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ORIGINAL ARTICLE

Comparison of plasma-thrombin, HistoGel, and CellGel cell block preparation methods with paired ThinPrep slides in the setting of mediastinal granulomatous disease

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Introduction Various cell block (CB) preparation methods are utilized by different laboratories, and not all laboratories perform CBs in tandem with ThinPreps (TPs). To compare the performance of different CB methods and their diagnostic value when used in conjunction with TP, we assessed the quantity and size of granulomas obtained from endobronchial ultrasound-guided transbronchial needle aspiration (EBUS-TBNA) of lymph nodes in the evaluation of granulomatous mediastinal disease.

Materials and methods A retrospective analysis of mediastinal lymph node EBUS-TBNA specimens that detected granulomas at our institution was performed. A total of 264 specimens from 124 patients had a TP followed by a CB (either plasma-thrombin, HistoGel, or CellGel) prepared from the residual material in the PreservCyt vial. The number and size of granulomas on each preparation was assessed using digital software.

Results Granulomas were detected only on the CB in 18.9% of cases and only on the TP in 5.3%. All 3 CB preparation methods showed significantly more and larger granulomas compared with the paired TP, with the plasma-thrombin and CellGel methods yielding more diagnostic material than the HistoGel method. In addition, the average number of granulomas (4.0 ± 0.4 versus 15.3 ± 1.1) and granuloma size (119.2 ± 3.2 μm versus 271.8 ± 7.3 μm) were significantly lower on TP compared with CB, respectively.

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Conclusions Plasma-thrombin and CellGel CB preparation methods had a higher granuloma yield compared with the HistoGel method. Additionally, significantly more numerous and larger granulomas were present on CBs compared with TP slides. Therefore, solely relying on TP slide evaluation may unintentionally overlook larger tissue fragments obtained during needle aspirations.

Introduction

Endobronchial ultrasound-guided transbronchial needle aspiration (EBUS-TBNA) is a minimally invasive, versatile technique that over the last 2 decades has become instrumental in sampling lesions of the mediastinum, hilum, and lung parenchyma. Although EBUS-TBNA is used most frequently for the diagnosis and staging of lung cancer,[1-4] it is also a useful diagnostic modality for a variety of other non-neoplastic and neoplastic thoracic diseases including sarcoidosis, infections, lymphomas, and metastases from extrapulmonary sites.[5-9] EBUS-TBNA has found particular importance during this time when minimally invasive tissue acquisition modalities are desired for initial diagnostic evaluation and subsequent ancillary testing, as many patients with lung cancer present at advanced stages that are not amendable to surgical intervention.

EBUS-TBNA is able to obtain cytologic material that can be used to prepare direct smears, liquid-based preparations such as ThinPrep (TP), and cell blocks (CBs). Tissue can be extracted from each of these methods that can then be used for ancillary studies such as immunohistochemical stains, cytogenetic testing, or molecular analysis, which is especially important in this era of targeted therapy. Different laboratories use one or more of these techniques for evaluation of their EBUS-TBNA specimens, and the decision of which preparation techniques to use is a complex one that depends on a number of institution-specific factors. Whereas CB has been shown to increase the diagnostic utility of a number of different specimen types[10-15] and has the additional benefit of being able to preserve tissue for subsequent studies, there are drawbacks to the use of routine CBs, not least of which include added costs and personnel time. Additionally, the variability in CB preparation techniques contrasts with the more standardized histologic processing of surgical pathology specimens, with CB preparation methods and cellular yields varying greatly between institutions.

At our institution, all EBUS-TBNA specimens have a TP liquid-based cytology slide prepared, followed by a CB made from any remaining residual material in the PreservCyt (Hologic, Marlborough, MA) vial. Over the last few years, our laboratory has used 3 different CB preparation methods (plasma-thrombin, HistoGel, and the modified HistoGel technique termed CellGel[16]). The primary objective of this study was to compare and contrast the diagnostic yield of these 3 different CB preparation techniques with a secondary objective being to evaluate the diagnostic value of routine CB preparation when used in tandem with liquid-based cytology. To accomplish this, we looked specifically at the performance of these 3 different CB preparation methods and TP liquid-based cytology in the focused setting of EBUS-TBNA sampling of mediastinal granulomatous disease. To our knowledge, this is the first study indirectly evaluating the performance of these 3 particular CB preparation methods to each other as well as the cell block diagnostic value when being performed in tandem with liquid-based cytology.

Materials and methods

Sample acquisition and preparation

At Beth Israel Deaconess Medical Center (Boston, MA), all EBUS-TBNA procedures are performed by experienced, board-certified interventional pulmonologists. A 22-gauge needle is used, with at least 3 needle passes of at least 15 needle excursions performed per sampled nodal station. The aspirates are collected directly into the methanol-based fixative CytoLyt (Hologic, Marlborough, MA), and sent to the cytology laboratory for processing. Rapid on-site evaluation is not routinely utilized at our institution for EBUS-TBNA of mediastinal lymphadenopathy, and as such, no direct smears are generated during these EBUS-TBNA procedures. Over the course of this study period, EBUS-TBNAs were performed by four different interventional pulmonologists, with no statistically significant difference in granuloma yield or size (the standardized outcome measures for this study) relative to the individual who performed the procedure (data not shown).

TP liquid-based cytology is utilized at our institution. Briefly, the procedure includes pouring the CytoLyt fluid into a 50-mL conical tube, centrifuging the sample, discarding the supernatant, and vortexing to resuspend the sediment. The sediment is suspended in PreservCyt solution, which is then used to make a single TP slide using a ThinPrep 2000 processor (Hologic, Marlborough, MA). A cell block is then immediately prepared from the residual material in the PreservCyt vial.

The plasma-thrombin CB preparation method was used in our laboratory until January 2017.[17-19] Briefly, the protocol includes adding 3 drops of expired blood bank plasma and subsequently 3 drops of saline-reconstituted thrombin to the cellular pellet after it has been centrifuged and the supernatant discarded. The specimen is gently agitated with a pipette, and allowed to sit for approximately 3 to 5 minutes

until a soft clot forms. This is then wrapped in tissue paper, transferred to a cassette, placed in formalin, and then processed as a routine formalin-fixed, paraffin-embedded histological sample per standard laboratory protocols.

The HistoGel (Thermo Fisher Scientific, Waltham, MA) method was adopted at our institution in February 2017.[20,21] Briefly, the protocol includes transferring the specimen to a 50-mL conical tube and centrifuging the sample for 5 minutes. The supernatant is then discarded and 10 drops of a warmed HistoGel solution is then added to the pellet. The gel is allowed to cool in the base of the tube, this congeals and results in a gel-like pellet at the bottom of the conical tube. This pellet is then dislodged with a disposable spatula, wrapped in tissue paper, and placed with the point of the cone-shaped pellet face down in a cassette before being placed in formalin and processed as a routine histological sample.

After its publication in the literature as a modification to the HistoGel method that resulted in an increased yield of cell block material and easier handling by the prep and histology laboratories,[16] the CellGel method was adopted in our laboratory in August 2017. The main difference in this modified preparation method compared with the HistoGel method is the use of disposable plastic base molds rather than a centrifuge tube for containing the sediment while it cools and solidifies. Use of the base molds allows for an even distribution of the cells over a well-defined surface area, the ability to concentrate the material by using either smaller or larger base molds, and improved embedding and sectioning.[16] For all EBUS-TBNA specimens, a 15 × 15 mm disposable base mold was used (Fisher Scientific Tissue Path Disposable Base Mold; Thermo Fisher Scientific).

Case selection and review

As part of laboratory quality assurance measures and validation of the CB preparation methods used in our lab, a retrospective search of the cytology database of our institution was conducted from July 2015 to April 2018. Using the SoftPath client platform, all reports were searched for lymph node EBUS-TBNA cases in which a finding of granulomas was indicated, regardless of underlying disease state (ie, sarcoidosis versus malignancy versus other or unknown).

In total, there were 268 EBUS-TBNA specimens from 126 patients that detected granulomas during the time interval searched. Four cases from 2 patients had either the TP or CB slide not on file, and were excluded from the study. All TP and CB slides, as well as any slides with ancillary testing such as special stains for organisms (acid-fast bacilli, Grocott's methenamine silver, Fite's acid fast, Gram stain), were reviewed for each case by a pathologist with fellowship training in cytopathology (VFT). The number of granulomas was assessed for each case by conducting a manual count of the granulomas found on each slide. An Olympus DP25 camera with cellSens image acquisition software (Core Version XV 3.13, Build 14,116, copyright year 2009 to 2015) was used to measure the size of the granulomas digitally.

For the purposes of this study, a granuloma was defined as at least 5 epithelioid histiocytes in a cohesive aggregate. Using the software measurement tool, the granulomas were measured on their longest axis. An average granuloma size was computed for each preparation method. For cases with greater than 10 granulomas, the lengths of 5 representative granulomas (2 largest, 2 smallest, and 1 intermediate-sized granuloma) were measured and averaged. All cases were analyzed twice, the second time being after a 3-month washout period. If the granuloma number or average size changed by more than 10% on the second reading, the new measurement was recorded (to ensure consistency between all of the samples over time). Additionally, because the granulomas often formed syncytial groupings, the sizes of the largest and smallest tissue fragments containing granulomas were also measured for each case in order to establish the total size range of tissue fragments detectable for each case on each preparation method.

Of note, only original slides used at the time of diagnosis were reviewed; deeper levels and/or additional stains were not performed as part of the study. The CB sections were all cut in 4 μm thick sections, which is the standard for all hematoxylin and eosin (H&E) histology sections performed at our institution, with two levels per slide. Only one TP slide was prepared for each sample. Conventional smears were not performed for any of the cases.

Institutional review board approval was waived for this project as it was part of ongoing quality assurance studies in our laboratory and did not require review of any additional patient information beyond basic patient demographics provided as part of the cytology laboratory specimen requisition.

Statistical methods

Descriptive analysis

Demographic and specimen information for 264 cases was summarized. Continuous variables were expressed as mean with standard error and categorical variables were measured as percentages.

Paired (matched) analyses

Overall comparisons between TP and CB methods: TP and CB observations were paired for each patient case. Average number of granulomas observed on TP and CB was compared. Value defaulted to zero if no granuloma was detected. For cases in which granulomas were observed on both TP and CB ($n = 200$), difference in average size of granulomas was further evaluated. Because of non-normality in the distributions of the data, paired analyses were conducted using Wilcoxon signed rank tests.

Unmatched analyses

Comparisons of 3 CB methods (plasma-thrombin, HistoGel, and CellGel): Multiple linear regression analyses were

applied to investigate the associations of 3 different CB methods with average number and size of granulomas observed, respectively. Given the possibility that the average number and size of granulomas detected by CB methods might be associated with the measurements on the paired TP (due to either technical differences in the specimen procurement by the proceduralist or to inherent qualitative differences in the disease process for each case), TP measurements were also included in the regression model to adjust for any confounding factors. Final multiple linear regression model contains only statistically significant parameters.

All tests were performed in SAS version 9.3 (SAS Institute, Cary, NC) with the level of statistical significance set at 0.05.

Results

A total of 264 cases from 124 patients were included in the study, which included 53.2% (66 of 124) men and 46.7% (58 of 124) women with an average age of 55.2 years (range, 20 to 90 years) (Table 1). Non-necrotizing granulomas were predominantly detected, with only 3.0% (8 of 264) of cases containing necrotizing granulomas. The majority of cases (70.8%; 187 of 264) had special stains for organisms performed, including acid-fast bacilli, Fite's acid fast, and/or Grocott's methenamine silver stains, only one of which was found to be positive for organisms (on Fite's acid fast stain).

Table 1 Patient demographics and overall specimen data.

Characteristic	Sample size
Demographics	
Female	58 (46.7%)
Male	66 (53.2%)
Average age, years	55.2
Specimen type	
Non-necrotizing granulomas	256 (97.0%)
Necrotizing granulomas	8 (3.0%)
Stain type	
Special stains	187 (70.8%)
Regular stains	77 (29.2%)
Specimen preparation method	
ThinPrep	264
Cell Block	264
Plasma-thrombin	97 (36.7%)
HistoGel	76 (28.8%)
CellGel	91 (34.5%)
Cases with granulomas only on ThinPrep	14 (5.3%)
Plasma-Thrombin	1 (7.1%)
HistoGel	12 (85.8%)
CellGel	1 (7.1%)
Cases with granulomas only on cell block	50 (18.9%)
Plasma-thrombin	23 (46.0%)
HistoGel	12 (24.0%)
CellGel	15 (30.0%)
Cases with granulomas on both ThinPrep and cell block	200 (75.8%)

During the study period, all cases had TP slides prepared and 36.7% (97 of 264) of the total cases had CBs prepared using the plasma-thrombin method, 28.8% (76 of 264) using the HistoGel method, and 34.5% (91 of 264) using the CellGel method. Representative images of granulomas from each preparation method are shown in Figure 1.

Overall, 75.8% (200 of 264) of cases had granulomas detected on both the TP slide as well as the CB slide. Granulomas were detected only on the CB slide in 18.9% (50 of 264) of cases. Of the 50 cases where granulomas were detected only on the CB and not on the TP slide, 23 were by using the plasma-thrombin method, 15 by using the CellGel method, and 12 by using the HistoGel method. Granulomas were detected only on the TP in 5.3% (14 of 264) of cases. Of the 14 cases where granulomas were detected only on TP and not on the CB slide, 12 of the corresponding CBs were prepared using the HistoGel method, 1 with the plasma-thrombin method, and 1 with the CellGel method (Table 1).

When assessing all CBs together during the entire study period (all three preparation methods combined), the average number of granulomas detected on CB was 15.3 ± 1.1 (mean ± standard error). This was significantly higher than the overall average number of granulomas detected on TP (4.0 ± 0.4), indicating CBs yielded significantly more granulomas compared with the concurrent paired TP slides ($P < 0.001$), as graphically illustrated in Fig. 2. The average number of granulomas observed on the H&E slide cut from each CB prepared using plasma-thrombin, HistoGel, and CellGel methods were 16.3 ± 1.6, 7.1 ± 1.2, and 20.9 ± 2.3, respectively (Table 2).

A multiple linear regression was calculated to predict the number of granulomas detected on CB based on number of granulomas detected on TP for each CB method used. A significant regression equation was found ($P < 0.001$), with an R^2 of 0.196. Predicted number of granulomas observed on CB is equal to 13.3 + 0.94 (number of granulomas observed on TP) + 3.26 (CellGel) − 10.21 (HistoGel), where plasma-thrombin method was used as reference. The regression results indicate a statistically significant difference in the number of granulomas detected among the 3 CB methods while controlling for number of granulomas detected in the paired TP. Compared with the plasma-thrombin method, the CellGel method slightly increased the number of granulomas detected on CB by 3.26 units ($P = 0.166$), and the HistoGel method significantly decreased the number of observation by 10.21 units ($P < 0.001$).

To determine a range of diagnostic tissue fragment sizes (not just individual granuloma size) that could be detected on both a TP sample compared with the paired CB sample, the largest and smallest tissue fragments containing granulomas were examined. The average size of the largest tissue fragment containing granulomas was found to be larger on the CB material compared with the average on TP (623.5

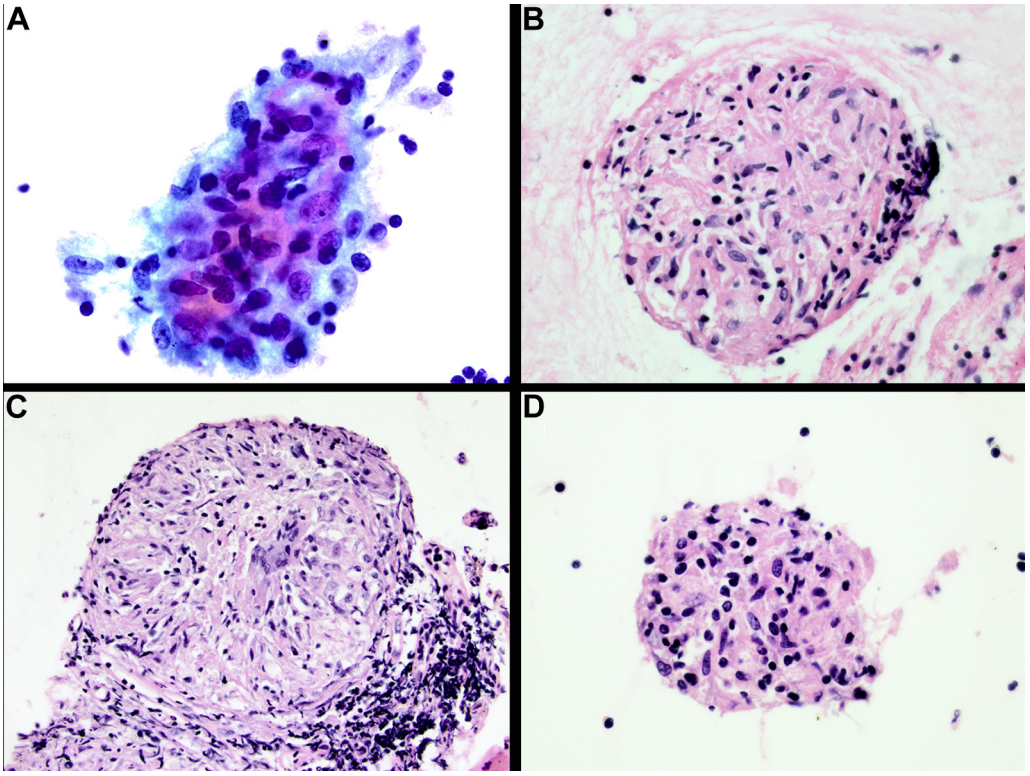

Figure 1 Representative high-power images of granuloma morphology as seen on Papanicolaou-stained ThinPrep (A) and hematoxylin and eosin−stained sections from the three cell block methods: plasma-thrombin (B), HistoGel (C), and CellGel (D). Note the overall similar cellular morphology on the 3 different cell block methods, with the faint eosinophilic fibrinous background of the plasma-thrombin (B) contrasting with the colorless backgrounds of the two HistoGel-based methods (C and D). Original magnifications: 1000x, 600x, 400x, and 600x, respectively.

μm versus 154.2 μm). The average size of the smallest tissue fragment containing granuloma was lower on TP compared with CB (81.9 μm versus 121.4 μm). The size of the largest granuloma-containing tissue fragment detected on TP was 635 μm and smallest detectable granuloma size was 37 μm. The largest granuloma containing tissue fragment detected on CB was 2592 μm and the smallest detectable granuloma size was 44 μm.

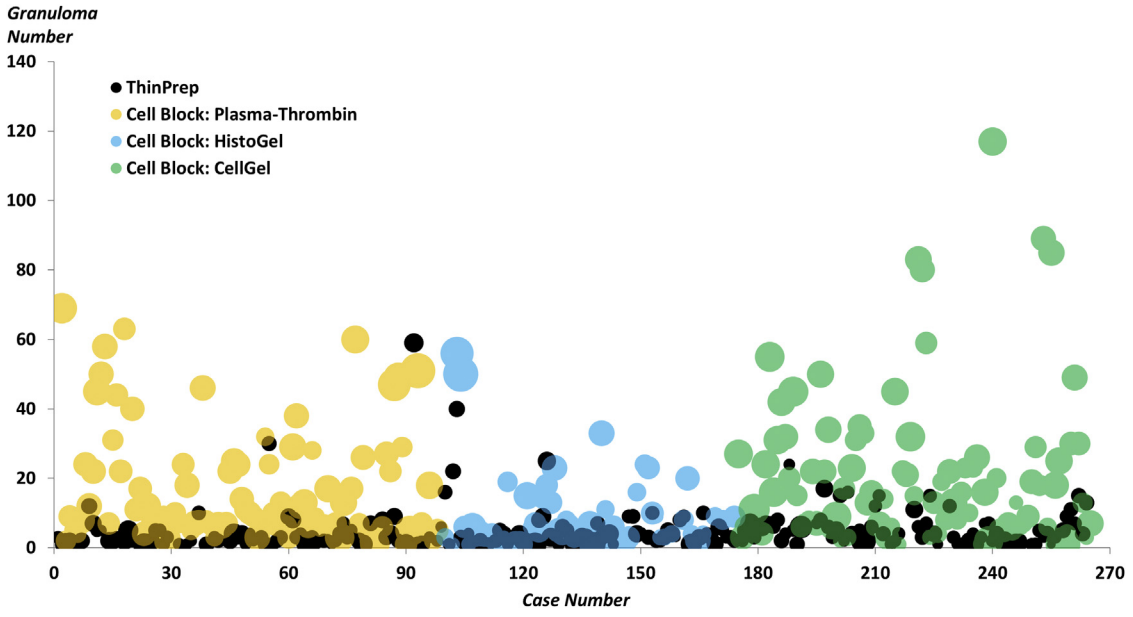

Figure 2 Dot plot illustrating granuloma number (*y* axis) and granuloma size (dot size) for each paired cell block and ThinPrep case.

Table 2 Granuloma number and size relative to preparation type.

	ThinPrep overall	Cell block overall	*P*	Plasma-thrombin	HistoGel	CellGel	*P*
Number of cases	264			97 (36.7%)	76 (28.7%)	91 (34.6%)	
Average number of granulomas observed, mean (SE)	4.0 (0.4)	15.3 (1.1)	<0.001	16.3 (1.6)	7.1 (1.2)	20.9 (2.3)	<0.001
Number of cases	200			73 (36.5%)	52 (26.0%)	75 (37.5%)	
Average size of granulomas (if observed in both TP and CB), mean (SE)	119.2 μm (3.2 μm)	271.8 μm (7.3 μm)	<0.001	293.8 μm (10.5 μm)	226.3 μm (15.8 μm)	281.9 μm (11.2 μm)	<0.001

If granulomas were detected on both TP and CB, the average size of granulomas observed by TP (119.2 μm ± 3.2 μm) was significantly smaller than their concurrent paired CB results (271.8 μm ± 7.3 μm). The values for average size of granulomas detected by plasma-thrombin, HistoGel, and CellGel were 293.8 μm ± 10.5 μm, 226.3 μm ± 15.8 μm, and 281.9 μm ± 11.2 μm, respectively.

A multiple linear regression analysis was also performed to predict average size of granulomas detected on CB based on number of granulomas detected on TP and CB, and CB method used. A significant regression equation was found ($P < 0.001$), with an R^2 of 0.351. Predicted average size of granulomas observed on CB is equal to 235.39 + 4.84 (number of granulomas observed on TP) + 1.97 (number of granulomas observed on CB) − 25.27 (CellGel) − 52.97 (HistoGel), where plasma-thrombin method was used as reference and size was measured in μm. After controlling for number of granulomas detected on TP and CB, the average size of granulomas observed by the 3 CB methods still has a statistically significant difference. Both CellGel and HistoGel methods resulted in smaller average size of granulomas observed on CB by 25.27 μm ($P = 0.069$) and 52.97 μm ($P < 0.001$), respectively, when compared with the plasma-thrombin method.

Discussion

The objective of this study was 2-fold: to compare the performance of 3 different CB preparation methods and to compare the diagnostic value of preparing CBs in conjunction with liquid-based cytology. To this end, we chose a very specific specimen type and outcome measure, namely, the number and size of granulomas from EBUS-TBNA specimens obtained from mediastinal lymph node aspirates. These outcomes were chosen as an overall surrogate for tissue yield in the various preparation methods examined.

We evaluated the cellular yield of 3 different CB preparation methods (plasma-thrombin, HistoGel, and CellGel) and analyzed them indirectly in relation to each other as well as directly to their paired TP specimens. We used EBUS-TBNA−acquired lymph node cytologic specimens that yielded granulomatous material as this provided an easily quantifiable measurement that could be compared between the different preparation types. The results of this study demonstrated the following findings. First, the plasma-thrombin and CellGel CB preparation methods overall performed better compared with the standard HistoGel preparation method with respect to yielding significantly more and larger granulomas than the HistoGel method. Second, we also found that the number and size of granulomas detected by CB were significantly greater than TP, regardless of the CB preparation method used. This is illustrated in Fig. 3. Finally, granulomas were only detected on the CB preparation in 18.9% of cases, meaning that if a

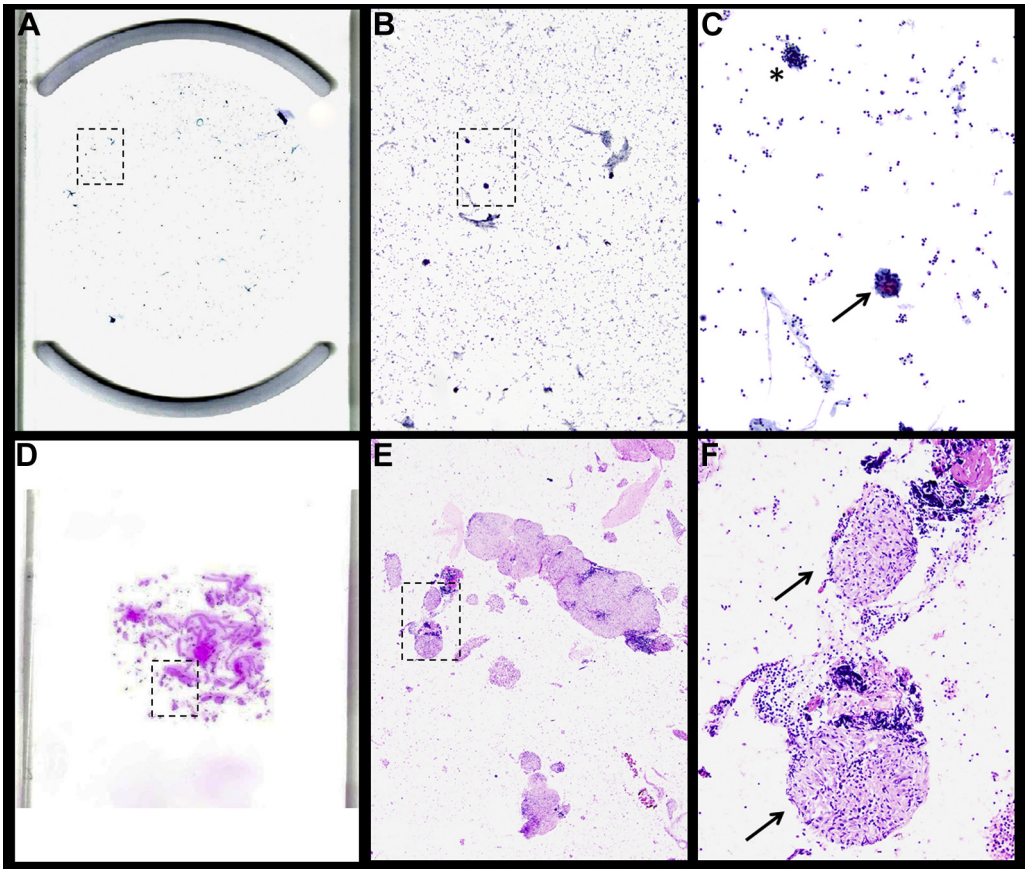

Figure 3 A to C, ThinPrep (Papanicolaou stain; 1x, 40x, 200x original magnifications, respectively) demonstrates few scattered small clusters of granulomas (C, arrow). For this case, seven granulomas were counted, measuring on average 65 μm, and ranging from 41 to 80 μm. Note the similar size of lymphoid aggregates (asterisk). D to F, Paired cell block (hematoxylin and eosin stain; 1x, 40x, 200x original magnification, respectively) prepared by the CellGel preparation method demonstrates significantly more granulomas that are also larger (F, arrows) compared with the preceding paired ThinPrep, with corresponding panels at same magnification. For this case, 50 granulomas were counted, measuring on average 371 μm, and ranging from 84 to 2520 μm.

concurrent CB had not been performed, nearly a fifth of cases would not have been diagnosed with granulomatous disease.

The plasma-thrombin and CellGel methods both performed similarly, with the quantity and size of granulomas detected not varying significantly between the 2 preparation methods. These both performed significantly better than the HistoGel method. Although the HistoGel method offers relatively good cellular and architectural preservation, there are a number of factors that may have contributed to the cellular yield being less than that of the other preparation methods. One potential contributing factor is that HistoGel is used to congeal the sediment material within the conical centrifuge tube. This can make it difficult to extract all the material from the base of the tube, with the spatula transfer potentially disrupting the sedimented material or leading to specimen loss in transfer. The irregular conical tip shape of the HistoGel pellet may also lead to an uneven distribution of cells and less predictable surfaces for histologic sectioning. These technical factors, which in theory could be optimized in different ways, likely explain the lower granuloma yield in the traditional HistoGel method compared with the plasma-thrombin and especially the CellGel methods, though the reason for average granuloma size difference between HistoGel and CellGel is less clear.

Despite our study showing that the plasma-thrombin method performed relatively well, there are issues with the plasma-thrombin method that should be considered. One issue with the plasma-thrombin CB preparation method is the potential variability in clotting ability from specimen to specimen, which thus leads to unpredictability in the time to clot formation and resultant clot size. This may cause issues with the cellular material both during the preparation of the cell block as well as with the final histologic section as it may create an uneven concentration of the fibrin-enmeshed cell population. Additionally, because saline is used in the preparation of plasma-thrombin–based CBs and not a fixative like formalin (since formalin interacts with clotting factors), the cellular yield, cytomorphologic detail, or architectural features may be compromised as there may be increased cell lysis with saline, which is not a perfectly isotonic cell medium.[22] Finally, because discarded human plasma is used in

this process, there is the theoretical risk of contaminating nucleic acids or other biologic factors that may confound sensitive downstream molecular testing analyses.

The CellGel preparation method offers several advantages over other CB preparation methods, which were outlined by the developers of the method,[16] and which we have found to hold true in our laboratory as well. In our experience, the most advantageous modification in this CB preparation method has been the adaptation of using disposable base molds rather than conical tubes as used in the HistoGel method. This allows not only for easier cell sediment transfer, which decreases the loss of cellular material, but also it allows for better and more predictable concentration of the cellular material. By virtue of using rectangular molds that have a flat surface, there is a more even distribution of the cellular material as well as a greater surface for histologic sectioning. Anecdotally, both our cytology preparatory laboratory as well as our histology laboratory prefer the current CellGel method, as it leads to easier handling and standardized processing and cutting of each specimen. Moreover, the size of the molds can be modified depending on specimen type and cellularity, to additionally concentrate the material.

As stated previously, CB has been shown to increase the diagnostic utility of a number of different specimen types.[10-15] A 2016 study by Horton et al examining the value of Cellient CBs in the evaluation of thyroid fine-needle aspirations found that the use of CB when used in addition to TP contributed to a change in the final diagnosis in 15% of their cases, with the greatest benefit in those initially classified in the nondiagnostic and atypia/follicular lesion of unknown significance categories, both of which tended to have sparse cellularity.[23] In line with these prior studies, in our experience significantly larger granulomas and tissue fragments were found in the CB preparations as compared with the TP liquid-based preparations. Although it not entirely clear why, it is likely related to how the TP slide is prepared. The filter-based transfer of a monolayer of cells to a glass slide may not be as effective to transfer larger cohesive tissue fragments compared with smaller tissue fragments or individual cells. Thus, larger tissue fragments may remain behind in the PreservCyt vial. This residual material containing the larger fragments is then centrifuged and entirely resuspended in the plasma-thrombin or HistoGel material, effectively transferring all remaining cellular materials to the CB preparation.

Although not the primary objective of this study, there are a few other interesting findings that are worth note. First, in our patient population, lymphadenopathy with necrotizing granulomas is a rare finding. Furthermore, although special stains for microorganisms by policy are generally always run when granulomas are identified on CB preparations, the rate of finding organisms, especially in the absence of necrosis, is exceedingly low (seen in only 1 of 187 cases where stains were performed).

There are a number of limitations to our study to be discussed. First, this was a retrospective study where each clinical specimen was prepared by the cell block preparation method used and validated in the laboratory at the time: We did not prospectively split samples and prepare CBs using the 3 different preparation methods from each sample, which would have allowed for a direct comparison of the 3 CB preparation methods. By retrospectively comparing the yield of CBs over the 3 time intervals in which the different methods were independently used, this study serves as an *indirect* way of comparing the different CB preparation methods. Although factors such as preparation technologist experience and familiarity with the preparation technique could have factored into the results, there were no changes in technical staff during the study time period and all technologists received training in and were familiar with each preparation method. Also, the number of cases per cell block preparation technique ($n = 76$ to 97 specimens per CB technique) ensured a large sample size to adequately detect any preparation method based differences. From a morphologic standpoint, by virtue of being histologic H&E sections, CBs can offer much by way of complementary cellular morphology and architectural evaluation, particularly when small tissue fragments are present, which can be of great value when it comes to things like tumor diagnosis and subtyping.[11-13,24] In this study, the main point of comparison between the different CB preparation methods was the diagnostic yield (the quantity and size of granulomas); although the morphologic and architectural quality of the yielded cellular material was not directly assessed, from a qualitative standpoint there were little if any differences appreciated in the granulomas between the three CB methods (Fig. 1). Thus, the applicability of these findings to other specimen types and disease states (such as tumor diagnosis and subtyping) is likely to hold true.

In conclusion, our study is the first to indirectly assess the performance of plasma-thrombin, HistoGel, and CellGel CB preparation methods with respect to diagnostic yield to each other and when used in tandem with liquid-based cytology. Their performance in the assessment of mediastinal granulomatous disease in EBUS-TBNA−acquired specimens was studied given that it served as a controlled and focused cohort of cases with easily measurable and comparable data points. In our experience, we found that the plasma-thrombin and CellGel CB preparations yielded significantly more material on the initial H&E section as compared with the HistoGel method. All 3 methods yielded significantly more and significantly larger granulomas compared with their paired TP specimens. Additionally, granulomatous disease would have been missed in nearly a fifth of cases if TP had been performed without a concurrent CB preparation. Further studies are needed to investigate whether these results are also applicable to other specimen types and other lesions, such as cancer specimens, as factors such as cellular morphology and architectural preservation may affect the diagnostic value or molecular testing success of the different cytologic preparation methods.

Funding sources and relevant conflicts of interest

None.

References

1. Colice GL. Technical standards for tracheal tubes. *Clin Chest Med*. 1991;12:433–448.
2. Groth SS, Andrade RS. Endobronchial ultrasound-guided transbronchial needle aspiration for mediastinal lymph node staging in non-small cell lung cancer. *Semin Thorac Cardiovasc Surg*. 2008;20:274–278.
3. Herth FJ, Krasnik M, Vilmann P. EBUS-TBNA for the diagnosis and staging of lung cancer. *Endoscopy*. 2006;38:S101–S105.
4. Medford AR, Bennett JA, Free CM, Agrawal S. Mediastinal staging procedures in lung cancer: EBUS, TBNA and mediastinoscopy. *Curr Opin Pulm Med*. 2009;15:334–342.
5. Cameron SE, Andrade RS, Pambuccian SE. Endobronchial ultrasound-guided transbronchial needle aspiration cytology: a state of the art review. *Cytopathology*. 2010;21:6–26.
6. Marshall CB, Jacob B, Patel S, et al. The utility of endobronchial ultrasound-guided transbronchial needle aspiration biopsy in the diagnosis of mediastinal lymphoproliferative disorders. *Cancer Cytopathol*. 2011;119:118–126.
7. von Bartheld MB, Dekkers OM, Szlubowski A, et al. Endosonography vs conventional bronchoscopy for the diagnosis of sarcoidosis: the GRANULOMA randomized clinical trial. *JAMA*. 2013;309:2457–2464.
8. Navani N, Molyneaux PL, Breen RA, et al. Utility of endobronchial ultrasound-guided transbronchial needle aspiration in patients with tuberculous intrathoracic lymphadenopathy: a multicenter study. *Thorax*. 2011;66:889–893.
9. VanderLaan PA, Wang HH, Majid A, Folch E. Endobronchial ultrasound-guided transbronchial needle aspiration (EBUS-TBNA): an overview and update for the cytopathologist. *Cancer Cytopathol*. 2014;122:561–576.
10. Sadullahoğlu C, Yaman B, Veral A, Nart D. The diagnostic value of cell blocks in Liquid-Based Cytology of bronchial aspiration and bronchial brushing materials. *Cytopathology*. 2018;29:156–162.
11. Collins GR, Thomas J, Joshi N, Zhang S. The diagnostic value of cell block as an adjunct to liquid-based cytology of bronchial washing specimens in the diagnosis and subclassification of pulmonary neoplasms. *Cancer Cytopathol*. 2012;120:134–141.
12. Calabretto ML, Giol L, Sulfaro S. Diagnostic utility of cell-block from bronchial washing in pulmonary neoplasms. *Diagn Cytopathol*. 1996;15:191–192.
13. Sanz-Santos J, Serra P, Andreo F, Llatjós M, Castellà E, Monsó E. Contribution of cell blocks obtained through endobronchial ultrasound-guided transbronchial needle aspiration to the diagnosis of lung cancer. *BMC Cancer*. 2012;12:34.
14. Flint A. Detection of pulmonary neoplasms by bronchial washings. Are cell blocks a diagnostic aid? *Acta Cytol*. 1993;37:21–23.
15. Nathan NA, Narayan E, Smith MM, Horn MJ. Cell block cytology. Improved preparation and its efficacy in diagnostic cytology. *Am J Clin Pathol*. 2000;114:599–606.
16. La Fortune KA, Randolph ML, Wu HH, Cramer HM. Improvements in cell block processing: The Cell-Gel method. *Cancer Cytopathol*. 2017;125:267–276.
17. Gill G. Cytopreparation Principles and Practice. In: *Essentials in Cytopathology*. Berlin: Springer; 2013.
18. Kulkarni MB, Desai SB, Ajit D, Chinoy RF. Utility of the thromboplastin-plasma cell-block technique for fine-needle aspiration and serous effusions. *Diagn Cytopathol*. 2009;37:86–90.
19. Jain D, Mathur SR, Iyer VK. Cell blocks in cytopathology: a review of preparative methods, utility in diagnosis and role in ancillary studies. *Cytopathology*. 2014;25:356–371.
20. American MasterTech. HistoGel. Available at: https://www.americanmastertech.com/instructions/emhge.pdf. Accessed September 2018.
21. Hecht SA, McCormack M. Comparison of three cell block techniques for detection of low frequency abnormal cells. *Pathol Lab Med Int*. 2013;5:1–7.
22. Balassanian R, Wool GD, Ono JC, et al. A superior method for cell block preparation for fine-needle aspiration biopsies. *Cancer Cytopathol*. 2016;124:508–518.
23. Horton M, Been L, Starling C, Traweek ST. The utility of Cellient cell blocks in low-cellularity thyroid fine needle aspiration biopsies. *Diagn Cytopathol*. 2016;44:737–741.
24. Michel RP, Lushpihan A, Ahmed MN. Pathologic findings of transthoracic needle aspiration in the diagnosis of localized pulmonary lesions. *Cancer*. 1983;51:1663–1672.