Cell block specimens applied to diagnostic routine of thyroid fine-needle aspiration biopsy

Aplicação do emblocado em parafina ou cell block na rotina diagnóstica de biópsia aspirativa por agulha fina de tireoide

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ABSTRACT

Introduction: Various preparations can be used in diagnostic cytology, including conventional smears (CS), liquid-based preparations (LBP) and cell block (CB). Objective: The aim of this study is to evaluate the quality of CB preparations in addition to conventional cytological specimens in cases of fine-needle aspiration biopsy (FNAB) of thyroid nodules in diagnostic routine. Method: One hundred and six consecutive cases of FNAB routine thyroid nodules were independently evaluated by two cytopathologists (Obs1 and Obs2) on the cellularity of SM, LBP and CB. Results: The cellularity was rich/moderate in 56 (52.8%) CBs for both observers. LBP showed rich/moderate cellularity in 86 (81.1%) cases for Obs1 and 91 (85.8%) for Obs2; among these cases, CB showed the same cellularity in 52/86 (60.4%) cases for Obs1 and 54/91 (59.3%) for Obs2. SM showed rich/moderate cellularity in 86 (81.1%) cases for Obs1 and 87 (82%) for Obs2; among these cases, CB showed the same cellularity in 48/86 (55.8%) cases for Obs1 and 54/87 (62%) for Obs2. CB cellularity was higher than that in LBP in only five cases for Obs1 and three for Obs2. LBP was assessed as low/absent in only five (4.7%) and six (5.6%) cases for Obs1 and Obs2, respectively. Conclusion: CB can be routinely used as additional specimen in material obtained from thyroid nodules FNAB, without adversely affecting LBP specimens, enabling the conduction of further immunohistochemical and molecular studies.

Key words: thyroid gland; fine-needle aspiration biopsy; cytological techniques.

INTRODUCTION

In diagnostic cytology, including fine-needle aspiration biopsy (FNAB), morphological diagnosis was initially based on conventional cytological preparations, characterized by air-dried and wet-fixed smears (conventional smears [CS]) stained with Romanowsky and Papanicolaou methods. Other techniques followed the traditional method, including cytocentrifuge preparations, liquid-based preparations (LBP) and cell block (CB). Each modality and stain offers its own advantages.

Preparations of the CB type are true microbiopsies and are recognized by their similarity to histology, representing an interface between cytology and histopathology. CB allows the identification of architectural patterns similar to those observed in histological sections, which, associated with morphological cellular details present in the other cytological preparations, enable a definitive diagnosis, with neoplasm classification similar or identical to histological classification. Besides, it permits performing additional studies, such as histochemical staining and immunohistochemical analysis, what increases diagnostic accuracy, as well as molecular tests.

At the laboratory of anatomical pathology of Hospital Sírio-Libanês, besides CS, we routinely use CB processed, as in histology, fixed in 10% buffered formalin and paraffin-embedded, therefore adequate for processing by means of pre-established protocols for additional studies. Recently we have introduced liquid-based cytology in our FNAB routine of thyroid nodules, besides CS and CB, not only as specimens with additional morphological findings, but also because they are a source for complementary molecular tests.

This study aims to evaluate the quality of CB preparations, besides conventional cytological specimens, such as CS and LBP, in cases of FNAB of thyroid nodules in diagnostic routine.
METhoD

One hundred six consecutive cases of FNAB of thyroid nodules at Hospital Sírio-Libanês between July and August, 2015, were evaluated on the cellularity of CS, LBP, and CB.

All cases were punctured by a trained cytopathologist, ultrasound-guided, employing a conventional technique, systematically performing three passes per nodule. Each of the passes was used for the conduction of two conventional smears, one fixed in 70% alcohol, the other air dried, stained with Papanicolaou and Romanowsky (Panótico Rápido LB©) stains, respectively. Besides conventional smears, the remaining material of one of the passes was washed in a liquid-based medium (Thinprep©), stained with Papanicolaou method. After preparation of direct smears, residual material of the two remaining passes was used to prepare CB, being washed with alcohol and placed into a Falcon conical tube. CB was processed with the Agar method© summarized as follows: specimen was centrifuged for 3 minutes, at 3,000 revolutions per minute (rpm), reserving the supernatant fluid at a new tube; 1% Agarose gel was added to the pellet (around 2× its volume), with homogenization, followed by centrifugation for 3 minutes at 2,000 rpm; the specimen was cooled in the refrigerator (4°C) for approximately 15 minutes; the material was removed from the conical tube with the aid of tweezers and cut in half vertically, discarding the portion containing just Agarose gel; the specimen was placed in filter paper in a cassette, fixed in 10% buffered formalin up to routine processing, as in histology, stained with hematoxylin and eosin (HE).

All cases were independently evaluated by two cytopathologists, observers 1 and 2 (Obs1 and Obs2), after cytotechnology evaluation. Specimen cellularity was assessed without considering macrophages, just epithelial elements and eventually lymphoid infiltrate, being classified as:

- absent (0): acellular;
- scanty (1+): less than six groups containing 10 or more follicular cells;
- low (2+): more than six groups containing 10 or more follicular cells, but borderline;
- moderate (3+): material exhibiting moderate cellularity, identified at low magnification (4×), with hundreds of cells;
- rich (4+): material showing rich cellularity, easily identified at low magnification (4×), with thousands of cells.

RESuLTS

Results are presented as a flowchart in Figure 1 for simplified visualization and overall analysis.

![Flowchart showing evaluation results of 106 cases of FNAB of thyroid nodule in diagnostic routine, including CS, LBP, and CB](image)

Preparations were analyzed on cellularity as rich (4+), moderate (3+), low (2+), scanty (1+), and absent (0).

No case was unsatisfactory, considering all cytological preparations. Among the 106 assessed cases, cellularity was rich/moderate in 56 (52.8%) CB for both observers. LBP showed rich/moderate cellularity in 86 (81.1%) cases for Obs1 and 91 (85.8%) for Obs2. Among the LBP cases with rich/moderate cellularity, CB presented the same cellularity in 52/86 (60.4%) cases for Obs1 and 54/91 (59.3%) for Obs2. SM showed rich/moderate cellularity in 86 (81.1%) cases for Obs1 and 87 (82%) for Obs2. Among the SM cases with rich/moderate cellularity, CB presented the same cellularity in 48/86 (55.8%) cases for Obs1 and 54/87 (62%) for Obs2.

Concerning LBP cases with low/scanty/absent cellularity, CB showed the same cellularity in 16/20 cases for Obs1 and 13/15 for Obs2. CB cellularity was higher than that of LBP in just five cases for Obs1 and three for Obs2. LBP was evaluated as scanty/absent in just five (4.7%) and six (5.6%) cases for Obs1 and Obs2, respectively.

Considering, individually, the five categories used for classification (4+/3+/2+/1+/0), 24 cases presented differences in the assessment of cellularity for the studied preparations between observers (22.6%), including nine CS, 15 LBP and 17 CB.

Analyzing the grouped categories, 3+/4+ versus 0/1+/2+, just 16 cases (15%) showed differences between observers, including nine CS, 13 LBP, and 10 CB.

The cases with discrepancy between observers can be analyzed in the Table.
TABLE — Cases with interpretation discrepancy between observers in evaluation of 106 FNAB cases of thyroid nodules in diagnostic routine, including CS, LBP, and CB

<table>
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<tr>
<th>Case</th>
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Preparations were analyzed based on cellularity as rich (4+), moderate (3+), low (2+), scanty (1+), and absent (0). The last column shows presence or absence of discrepancy when one assesses the grouped categories, rich/moderate (4+/3+) versus low/scanty/absent (2+/1+/0).

FNAB: fine-needle aspiration biopsy; CS: conventional smear; LBP: liquid-based preparations; CB: cell block; Y: presence; N: absence.

Figure 2 presents a case of papillary carcinoma, comparing the most habitually observed cellularity in the different preparation methods (Figure 2A-D).

FIGURE 2 — An illustrative case showing most habitual cellularity in different preparations
A) FNAB of thyroid nodule with final diagnosis of papillary carcinoma, with rich (3+/4+) cellularity in CS, presenting papillary arrangement at low magnification (Panótico Rápido LBP©, 4×); B) detail revealing nuclear characteristics, with presence of nuclear pseudoinclusion and irregular nuclear membrane (Panótico Rápido LBP©, 20×); C) LBP demonstrating moderate cellularity and papillary arrangement (Papanicolaou, 4×); D) CB containing small papillae, with clear nuclei (HE, 20×).

Figure 2B presents a case of papillary carcinoma, comparing the most habitually observed cellularity in the different preparation methods (Figure 2A-D).

DISCUSSION

Different cytological preparations have been routinely used for many years in the processing of material aspirated by fine needle of thyroid nodules in our service, including CS and CB. We have recently opted for liquid-based cytology, and since then, there was the concern in confirming the quality of CB material, without jeopardizing LBP.

Our results confirm that CB can be used routinely as an additional preparation in the material obtained from thyroid nodules FNAB, with no harm to LBP. Around 80% of the LBP showed rich/moderate cellularity; in 60% of these cases, CB is also abundant. It is interesting to note that preparations of CB, CS, and LBP were equally abundant in around 60% of the cases.

CB displayed higher cellularity than LBP in less than 5% of the cases. Among the cases with low/scanty/absent cellularity, similarity was also observed between LBP and CB, higher than 80%.

Interobserver variability is a well-known fact in anatomic pathology, as well as in other medical fields. Although our study reveals individual differences in cellularity assessment of the diverse cytological preparations in around 20% of the cases, the resulting sums of the three concomitant methods (CS, LBP, and CB) were similar between observers.

Although CS and LBP show rich/moderate cellularity in more than 80% of the cases, in comparison with close to 50% of CB, our adequate portrayal rate justifies the conduction of multiple preparations. Our aim was not to determine which preparation is more efficient, so as to favor a method over another, but to evaluate the possibility of obtaining representative samples in all, aiming at obtaining the most morphological information and cellularity for conventional diagnosis and eventual ancillary studies.

At diagnostic cytology and FNAB, each preparation used in processing, CS – dry and wet –, LBP and CB, offers its own advantages, with complementary morphological information, if used together.
Conventional smears are the widely recognized gold standard of cytological study, with well-established diagnostic criteria used worldwide. They also represent the preparations used for rapid specimen assessment during the rapid on-site evaluation (ROSE), in general air-dried, because they can become quickly stained.

Liquid-base cytology has as an unquestionable advantage its practicality and simplicity, for all the collected material is placed in a single vial with the preservation medium, with no need of other care. The use of LBP and CS together helps in the diagnosis of thyroid nodules\(^{(9)}\). In the cases whose smears show artifacts, most times due to an inexperienced performer, LBP contributes most to a diagnostic conclusion.

The CB technique gained wide recognition as a diagnostic method in the decade of 1950\(^{(4)}\). The use of CB in concert with other preparations permits the association of architectural and cytomorphological features, optimizing the definitive diagnosis. The combination of CS with CB preparations increases sensitivity and specificity in cases of endobronchial ultrasound-guided (EBUS) FNAB, with greater diagnostic accuracy\(^{(10)}\). CB and CS complement each other, what permits the evaluation of morphological findings and further immunohistochemical studies\(^{(11)}\).

Liu et al. (1998)\(^{(12)}\) assessed CB cost-effectiveness, comparing it, alone, with other methods, CS and LBP, that is, the diagnostic accuracy of each preparation individually. They concluded that the addition of other methods to CS is not justifiable, recommending its use just when the immediate evaluation of a specimen is not satisfactory. Although our work does not focus on this question, we know from experience that the use of different preparations together, confirming or adding morphological findings, contributes to diagnostic conclusion, besides providing additional material for the conduction of further studies. Therefore, CB does not replace the other preparations, but complements them.

Despite the additional cost with the use of multiple cytological preparations, CS, LBP, and CB, which in most cases is covered by private health plans, this approach enables maximum benefit with technical excellence in specimen processing.

Regardless of the numerous advantages that CB offers, results vary widely. Many cytopathologists report inconsistent results\(^{(13)}\), due to variable processing, and many times, to absent or poor representation of the lesion.

A larger number of passes, in order to obtain specimens with adequate cellularity, draws criticism. Our experience proves that standardizing collection and processing of FNAB of thyroid nodule enables obtaining satisfactory specimens and good results, what increases availability of cytopathologist and cytotechnician\(^{(14)}\). Therefore, all our cases, punctured by pathologists and radiologists, are collected and processed in the same manner, in general by means of three/four passes; part of each of the three passes is used to make two smears, one dry and one wet; the remaining material of a pass, in general the first, is processed as LBP; in the end, the remaining material of the other two passes is processed as CB. Our rate of insufficient material with the use of this technical standard is 4%-5%, confirming the efficient cellular representation, even without ROSE. The literature suggests that the ideal rate of unsatisfactory specimens must not surpass 10%, for the malignancy percentage of these nodules is similar to or higher than that of patients with conclusive diagnosis, with adequate cellularity, in the initial biopsy\(^{(15-17)}\).

Several studies point that the exclusive employment of CS and LBP in FNAB cases of thyroid nodules, associated with ROSE, require fewer passes through the lesion. However, in our experience, this is the cause of preparations such as CB with low cellularity, as some authors report\(^{(18)}\). Actually, ROSE is not fundamental when multiple passes through the nodule are done\(^{(19)}\), especially if the performer is experienced. At around 90% of the cases in our study, CS and LBP were rich, demonstrating that most of times the pass used for processing in liquid medium was adequate, what proves that ROSE is not necessary. We recommend that ROSE be restrict to cases with unsatisfactory or insufficient previous result.

Delay in diagnosis and higher demand for technical training have been discussed as disadvantages of CB, what in our laboratory routine, has no significant impact on time of result release, in general one or two days.

In the same way that histological specimens, CB processing, with formalin fixation and paraffin embedding, permits putting tissue into storage for future studies. Although the literature reports that CB little contributes to diagnoses of thyroid nodules aspirates, it is useful to demonstrate the cell line involved in cases with oxyphilic pattern and atypia, for example, when confirmation of follicular origin is desirable due to prognostic and management implications associated with medullary carcinoma, the main differential diagnosis to be considered. Another possible application is the conduction of an immunohistochemical panel predictive of malignancy, including cytokeratin 19, galectin-3 and HBME-1 or the detection of \textit{BRAF}\textsubscript{v600} gene mutation with a mutation-specific antibody\(^{(20,21)}\).
At last, molecular tests become increasingly valuable in the evaluation of variable lesions, with diagnostic, prognostic and predictive applications. It is possible that their use is incorporated in the routine investigation of thyroid nodules with undetermined diagnosis, such as atypia of undetermined significance/follicular lesion of undetermined significance, suspect both for follicular neoplasm and carcinoma(6, 22).

LBPs are a verified source for conduction of complementary studies, including molecular analysis, with diagnostic, predictive and prognostic implications(13, 23). The molecular analysis for investigation of specific mutations in thyroid nodules is possible in residual material of LBP, especially helping management decisions in cases with undetermined diagnosis(24). Additionally CB is one more specimen to be used in these studies, allowing perhaps better control of the studied cells and, consequently, a more adequate result interpretation(6, 22).

**CONCLUSION**

Although a sample obtained with CS and with LBP is in general, more representative, CB can be used routinely as an additional method for the material obtained by FNAB of thyroid nodules. Also, it can contribute to elucidate the definitive diagnosis in cases that need immunohistochemical and molecular additional studies.

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