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ORIGINAL ARTICLE

Characterizing specimen quality of cell block samples in an era of personalized diagnostics: analysis of 221 lymph node fine-needle aspirations

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Introduction Cell block (CB) preparations of fine-needle aspirates (FNAs) are utilized for patient management, which requires retention of representative material on slides. Personalized medicine demands quality CB specimens. There is no standard protocol for CB preparation, often resulting in suboptimal slides. The utility of using two CB slides in lymph node (LN) FNA cases was investigated.

Materials and methods We cut 10 serial sections from each CB, slides 1 and 5 are stained and considered the first and second cuts, respectively; the remaining slides are reserved for ancillary studies. Hematoxylin and eosin–stained CBs of 221 consecutive LN FNA cases were reviewed; qualitative and quantitative assessment of diagnostic value was made on sections 1 and 5.

Results Of the 221 cases, 46.1% (102) had comparable diagnostic cellularity (equally representative) on both slides, whereas 26.7% (59) and 27.1% (60) had more representative material on the first and second cuts, respectively ($P = 0.52$). Differences between the representativeness of first and second CB cuts of intrathoracic lymph nodes were minor (n = 192, $P = 0.065$). Differences between the first and the second slide representativeness of superficial (n = 22, $P = 0.98$) and intra-abdominal lymph nodes (n = 7, $P = 0.38$) are limited because of small sample sizes.

Conclusion One CB cut can be suboptimal for diagnosis. In our study, inclusion of a second slide increases equal representativeness from 46.1% to 73.2%. These limited observations recognize the need for additional

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investigations regarding the collection and preparation of CBs.
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Introduction

Fine-needle aspiration (FNA) cytopathology specimens are increasingly being utilized in the current era of personalized medicine requiring molecular investigations and other ancillary tests; they offer vital, representative, and minimally distorted samples suitable for subcellular studies.[1,2] Advances in personalized medicine, molecular diagnostics, and minimally invasive FNA warrant the need to preserve precious cellular material by improving specimen collection methods and cell block (CB) processing.

Various cytology preparations including stained and unstained smears, cytospins, filters, and CB slides, all prepared using a number of preservatives, fixatives, and media, have been used for ancillary investigations.[3,4] Utilization of CB material has become the "standard of care".[5-8] The issue of CB qualitative and quantitative adequacy is critical because carefully prepared FNA cell block slides, often paucicellular, are extremely variable in their representative cellular contents and appropriateness for additional studies.[1]

CBs are versatile, they are relatively small, and can provide abundant diagnostic information. Like histologic resections and biopsies, they provide multiple substrates for ancillary tests, including special stains, immunohistochemistry (IHC), and molecular assays. Their semblance to histologic sections enables the identification of architectural features.[1-3] They also enable long-term archiving of cellular material for future diagnostics and research.[5,9]

Although there are multiple methods of CB preparation, there is no standardized protocol. Many institutions are unsatisfied with their current preparation practices, with low cellular yield being the leading cause of dissatisfaction.[1] Although a universal method of CB preparation is unlikely to be accepted, careful attention to the optimal number of stained slides and processing technique for each situation is necessary. Studies comparing the diagnostic value of separate hematoxylin and eosin (H&E)-stained CB sections are lacking.

In this retrospective investigation we assessed the efficacy of using 2 stained CB cuts by discerning their cellular representativeness from a cohort of 221 lymph node FNA cases. Although CB specimens are used for diagnostic purposes across multiple anatomic sites, our study utilized lymph nodes as a model because these are most important for tumor staging and patient care decisions.

For FNA cases with CB slides, we put 10 serial paraffin sections on separate slides. Slides 1 and 5 are stained with H&E and considered the first and second cuts, respectively; the remaining preparations are kept for ancillary studies. This protocol saves precious cellular material that is often shaved off when re-cutting CBs and also improves turnaround time and patient care.

Materials and methods

After obtaining approval from the institutional review board of the University of Pennsylvania, an electronic search of the Cytopathology records at the University of Pennsylvania Medical Center identified 221 consecutive lymph node FNA cases with CB preparations accessioned during the months of January and February 2013. The diagnostic cellularity of separate cuts was compared visually; one slide was considered more representative than the other if it had 100 or more cells of interest. We qualitatively selected this threshold to account for multiple variables (see Discussion) and facilitate an investigation focused on the utility of using 2 stained CB cuts. The pairs of CB slides were assessed on their own merits, regardless of the cytological diagnoses, which are based on additional liquid-based preparations and/or smears. For example, if CB slides were negative with lymphocytes and the cytological diagnosis was not, the observed cells would be considered to be those of interest (we did not encounter this situation). Statistical analysis was completed via chi-squared tests using 0.10 significance and 2 degrees of freedom.

The lymph node FNA specimens were collected in Normosol-R (Hospira Inc., Lake Forest, Ill) or ThinPrep CytoLyt solution (Hologic Inc., Marlborough, Mass); after centrifugation they underwent qualitative visual assessment for CB preparation. During the timeframe of this study we simultaneously used 3 processors for paraffin embedding: Leica TP 1050 (Leica Microsystems, Buffalo Grove, Ill), Thermo Scientific STP 420 Tissue Processor (Thermo Fisher Scientific, Waltham, Mass), and Sakura Tissue-Tek VIP 2000 (Sakura Fineteck USA Inc., Torrance, Calif). These 3 processors operated simultaneously, and specimens were randomly subjected to paraffin-embedding via any available method. Recently, the Cellient Automated Cell Block System (Hologic Inc.) has been introduced into our laboratory use in selected specimens. Subcarinal lymph nodes include those accessioned as subcarinal and/or level 7. Whereas the hilar lymph nodes include those accessioned as hilar and/or levels 10 or 11, the paratracheal lymph nodes were accessioned as paratracheal and/or level 4. Neck lymph nodes include those accessioned as neck and/or cervical or supraclavicular.

Results

From our cohort of 221 lymph node FNA cases with CB slides (Table 1), 46.1% (102) had comparable diagnostic cellularity (equally representative) on both preparations (section 1 and section 5) (Fig. 1), whereas 26.7% (59) and 27.1% (60) had more representative first (Fig. 2) and second cuts (Fig. 3), respectively ($P = 0.52$). Differences between

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