparison to the traditional one based on the preparation of air-dried slides.

Our results showed no significant difference between the two procedures. Specifically, we found no differences in the number of diagnoses classified as C1 and quality or quantity of cells. Importantly, for lesions suggestive of malignancy (C4 and C5), the concordance between cytological and histological results was excellent in both protocols.

Therefore, at least in our initial experience, these results suggested the reliability of alcohol-based procedures in terms of diagnosis and safeness.

Moreover, the overall number of breast FNAC procedures during the COVID-19 period was slightly lower compared to that of the pre-COVID-19 period (75 vs 100, respectively), and this was clearly related to the restrictions imposed by the Italian Government. In fact, the percentage of malignant results was higher during the COVID-19 period than that of the pre-COVID-19 period (52% vs 40%, respectively). Such restrictions ended in decreasing number of diagnostic procedures for both screening and follow-up programs, favouring those dedicated to symptomatic patients.

A still safer opportunity is given by Liquid Based cytology. In this case, the specimen is directly collected in the fixative which is warranted to inactivate the virus and processed in a closed system. Furthermore, centrifugation of aspirates allows to obtain cell blocks which can be submitted to histologic evaluation and also immunohistochemical studies.

This is the only procedure we still use for head & neck and lung specimens. The limitation is related on the need of having the instruments and experience in interpreting the slides.

CONCLUSIONS

Given the risks associated with air-dried slide production, our results showed that this new protocol provided safe FNAC procedures during COVID-19 pandemic without compromising diagnostic conclusions.

Emergency management and infection-control measures performed with FNAC in our hospital protected both patients and operators, making this experience useful for other departments dealing with pandemic.

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TABLES

Table 1. Clinico-radiological features

	Pre Covid 19 period	Covid 19 period
Patients (number)	100	75
Age at VABB, mean (years)	50 (25-80)	50 (35-65)
Mean diameter of the lesion (mm)	12 (5-24)	10 (4-25)
Non palpable lesion	91 (91%)	68 (90.6%)

Palpable lesion	9 (9%)	9.3%
Side and location of the lesion		
Right breast	48 (48%)	49 (65.3%)
Left breast	52 (52%)	26 (34.7%)
Upper quadrants	69(69%)	44 (58.6%)
Lower quadrants	31 (31%)	31 (41.4%)
BIRADS [16]		
BIRADS 3	4 (4%)	1 (1.3%)
BIRADS 4a	59 (59%)	40 (53.3%)
BIRADS 4b	9 (9%)	9 (12%)
BIRADS 4c	24 (%)	6 (8%)
BIRADS 5	4 (%)	19 (25.4%)

Table 2. Cytological characterization of samples [17]

CYTOLOGICAL RESULTS		
C1	3 (3%)	2 (2.7%)
C2	55 (55%)	33 (44%)
C3	2 (2%)	1 (1.4%)
C4	10 (10%)	7 (9.3%)
C5	30 (30%)	32 (42.6%)

Table 3. Comparison between FNAC and histopathological final surgical results

A) Pre-Covid 19 Period

	Histology		
FNAC	**Benign**	**Carcinoma**	**Total**
C4	1	9	10
C5	0	30	30
Total	**1**	**39**	**40**

Observed agreement = 97.5% (39/40)
Diagnostic overestimation rate: 2.5%

B) Covid 19 Period

	Histology		
FNAC	**Benign**	**Carcinoma**	**Total**
C4	1	6	7
C5	0	32	32
Total	**1**	**36**	**39**

Observed agreement = 97.4% (38/39)
Diagnostic overestimation rate: 2.5%

GRAPH AND FIGURE LEGENDS

Graph: distribution of C categories in PRECOVID and Covid periods.

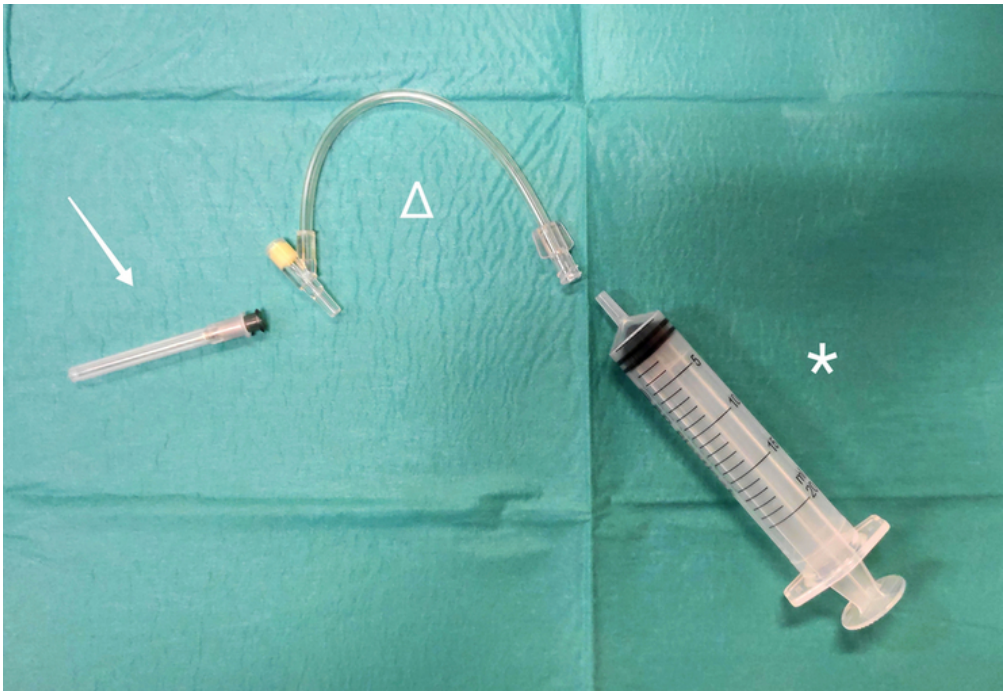
Figure 1. A 23 Gauge needle (arrow) connected to a 20 ml syringe (arrowhead) with extension tubing (asterisk).

Figure 2. Slide fixing in 95% alcohol solution

Figure 3. Air-dried slide

Figure 4. ThinPrep CytoLyt®: solution for subsequent automated processing with ThinPrep 5000 Processor®.

Figure 5a,b. Details of monomorphic large cell population in three-dimensional clusters with pleomorphic nuclei and prominent nucleoli offered by air-dried slides and useful for a definite diagnosis of malignancy (5a: Papanicolau stain, original magnification 20x; 5b: May-Grunwald Giemsa stain, original magnification 40x).

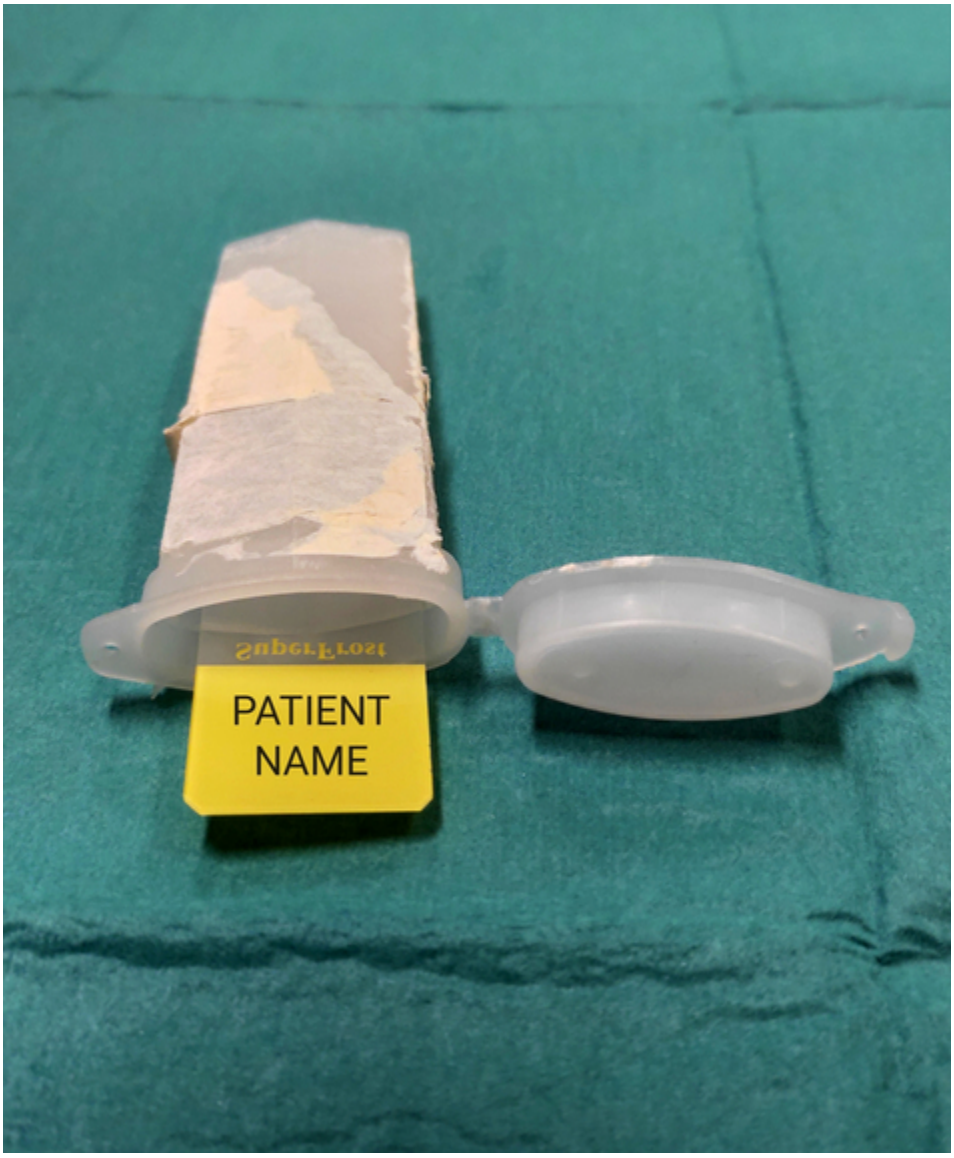


A 23 Gauge needle (arrow) connected to a 20 ml syringe (arrowhead) with extension tubing (asterisk).

62x42mm (300 x 300 DPI)

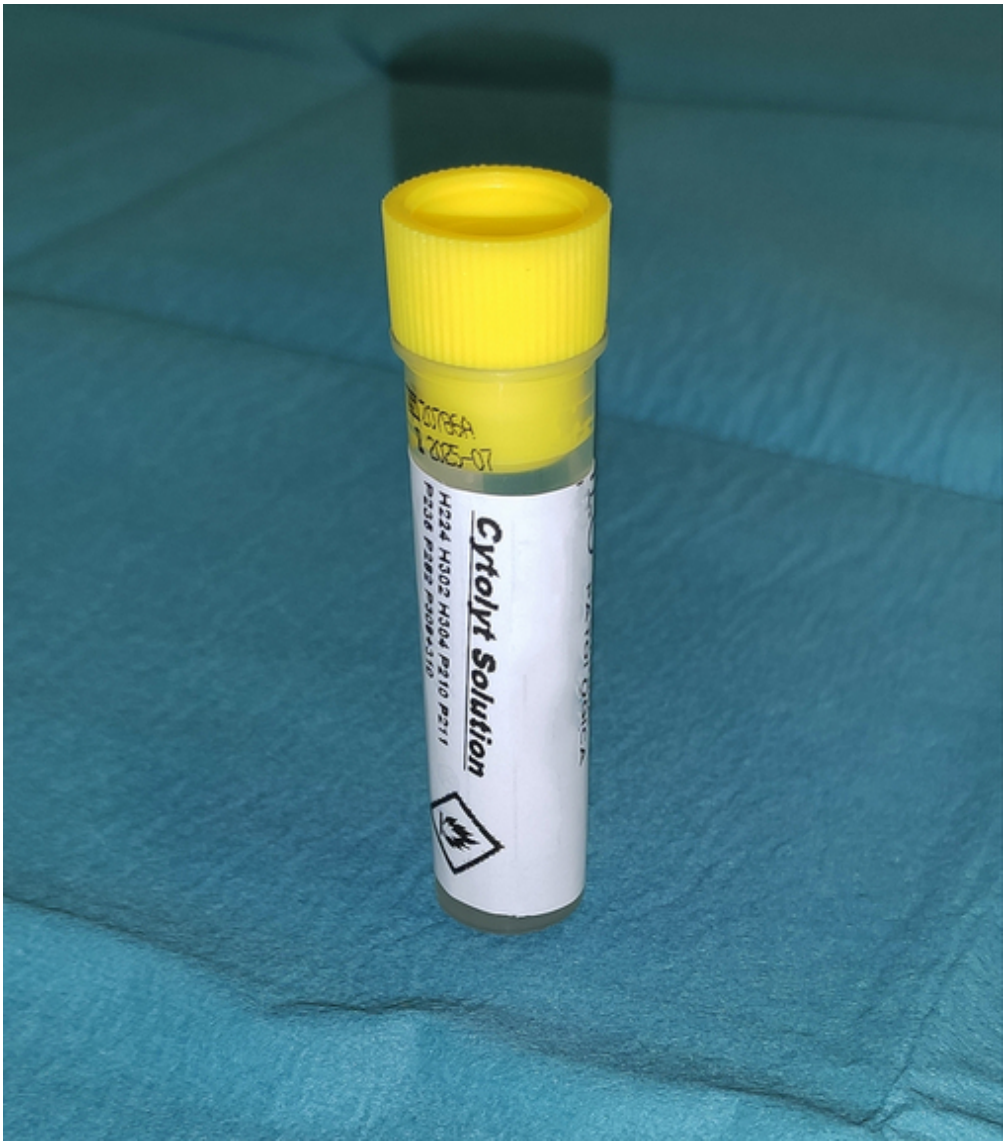
Slide fixing in 95% alcohol solution.

33x48mm (300 x 300 DPI)



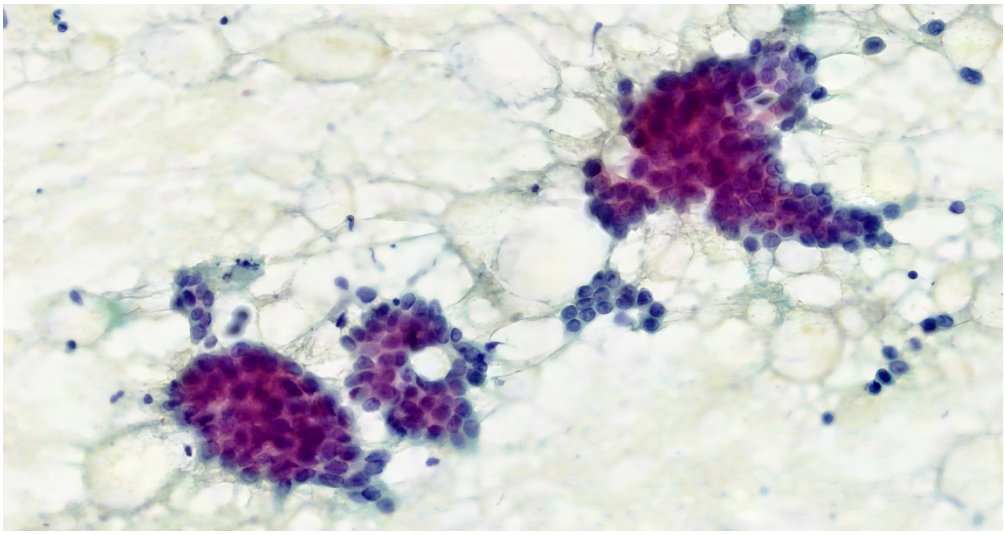
Air-dried slide.

40x48mm (300 x 300 DPI)



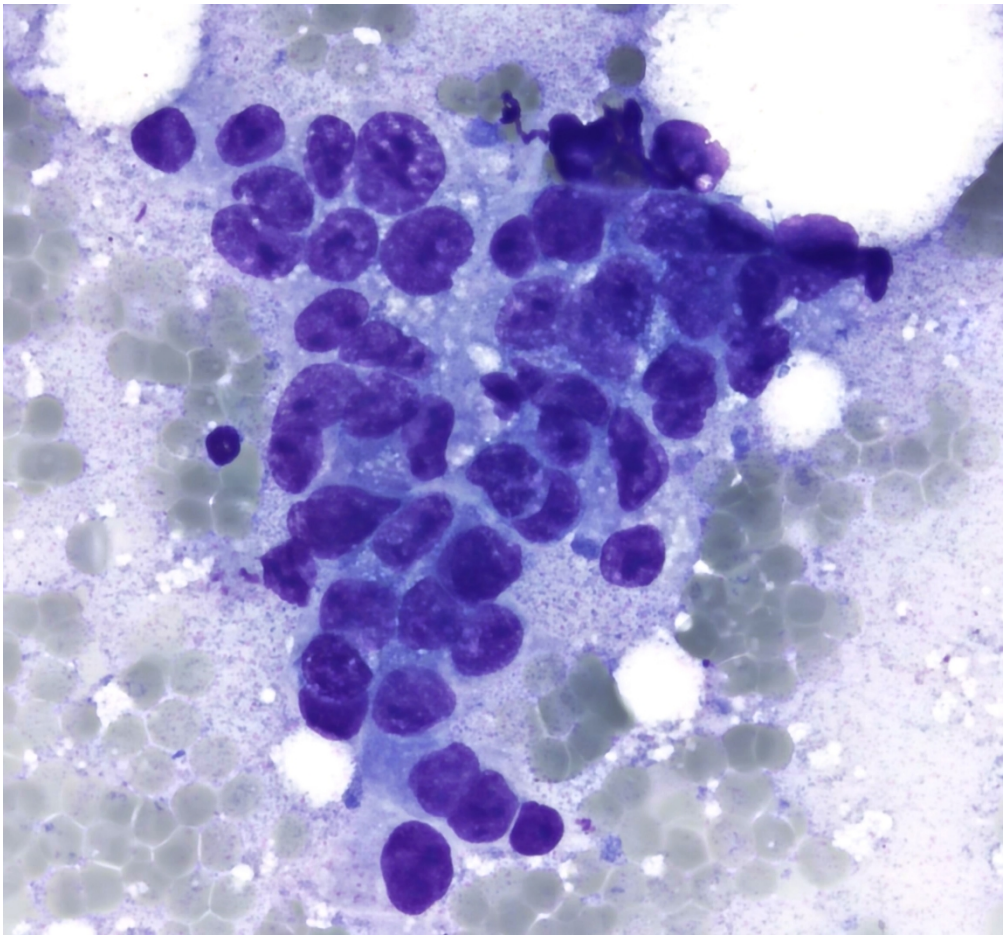
ThinPrep CytoLyt®: solution for subsequent automated processing with ThinPrep 5000 Processor®.

42x48mm (300 x 300 DPI)



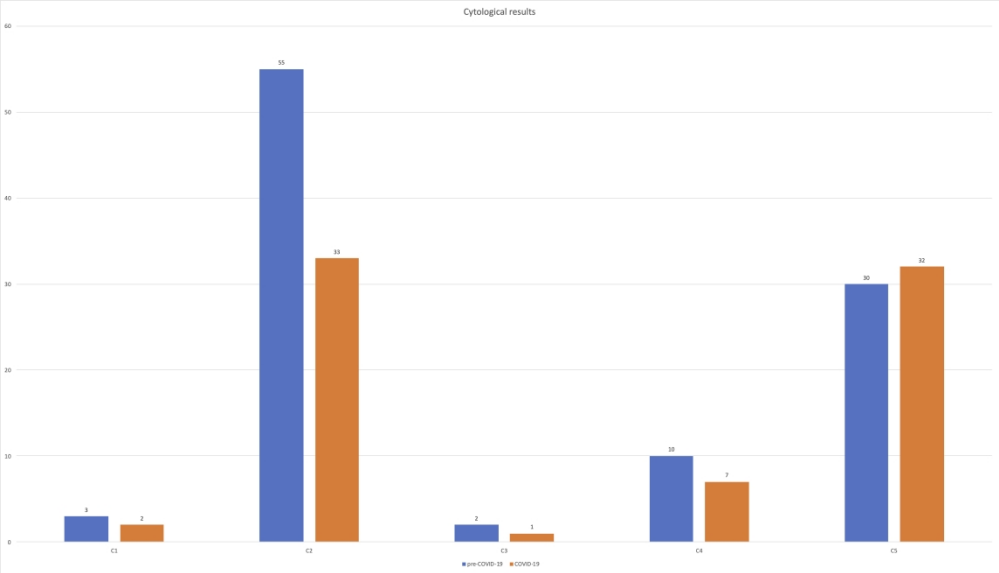
Papanicolau stain, original magnification 20x.

1352x711mm (72 x 72 DPI)



May-Grunwald Giemsa stain, original magnification 40x.

818x762mm (72 x 72 DPI)



Cytological results.

504x289mm (300 x 300 DPI)