An evaluation of the diagnostic adequacy and immunocytochemistry of manual liquid-based smears in breast aspirates

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The aim of this study was to determine if the Syner-Med®/Cell-Solutions® liquid-based cytology (LBC) technique would provide adequate diagnostic material when applied to breast fine-needle aspiration biopsy (FNAB) specimens and to determine its suitability for immunocytochemistry. A prospective study was undertaken of 38 consecutive patients who underwent FNAB of breast masses in the Fine Needle Aspiration Clinic at Tygerberg Hospital, Cape Town, over a period of six months. Conventional smear cytology slides (CSC) were formulated and the material that remained in the needle was used to prepare the LBC Syner-Med®/Cell-Solutions® slides. The CSC and LBC slides were evaluated by two pathologists. The assessed parameters were cellularity, background and representative diagnostic material. Immunocytochemical stains for pancytokeratin (MNF-116) and oestrogen receptor were performed in each case. In 33 cases (87%), LBC compared favourably with CSC. Adequacy rates of 84.2% for CSC and 76.3% for LBC were found. A diagnosis was made in 78.9% of the CSC cases and in 71% of the LBC cases. The LBC slides showed excellent results, with immunocytochemical staining for MNF-116 and oestrogen receptor. The Syner-Med®/Cell-Solutions® LBC fixative and preparation method provides an alternative technique for obtaining well fixed and prepared slides that are suitable for diagnostic cytology and immunocytochemistry.

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Introduction

Breast cancer has become the most frequent cancer in woman in developed and developing countries.[1] The world breast cancer incidence rate (age-standardised incidence) is given as 39 per 100 000. It is 30 per 100 000 population in South Africa, with a lifetime risk of one in 29.[1,2] At present, South Africa does not have an established breast cancer screening programme. As a result, the majority of women who have palpable breast lumps and who present to their healthcare provider do not have easy access to mammographic services. Fine-needle aspiration biopsy (FNAB) provides a diagnostic modality for many women. It is a technically easy and relatively cheap technique, does not require expensive equipment and can be performed at all levels of health care. We undertook a study of a new liquid-based cytology (LBC) technique to explore its diagnostic utility potential for FNAB services.

LBC has been in use for more than seven years in exfoliative cytology.[3,4] It is only in the last few years that it has been applied to general cytology and FNAB specimens. Initial reports suggested that LBC was less than optimal for FNAB of breast lesions, although some studies achieved a sensitivity and specificity of over 90%.[5] Later studies obtained better results using the AutoCyte Prep® (AutoCyte, North Carolina, USA) method in aspirates of breast lesions.[6,7] These studies showed that LBC had good correlation with conventional smear cytology (CSC), with the advantages of easier evaluation of cellular morphology, being less time-consuming, having superior reproducibility, and additional material remaining for possible adjunctive investigations such as immunocytochemistry and flow cytometry.

Using the Auto Cyto Fix 1000® (ACF, Chiba, Japan) method, Yamashita et al[8] found that a rather inexpensive method of LBC yielded results similar to those of CSC and stated that the problems of low cellularity, air drying and blood in the background could be alleviated using their LBC method.

LBC has shown similar results with thyroid lesions, matching CSC for adequacy.[9,10] There may be slight alterations in the cellular morphology in LBC material compared to that of the CSC because of the use of different fixatives.[9]

All the previous studies were performed using LBC from either Cytyc®, AutoCyte® and Auto Cyto Fix 1000®. The first two processes are relatively expensive and require costly equipment for the preparation of the material. By contrast, Syner-Med®/Cell-Solutions® offers a liquid-based cytological preparation that is not automated and is less expensive and therefore more appealing to resource-poor environments. However, this is a relatively new product and few formal trials have been undertaken to validate its use.

The Syner-Med®/Cell-Solutions® fixative has a long shelf life and does not require refrigeration, even after the aspirate has been added to the vials that contain the fixative. This makes it useful in peripheral and rural clinics that might not have optimal storage facilities. Aspirates from these centres are frequently degenerate and poorly fixed. LBC might alleviate this problem as the aspirated material is placed directly into a liquid fixative medium, rather than onto a slide.

The purpose of this study was to determine whether or not the Syner-Med®/Cell-Solutions® LBC preparation, when applied to breast aspirates, would provide adequate, well fixed diagnostic material. Secondly, as immunocytochemistry is required for both diagnostic and prognostic purposes, it was necessary to determine whether immunohistochemistry could be applied successfully to cells that were fixed in this medium. This would be invaluable as more slides could be prepared from a vial than by making smears directly, thereby providing adequate material for immunocytochemical stains using a manual liquid-based preparation method.

Method

Patient population

After obtaining ethical consent from Stellenbosch University, a prospective study that compared two cytological preparation methods was carried out at the Division of Anatomical Pathology of Tygerberg Hospital, Stellenbosch University and the National Health Laboratory Service.

Sequential patients who had been referred to the Department's Fine Needle Aspiration Clinic for aspiration of a palpable breast mass, who were 16 years or older, and who consented to FNAB and the research project, qualified for the study.

Procedure

A split sample technique was used to compare the CSC and manual LBC methods. FNAB, using a 22-G or 23-G needle and a 10-ml syringe, with no local anaesthesia, was performed after the skin had been cleaned with an alcohol swab. On average, two needle passes were performed according to standard procedure. Both needles were rinsed in the same vial of LBC medium, unless the aspirator decided that it was not possible to perform two passes for patient- or safety-related considerations. Conventional smears were prepared from each aspirate by expressing the material onto glass slides, spray fixing one slide for each pass with commercial cytology fixative for Papanicolaou staining, and air drying the other slide for Giemsa staining. The needle and syringe were then rinsed in a vial that contained Syner-Med®/Cell-Solutions® LBC medium. The Syner-Med®/Cell-Solutions® LBC slides were prepared as per the Syner-Med®/Cell-Solutions® manual technique. A slide from every FNAB that was performed was stained on site to determine the adequacy of the FNAB sample.

Two pathologists evaluated the FNAB CSC and LBC slides independently of each other. The pathologists were blinded to the LBC results when reviewing the CSC cases. The methods were compared for cellularity, obscuring factors, informative background and representative diagnostic material. After evaluation of the slides and the issuing of a diagnostic report by the pathologist, immunocytochemical stains were performed on both the CSC and LBC slides using a pancytokeratin antibody (MNF-116 DAKO, 1:50) and the oestrogen receptor antibody (Novocastra, 1:100). The immunocytochemical stains were also evaluated for the proportion and intensity of the staining reaction pattern (Table I).

Table I: Grading of immunocytochemistry for oestrogen and progesterone

Intensity	Proportion
0: Negative	1+: < 1/3 of cells staining
1+: Faint positive staining	2+: > 1/3 < 2/3 of cells staining
2+: Moderate positive staining	3+: > 2/3 of cells staining
3+: Strong positive staining	

Oestrogen- and progesterone-receptor staining were graded based on the system described by Elston and Ellis. This focuses on the intensity of staining and the proportion of positive nuclei.[11]

Grading system

The CSC and LBC slides were graded using a previously described grading system (Table II).[12]

Results

A total of 38 patients were recruited for this study, of whom 37 (97.37%) were women and 1 (2.63%) a man. The mean age was 43.2 years, with an age range of 16-74 years. Two needle passes were performed on 30 patients (78.95%), one needle pass on 7 patients (18.42%), and three needle passes on 1 patient (2.63%). Four slides were made for 29 patients (76.32%), two slide smears for 7 patients (18.42%) and six smears for CSC for 2 patients (5.26%).

Adequacy

With the CSC technique, there was adequate diagnostic material for 30 patients (78.95%), and it was present on the first pass for 27 patients (87.1%) (Table III). The aspirate was

Table II: Adequacy and cellularity grading of the cytology smears[12]

Grading	CSC	LBC
Adequacy		
I	Inadequate, or not representative of lesion.	Inadequate, or not representative of lesion.
II	Suspicious for, but not diagnostic.	Suspicious for, but not diagnostic.
IIIa	Diagnostic of lesion. < 30% of slide covered by material.	Diagnostic of lesion. < 20% per 400 x magnification.
IIIb	Diagnostic of lesion. > 30% of slide covered by material.	Diagnostic of lesion. > 20% per 400 x magnification.
Cellularity		
0	No blood.	No groups of cells.
1	Occasional red blood cells, not obscuring.	Less than 10 groups of cells.
2	Less than 30% of material obscured by blood.	Less than 10, but not more than 20 groups of cells.
3	More than 30% of material obscured by blood.	More than 20 groups of cells.

CSC: conventional smear cytology, LBC: liquid-based cytology

inadequate for diagnosis in 6 of the 38 patients (15.79%), while for 2 cases (5.26%) sufficient cells were present to enable the pathologist to alert the clinician to the possibility of a problematic lesion requiring re-aspiration or referral to a specialist clinic (suspicious for, but not diagnostic of, a malignant lesion). In the LBC group, 27 cases (71.05%) were diagnostic, 10 (26.32%) had inadequate material for a diagnosis, while in 1 (2.63%), a diagnosis of lesion requiring further investigation was made (suspicious for, but not diagnostic of, a malignant lesion).

Table III: Adequacy rates, n = 38

Grade	LBC adequacy	CSC adequacy
I: Inadequate	10 (26%)	6 (16%)
II: Suspicious	1 (3%)	2 (5%)
IIIa: Diagnostic	9 (24%)	19 (50%)
IIIb: Diagnostic, good material	18 (47%)	11 (29%)
Total	**38 (100%)**	**38 (100%)**

CSC: conventional smear cytology, LBC: liquid-based cytology

Cellularity

More than 30% of the slide was covered by material in 13 cases (34.21%) using the CSC technique, less than 30% of the slide was covered by material in 11 cases (28.95%), occasional red blood cells were present, but did not obscure the material in 9 cases (23.68%), while in 5 cases (13.16%) no blood obscured the material. There were more than 20 groups of cells in 10 cases (26.32%) in the LBC slide group, more than 10 but less than 20 groups of cells in 8 cases (21.05%), less than 10 groups of cells in 10 cases (26.32%), while no cells were present on the slides in 10 cases (26.32%).

Immunocytochemistry

Immunocytochemical stains for MNF-116 and the oestrogen receptor protein were performed for 23 cases of CSC and 25 cases of LBC. In the CSC group, 21 cases (91.30%) were positive and 2 (8.70%) were negative for MNF. Of the 21 positive cases, 20 (86.96%) showed strong positivity and 1 (4.35%) weak positivity. The proportion of cells that stained positive was more than two thirds in 11 of the cases (52.38%), more than one third but less than two thirds in 5 cases (23.81%), and less than one third in another 5 cases (23.81%).

In the LBC group, 23 cases (92%) were positive for MNF and 2 (8%) were negative. Seventeen cases (68%) were strongly positive and 6 cases (24%) were weakly positive. The proportion of cells that stained positive was more than two thirds in 12 cases (52.17%), more than one third but less than two thirds in 6 cases (26.09%), and less than one third in 5 cases (21.74%).

Oestrogen receptor in the CSC slides showed positive staining in 13 cases (56.52%) and negative staining in 10 cases (43.48%). There was strong nuclear positive staining in 3 of the cases (13.04%) and weak positive staining in 10 (43.48%). The proportion of nuclei that stained positive was more than two thirds in 1 case (7.69%), more than one third but less than two thirds in 1 case (7.69%), and less than one third in 11 cases (84.62%). In the LBC group, oestrogen receptor was positive in 11 cases (44%) and negative in 14 cases (56%). Three cases (12%) were strongly positive and 8 (32%) were weakly positive. The proportion of nuclei that stained positive was more than two thirds in 1 case (9.09%), more than one third but less than two thirds in 4 cases (36.36%), and less than one third in 6 of the cases (54.55%).

Results

LBC has been used in exfoliative cytology[3,4] for more than seven years. The initial results suggested that LBC was less than optimal for FNAB of breast lesions, although studies showed an overall sensitivity and specificity of over 90%.[5] Later studies showed that LBC had a good correlation with CSC. The advantages of LBC include the fact that it is easier and less time consuming to evaluate cellular morphology, the results are more reproducible and it is easier to provide material for adjunctive investigations, such as immunocytochemistry, flow cytometry and nucleic acid amplification techniques.[13]

This prospective comparative study of CSC and LBC showed adequacy rates of 84.21% for CSC and 73.68% for LBC. A diagnosis was achieved using CSC in 78.95% of cases and using LBC in 71.05% of cases (Table III). Although the small difference in adequacy between the CSC and LBC slides is not significant, this could have been affected by the fact that the majority of the reviewed FNA specimens contained only one LBC slide. The additional slides were processed for immunocytochemistry. A further important factor is that in

order not to subject patients to additional needle passes, the aspirated material was first used for the preparation of the CSC, and the residual material in the needle for the LBC medium. As observed in previous studies,[5] it is believed that this practice would result in paucicellular liquid-based preparations which could account for the rate of suboptimal and unsatisfactory specimens that were observed. If dedicated needle passes are performed for the LBC medium, this will almost certainly increase the adequacy and diagnostic yield.

Cytomorphology compared relatively well between CSC and LBC (Figures 1 and 2). LBC processing offers certain advantages over conventional techniques, such as the absence of obscuring background inflammation and blood, which allows for more rapid screening. The ability to make multiple slides for purposes of immunocytochemistry, particularly automated immunocytochemistry, and nucleic acid amplification tests (NAAT) is a major benefit.[6,14] However, there was decreased cellular preservation on the LBC-processed slides in some cases, compared to that on the conventionally prepared slides. This resulted in a blurring of the nuclear detail (Figures 1 and 2), which was particularly noticeable on the Papanicolaou smears. The fine nuclear detail is important for the diagnosis of well-differentiated duct carcinomas. Therefore, the present study suggests that the utilisation of LBC alone may have reduced diagnostic value with regard to the cytomorphologic interpretation of breast FNA specimens.

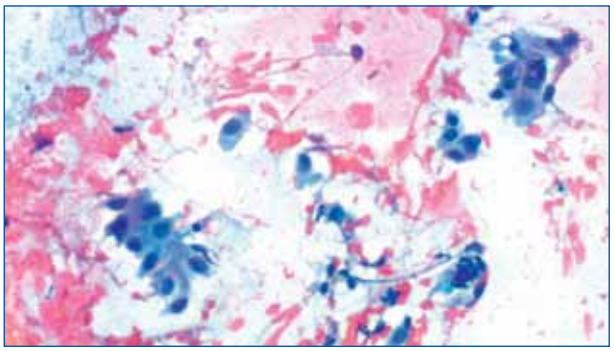

Figure 1: Duct carcinoma. Conventional smear cytology slide showing a discohesive group of ductal cells with hyperchromatic, irregular nuclei, a high nuclear to cytoplasmic ratio, and single cells with intact cytoplasm (Papanicolaou stain x 400)

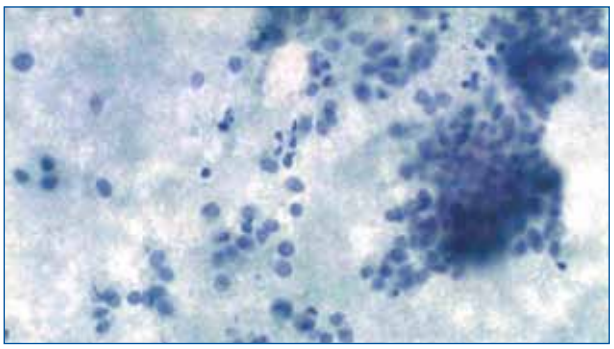

Figure 2: Duct carcinoma. Discohesive groups of ductal cells with very hyperchromatic nuclei and single cells with intact cytoplasm. Liquid-based cytology slide (Papanicolaou stain x 400)

Fine-needle aspirates that are performed by untrained and inexperienced aspirators often result in poorly fixed, bloody aspirates in which the cellular material is not spread across the slide, thus rendering the slides far less interpretable than a decrease in nuclear detail. An affordable liquid-based medium would permit clinicians to rinse their aspirates into a vial containing a liquid fixative. This would allow better harvesting of material from the needle, better fixation and preservation, and diminution of blood.

There was no difference between the staining pattern of CSC and LBC smears with regard to the cytoplasmic (MNF-116) and nuclear (ER) antibodies (Figures 3, 4, 5 and 6). Therefore, this LBC technique would not negatively affect immunohistochemistry staining.

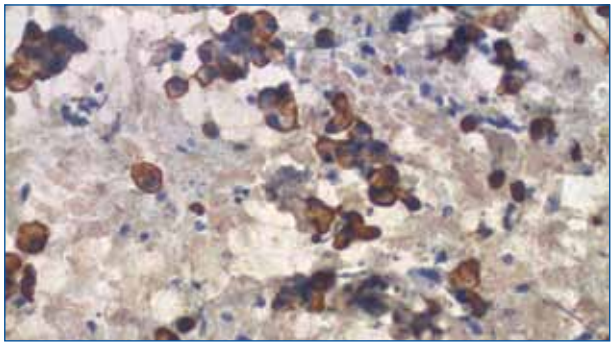

Figure 3: Conventional smear cytology slide. Positive cytoplasmic staining for MNF-116 (x 400)

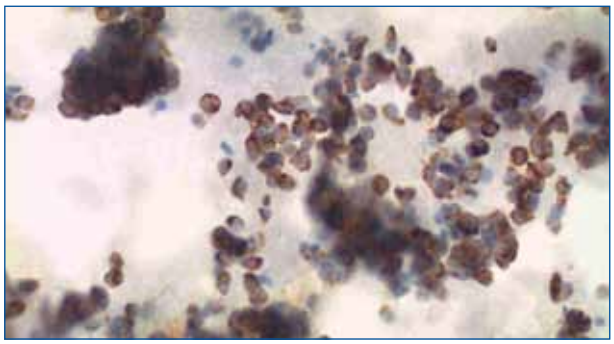

Figure 4: Liquid-based cytology slide. Positive cytoplasmic staining for MNF-116 (x 400)

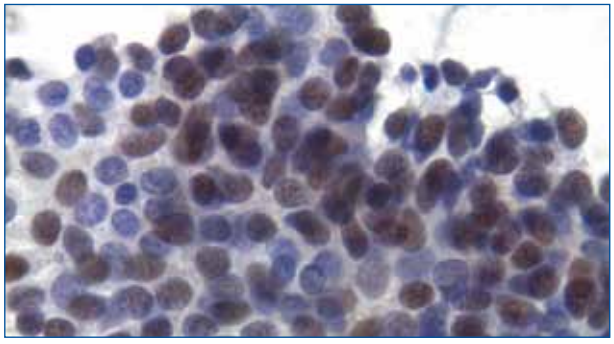

Figure 5: Conventional smear cytology slide. Positive nuclear staining with oestrogen receptor (x 1 000)

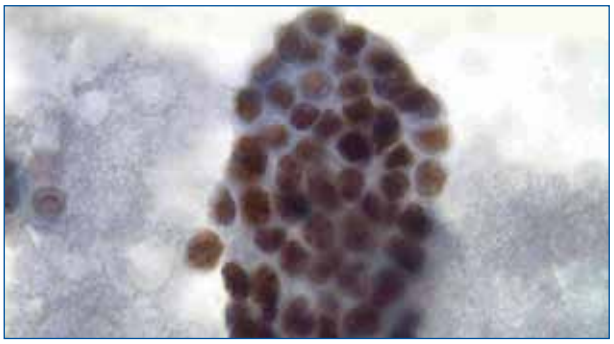

Figure 6: Liquid-based cytology slide. Positive nuclear staining with oestrogen receptor (x 1 000)

Conclusion

In our experience, the Syner-Med®/Cell-Solutions® LBC fixative and preparation method performed well compared to conventional cytology. Syner-Med®/Cell-Solutions® offers an alternative manual LBC method that may appeal to laboratories that are unable to employ automated systems.

Conflict of interest

There is no conflict of interest to declare. The authors have no interest, financial or other, in Syner-Med®/Cell-Solutions® or any of its associated companies.

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