Core Needle Biopsy Wash Optimization: Enabling Specimen Integrity for both Cytological and Histological Evaluation

WILFRIDO D. MOJICA, NORA K. FRISCH and JOYCE K. PATERSON

Department of Pathology and Anatomical Sciences, State University at Buffalo, Buffalo, NY, U.S.A.

Abstract. *Background. The recovery of cells after washing core needle biopsies represents an under-utilized approach to extend the diagnostic capacity of these diminutive specimens. Recovery of these cells can be dedicated for molecular studies so that the biopsy itself can be used apropos for its intended purpose, diagnosis. Non-enzymatic and enzymatic reagents have the potential to increase the number of cells dissociating from the tissue core, but can also negatively impact the quality of the tissue itself. Materials and Methods: Three different means (phosphate-buffered saline, a non-enzymatic and an enzymatic solution) were used to wash core needle biopsies. The washed cells were recovered by traditional preparatory methods and evaluated for cellularity and cytomorphology. The post-washed cores were processed by formalin fixation, paraffin embedding and evaluated for integrity and morphological quality. Results: The enzymatic solution damaged both the cytological and tissue specimens, while the saline and non-enzymatic process allowed for the comparable recovery of cells and tissue for evaluation. Conclusion: Adequate numbers of cells are dissociated from the tissue core when needle biopsies are washed. The recovery and preservation of cells and tissue for morphological interpretation was optimal when solutions devoid of enzymes were used for washing.*

Core needle biopsies (CNBs) have become an integral component in the management of patients with recently identified tumorous lesions. CNB is indicated for a suspected tumor lesion to determine whether it is benign or malignant and to help stage a patient's neoplastic disease process, determining the extent of local and metastatic spread (1). The increased number of CNBs being performed corresponds to reports of diagnostic accuracy rates similar to those of open biopsies but with much lower complication rates (2). The recent push for the incorporation of molecular testing to further guide patient treatment has increased the importance of the CNB in the burgeoning field of Precision Medicine (3, 4). Increasingly, the performance of additional assays from already diminutive starting material is being requested from treating physicians. These limitations have raised awareness of the difficulty of performing more tests on smaller biopsies (5, 6). This issue of having to do more with less is a growing concern for pathologists (7). A not so infrequent scenario encountered in laboratory medicine is the loss or exhaustion of diagnostic tissue after morphological examination and the performance of a battery of immunohistochemical stains, leaving insufficient tissue for molecular analysis.

Investigators have looked at performing various manipulations on the CNB with the hope of extending the diagnostic capabilities of these small-tissue specimens. Touch imprint cytology was recently described as a means of recovering cells from CNBs for molecular analysis (8). This approach has been most often reported as a method in establishing an on-site, rapid diagnosis for patients in the clinic setting (9, 10). However, this technique has also been reported to detrimentally affect the cellularity of the CNB, if performed too vigorously (11). Another method, namely washing of the CNB, has been investigated but only with the intent of determining the adequacy of the CNB or in obtaining a rapid diagnosis (12). Herein, we sought to determine if washing of the CNB would be a suitable approach of extending the diagnostic capabilities of the material retrieved from the procedure. We sought to determine if the material taken from a CNB wash would yield cells of reasonable quality and quantity without depleting the post-wash CNB tissue of diagnostic cells or damaging them in any way. Washing a CNB, we reasoned, may be able to recover diagnostic cells in sufficient numbers that could be dedicated for molecular analysis, all the while preserving the CNB for traditional formalin-fixed, paraffin-embedded (FFPE) morphological examination. In the present

Correspondence to: Wilfrido D. Mojica, MD, University at Buffalo, Department of Pathology and Anatomical Sciences, 100 High Street, Buffalo, NY 14203, U.S.A. Tel: + 1 7168592140, e-mail: mojica@buffalo.edu

Key Words: Core needle biopsy, core needle wash, PBS, microfluidics, cytology.

study, we report the findings after testing a number CNBs washed with three different solutions, and speculate on the importance of the wash in retrieving these cells.

Materials and Methods

Approval of this project was given by the University at Buffalo Institutional Review Board [416540-3]. Consent was waived as the material that was obtained was excess in nature, did not destroy the specimen, and came from specimens resected for medical indications. No personal health information was retained. A CNB was obtained from 26 cases of surgically resected tumors (Table I). Some of these cases would not have been traditionally sampled by CNB for diagnosis but were sampled in this experiment because of availability of adequate tissue and for proof of concept/demonstration purposes. These cases were delivered to the Department of Pathology unfixed, with the expectation that pathology personnel would add fixative. Upon receipt, a technician informed a pathologist participating in this project (WDM) of the arrival of a specimen resected for medical indications. The pathologist then inspected the specimen and identified by visual and palpable examination the presence of a tumorous lesion. Cases with lesions that were small and did not have ample excess tissue were excluded from this study.

Table I. *Breakdown of types and numbers of tumors from which core needle biopsies were taken.*

Tumor type	Number of cases	Case numbers
Renal cell carcinoma	6	4, 8, 10, 14, 16, 25
Colonic adenocarcinoma	4	2, 5, 18, 21
Breast carcinoma (ductal)	3	6, 20, 24
Ovarian serous carcinoma	2	1,3
Sarcoma NOS	2	12, 15
Endometrial carcinoma	2	9, 26
Adenomatoid thyroid nodules	2	22, 23
Renal mass (Angiomyolipoma)	1	13
Adrenal mass (Pheochromocytoma)	1	11
Ovarian mucinous carcinoma	1	17
Pancreatic carcinoma	1	7
Squamous cell carcinoma	1	19

For those cases selected, a total of 12 biopsies were taken from each case using an 18-gauge core needle biopsy instrument [Adjustable Coaxial Temno® biopsy device; CareFusion (a division of Becton, Dickinson and Company), San Diego, CA, USA]. The core needle biopsy instrument was inserted directly into the lesion and two cores procured six times (Figure 1). Of the resulting 12 cores, the first two were placed into a biopsy bag for subsequent fixation; these cores were intended to serve as standards from which subsequent cores were to be compared with at the morphological level. The next 10 cores were then dislodged, two each, into one of five separate 1.5 ml microcentrifuge tubes. The tubes held different solutions, namely 1X phosphate buffered saline (PBS; diluted to one-tenth the concentration from a starting stock solution of 10X PBS), Cell Stripper [MediaTech (a division of Corning), Manassas, VA, USA], a non-enzymatic cell dissociation reagent, and three decreasing concentrations [2%, 1% and 0.5% (w/v)] of collagenase (13) in RPMI 1640. This approach was used for the first six cases, with the two higher concentrations of collagenase discontinued after review of the initial cytomorphology. For the next eight cases, only eight core biopsies were procured, and similarly processed as above in the solutions not discontinued. For the final 12 cases, only six core biopsies were taken from each tumor mass, with two cores dedicated for formalin fixation, two for washing with PBS and two for washing with Cell Stripper.

The two cores dedicated for formalin fixation were immediately fixed in 10% neutral buffered formalin for 8 or more hours and then processed and ultimately sectioned after being embedded with paraffin wax for hematoxylin and eosin staining. Each of the other two cores for a specific solution (PBS, Cell Stripper, *etc.*) were placed into the microcentrifuge tube holding approximately 1 ml of solution by dislodging them from the needle with gentle agitation. All the cores were then incubated at 37˚C for 15 min with rotational mixing. After the mixing, a probe was used to extract the cores from the solution. The cores were then placed into a biopsy bag for formalin fixation as described above. The leftover solution from each core wash was then spun down and captured on glass slides using the cytospin method. The glass slides were then sprayed with a fixative (Cytology Fixative; Leica Biosystems, Buffalo Grove, IL, USA), allowed to air dry and then fixed for 10 minutes in 95% alcohol before undergoing a pre-programmed staining protocol using the Papanicolau staining method.

The cytospin slides were then examined by a Board-certified cytopathologist (NF) who evaluated a set number of characteristics for each case. These features included the quality of the cellular morphology, the quantity of the cells present, and a diagnostic assessment of the cells (if quantity and quality allowed). The quality of the cellular morphology was graded on a three-tier scale: 1=cells of high quality with crisp nuclear detail and sufficient numbers for diagnostic evaluation; 2=cells of moderate quality with some loss of nuclear detail and suboptimal quality for diagnostic interpretation; and 3=cells of poor quality, with the morphology of cells hindered in such a way as to make an adequate diagnosis difficult. The quantity of cells present were also graded according to a three-tiered method: 1=high cellularity [>5 cells/high power field (hpf, ×40)]; 2=moderate cellularity (3-5 cells per/hpf; and 3=paucicellular (<3 cells/hpf at ×40). The diagnostic assessment was divided into cases that were either non-diagnostic, negative, atypical, suspicious or overtly malignant. The cells were categorized as either being present as single cells, groups of cells or a mixture of single and groups of cells. This portion of the evaluation of the cytological specimens was intended to help assess if any of the solutions were more effective in dissociating the cells apart. Additionally, we sought to determine if single cells, groups of cells or a mixture of both would have an impact on the ability to make a diagnosis.

The core biopsy specimens recovered after being washed in the separate solutions were evaluated by two pathologists (WDM and JP) as to their integrity, the cellularity of the core, the proportion of the tumor present in the core and whether a diagnosis could be rendered based on the morphology. The integrity of the core was evaluated as being either intact or fragmented. The cellularity of the core was graded as either being high, moderate or low. The proportion of the core with tumor was categorized as being either high, moderate, low, or absent. The ability to make a diagnosis on the tissue present in the core was classified as either diagnostic or non-diagnostic.

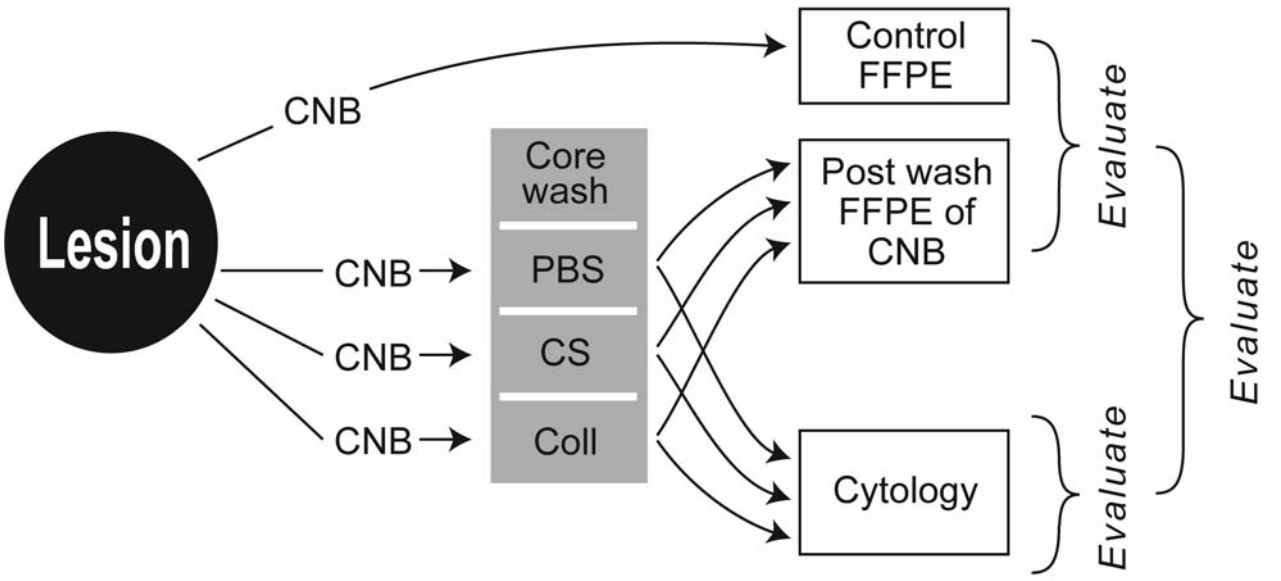

Figure 1. *Schematic of experimental design. Core needle biopsy (CNB) pairs were procured from unfixed resection specimens received by the Department of Pathology. One pair was dedicated for immediate formalin fixation, to be followed by paraffin embedding (FFPE). The remaining cores were either washed in phosphate-buffered saline (PBS), Cell Stripper (CS), or one of three dilutions of collagenase (Coll). After washing, the cores were placed into cassettes for FFPE while cells were spun-down for evaluation. The cytological specimens were evaluated primarily for cellularity, while the fixed tissue cores were evaluated for diagnostic integrity. The cytological and CNB specimen results were later cross-referenced to determine the source of problem in any of the cases.*

After these assessments were completed, the results for each case were collated between the cytospin and core biopsy specimens with these resultant findings compared as a whole for the entire experiment to determine if trends could be identified. Additionally, these results were compared to the morphology of the tumor in the unwashed cores which were submitted for FFPE sectioning to ascertain if tumor architecture was complicit in creating the experimental results.

Results

After preliminary review of the first six cases, the fact that the collagenase solutions at 2% and 1% concentrations extensively damaged the morphology of the cells and tissue was noted. In cytospins, the cells showed extensive degeneration artifacts, with smudged chromatin and cytolysis (Figure 2). In the CNB specimens, there was cell damage with smearing of nuclear material, most notably at the tissue periphery (Figure 3). Because the goal was to identify a method of cell recovery with minimal damage to the CNB specimen, these findings were considered unacceptable and the use of collagenase at these concentrations was discontinued. The wash solution with collagenase at its most diluted concentration was continued for another eight cases. This lower concentration of collagenase, however, was also discontinued after these additional eight cases because it was found to result in continued poor cellular morphology and a lower number of cells recovered in the cytospin specimens compared to the washes using PBS and Cell Stripper.

The cells examined by the cytospin approach after washing with either PBS or Cell Stripper were comparable in terms of quality of the cell morphology and quantity of cells recovered (Figure 2). No evaluation of the cell wash specimens were made on three cases for washes using PBS due to absence of adequate numbers of cells recovered in the cell wash [cases #6 (breast cancer), #12 (high-grade undifferentiated pleomorphic sarcoma), and #13 (renal angiomyolipoma)]. The number of recovered cells (cellularity) in the cell washes was moderate to high in 22 out of 26 (85%) cases washed with PBS. Four cases of CNB tissue washed with PBS recovered low numbers of cells in the cytospin specimens. These cases were #6 (breast cancer), #12 (sarcoma), #13 (renal angiomyolipoma) and #20 (breast cancer). In the latter case, even though the number of cells recovered was low, their quality was sufficient to consider them atypical and render a diagnosis.

No evaluation of cell wash specimens was made on six cases for washes using Cell Stripper. Two cases were for the same reason as for PBS – an absence of adequate numbers of cells [case #6 (breast cancer) and case #12 (sarcoma)]. In three cases, the cellular morphology was sub-optimal despite adequate numbers of cells [cases #20 (breast cancer), #24 (breast cancer) and #26 (endometrial cancer)]. One case was

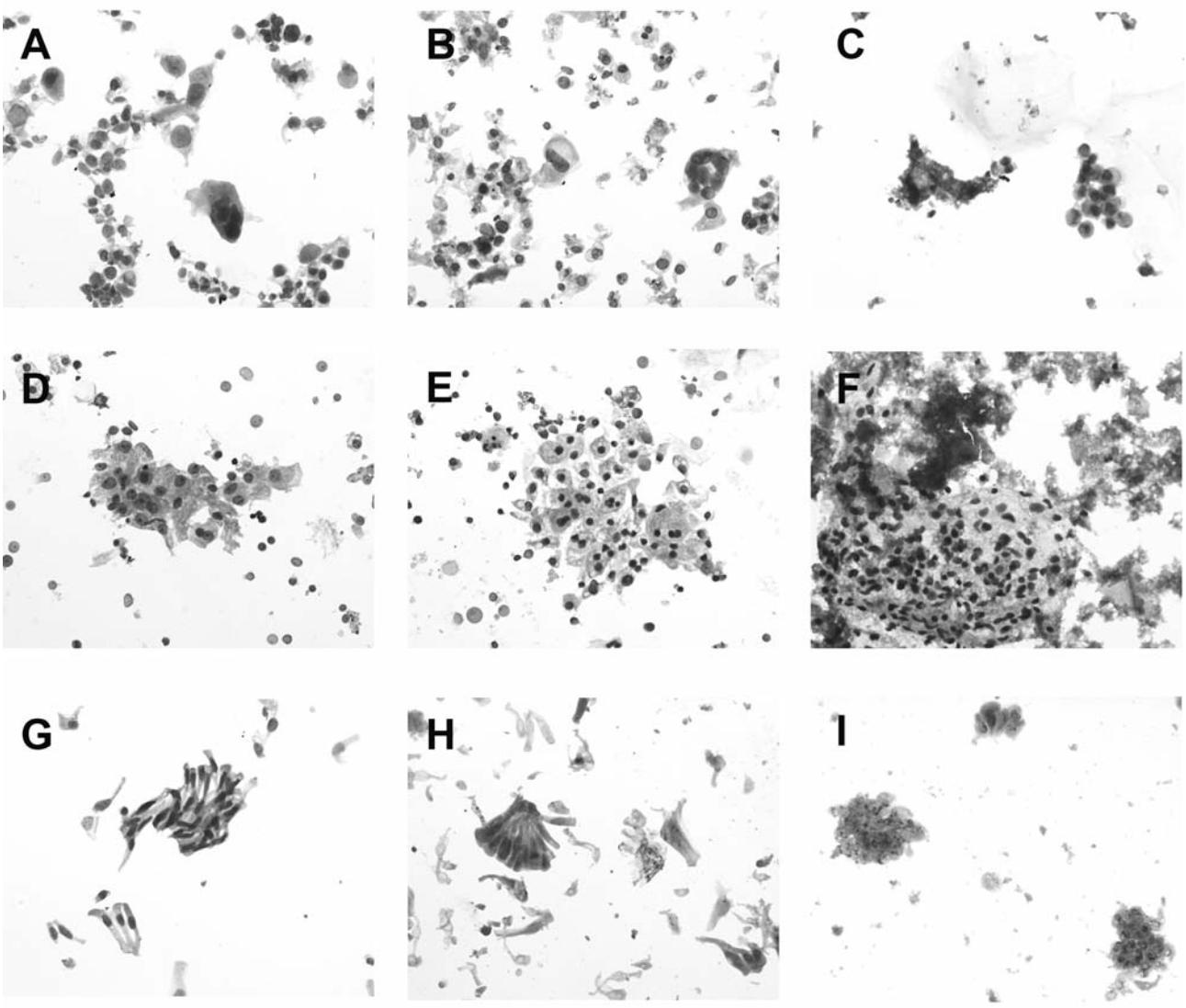

Figure 2. *Comparison of core needle biopsy washes using different solutions: A, D and G: Phosphate-buffered saline (PBS); B, E and H: Cell Stripper; C, F and I: collagenase at the 2% w/v concentration. The first row (A-C) were taken from a metastatic ovarian carcinoma. The second row (D-F) were taken from a clear cell renal cell carcinoma. The third row (G-I) were taken from a colonic adenocarcinoma. The quality of the cytomorphology was comparable between the wash solutions PBS and Cell Stripper. Damage to the washed cells was noticeable, and in some cases prominent, in the cells washed with dilutions of collagenase. Original magnification ×20.*

due to sampling error (tumor tissue not present in the CNB taken for washing with the Cell Stripper, case #2). The number of cells recovered (cellularity) in the cell washes was moderate to high in 19 out of the 26 (73%) of the cases washed with Cell Stripper. The cases with a paucity or no cells recovered in the cytospin specimens consisted of cases #2 (colon cancer), #6 (breast cancer), #8 (renal cell cancer), #12 (high-grade undifferentiated pleomorphic sarcoma), case #15 (high-grade osteosarcoma), #19 (metastatic focus of squamous cell carcinoma) and #20 (breast cancer).

The characteristics of the cells, *i.e.* whether they were present as single cells, groups of cells or a mixture of the two, was evaluated as an additional metric but did not impact on the ability to make a diagnosis. Originally, we intended to determine if a diagnosis could be rendered from the recovered cells of the washed CNB. However, we later deemed this as unnecessary because the post-wash CNB was adequate for evaluation in the majority of cases. Hence, the only evaluation actually needed for the washed samples was if tumor cells were recovered.

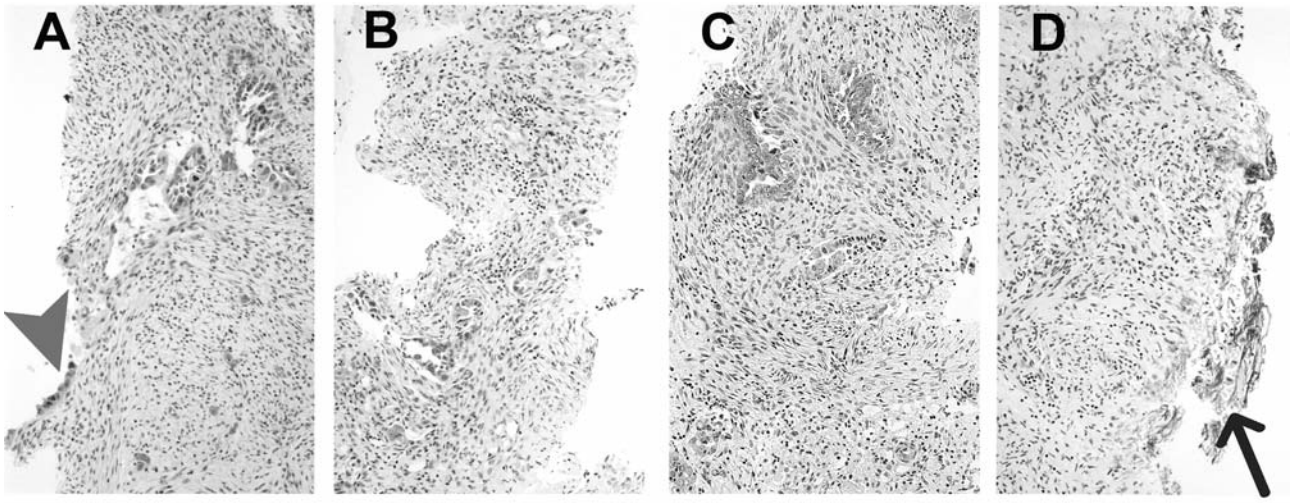

Figure 3. *Images of the core needle biopsy (CNB) post-wash and after formalin fixation and paraffin embedding. A: CNB from one case immediately fixed in formalin and utilized as a control. This image demonstrates tumor cells at the periphery of the CNB, which we hypothesize dissociated off during the wash step, but were recovered in the cytospin preparations (arrowhead). B-D: Images from different cores taken from the same tumor but washed with PBS, Cell Stripper and Collagenase, respectively. In D, there is damage to the cells as evidenced by the smearing of cellular material and loss of morphologic detail (arrow). Original magnification ×10.*

The core biopsy specimens washed with dilutions of collagenase showed varying levels of cell damage most pronounced at the periphery of the core (Figure 3D). As previously mentioned, these cases were eliminated from consideration as an optimal solution for the recovery of cells from a CNB wash due to the combined findings of cellular damage in both the cytospins and fixed CNB specimens. Examination of the core biopsy specimens washed with either PBS or Cell Stripper, then submitted for FFPE and H&E staining were considered diagnostic for tumor in 22 out of the 26 cases (85%) in cases washed with PBS, and 21 out of 26 cases (81%) in cases washed with Cell Stripper. For the non-diagnostic (*i.e.* the diagnosis of a tumor could not be confirmed) cases washed with PBS, the non-diagnostic nature was not due to cell depletion as a result of cell washing in one case, but rather the innate characteristics of the tumor. In case #6, the biopsy was taken from a palpably nodular area of a mastectomy specimen after chemo- and radiation-therapy. The histology of the mastectomy specimen demonstrated extensive fibrosis, with scattered, rare, residual, entrapped tumor cells (Figure 4A and B). The corresponding CNB consisted only of the fibrous tissue. When the CNBs were compared to the corresponding surgical resection blocks of tumor, it was found that the three cases with non-diagnostic CNB material could be attributable to sampling error. In case #17, the CNB was taken from an ovarian mucinous lesion, with the preponderance of the lesion volume attributable to acellular mucin. The corresponding control CNB did demonstrate some floating tumor cells, but the sampled CNB for the PBS and Cell Stripper washes did not (Figure 4C and D). The third and fourth cases not diagnostic for tumor and washed with PBS came from thyroid nodules that were ultimately diagnosed as adenomatoid nodules (cases #22 and #23). In the remaining 22 cases, the cellularity and preservation of the architecture were adequate in the CNB after the wash, and comparable to the CNB dedicated for immediate FFPE. There were five cases where the CNB specimens fixed after washing with Cell Stripper, that were non-diagnostic (*i.e.* a diagnosis of tumor could not be confirmed). Four of the cases (cases #6, #17, #22 and #23) had the same problems as previously described. In two cases, no tumor could be identified due to sampling error (cases #2 and #8). In cases #2 (colon cancer) and #8 (renal cell cancer), the cores taken for the Cell Stripper wash missed the tumor completely in both instances, as evidenced by cores consisting of only normal colonic glands, submucosa and muscularis in case #2, and normal renal parenchyma in case #8.

In eight of the CNB washed with PBS (cases #1, #2, #3, #5, #7, #16, #17 and #19), the proportion of tumor present in the tissue was considered to be low. The cells recovered in the CNB washes was moderate-to-high in all eight of these cases. The CNB tissue was adequate in quality to provide a diagnosis in seven of these cases. The only CNB tissue specimen that was washed but not considered adequate was case #17, which was the mucinous neoplasm of the ovary. The corresponding cytological wash specimen was considered to have atypical cells. For the CNBs washed with Cell

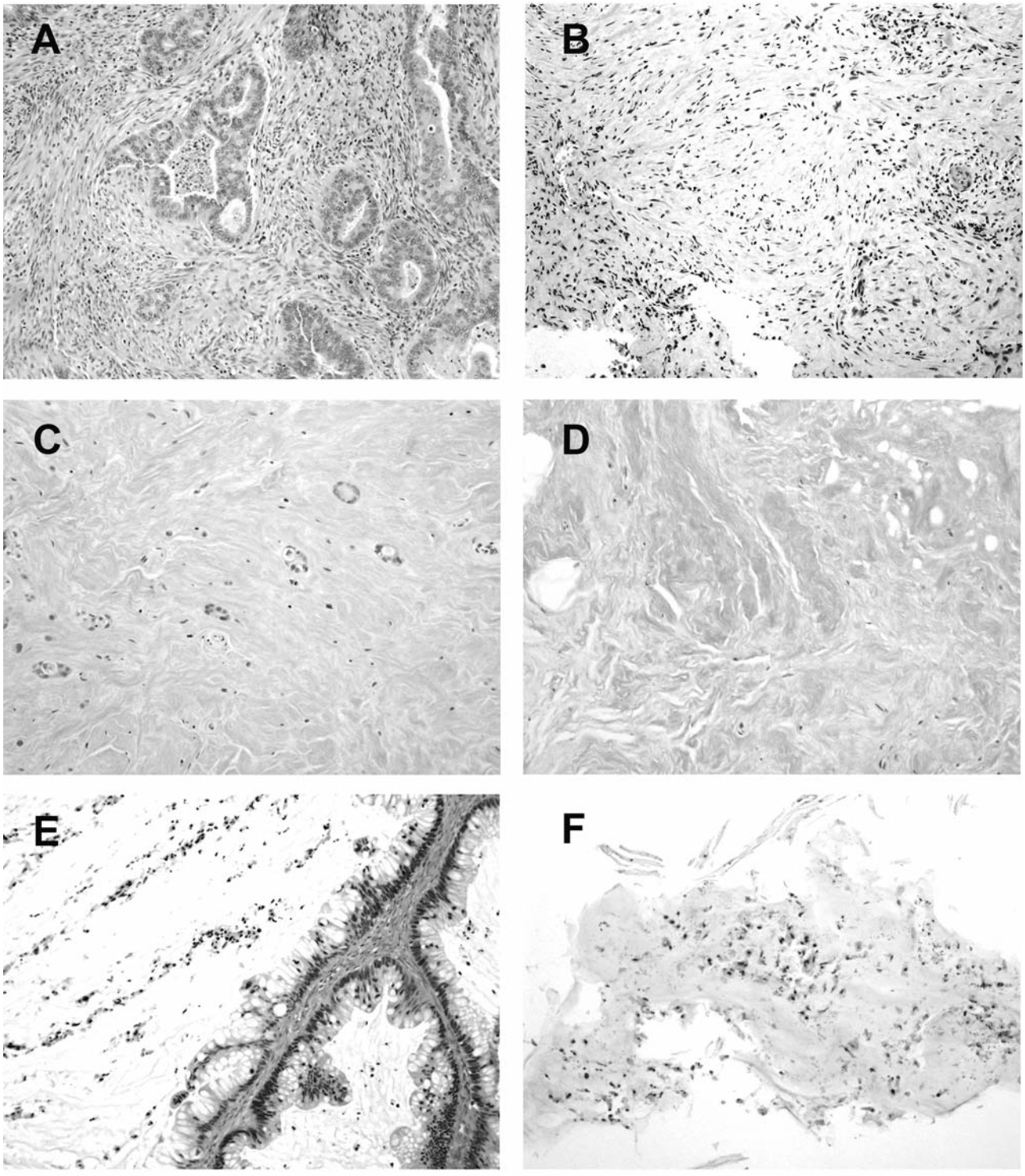

Figure 4. *Correlation between final surgical tumor sections and core needle biopsy (CNB) to determine the reasons for absence or paucity of tumor cells in the washed cytospin specimens. A: Representative image of the colonic tumor resected in case #2. B: Tissue taken from corresponding CNB, in which the tumor was not properly sampled. C: Representative image of the ductal breast tumor in case #6. D: The corresponding CNB, which sampled an area devoid of the sparsely populated tumor cells seen in C. E: Representative image of the mucinous tumor sampled in case #17. F: The corresponding CNB, which consisted predominantly of mucus. Original magnification ×10.*

Stripper, six cases were considered to have a low proportion of tumor cells present. The number of cells recovered in the corresponding CNB washes was moderate to high in five cases, with only case #19 (metastatic squamous cell carcinoma) having corresponding low numbers of cells. The CNB tissue was considered adequate in quality to provide a diagnosis in five one of these cases. The only CNB tissue specimen that was washed but not considered adequate was the same that posed problems with PBS wash (case #17), which was the mucinous neoplasm of the ovary.

Discussion

This project was undertaken to determine if a CNB could provide a source of additional diagnostic material from the lesion being biopsied. Our main hope was to obtain sufficient cells for use in molecular diagnostic testing. By washing these CNBs, dissociated cells could theoretically be recovered and dedicated for molecular testing while the post-wash CNB tissue specimen would be processed for FFPE and evaluation by traditional methods for diagnostic morphology. Currently, this is not an available modality, as CNBs at our Institution are only processed by FFPE. Some investigators have researched whether pre-fixation approaches such as touch preparations of the CNB could yield diagnostic cells, with mixed results (9-12, 14). Their goal, however, was to provide a rapid diagnosis of the cells before the slower turn-around time of the FFPE-processed CNB. In contrast, our approach used a more gentle method, namely washing of the CNB, and the cells recovered were intended for what currently is ancillary molecular testing. In the near future, these tests may become part of the diagnostic requirement for many types of tumors, especially lung carcinomas (5). To the best of our knowledge, this approach has not been investigated in relation to our intended purpose. Our hypothesis in undertaking this project was to determine if diagnostic cells from the CNB could be prompted to dissociate from the tissue aided by either non-enzymatic or enzymatic solutions. Originally, the idea of washing the cores with PBS was to provide a baseline from which to compare against, with respect to the numbers of (additional) cells that the non-enzymatic and enzymatic solutions were able to dissociate. A major requirement for selecting which solution was optimal for the recovery of cells washed-off a CNB was the preservation of the dissociated cells and the quality of the tissue morphology of the post-washed CNB. It became quickly apparent that despite serially diluting the collagenase solutions, none of the concentrations tested were viable options due to the poor results noted in both the cytology and fixed-tissue preparations. While the damage incurred by the cells in the cytospin specimens using the collagenase solutions were not universal (did not affect all cells), the requirement for there being no cellular damage was necessary, knowing that in some instances the numbers of recovered cells may be low. In these cases, the proportion of affected cells would be higher, possibly yielding biased results.

The experimental results of CNBs washed with either the non-enzymatic cell dissociator Cell Stripper were comparable to the results obtained by washing CNBs with PBS. Additionally, both solutions preserved the architecture and morphology of the corresponding cytospin specimens respective post-wash FFPE CNB. The PBS solution may be more favorable due to cost and availability. We speculate that the majority of cells recovered from the wash may not necessarily be due to cells dissociating from the core due to the intrinsic properties of each solution, but rather be due to the mechanical trauma associated with performing the CNB wash procedure. Additionally, it is possible that the cells recovered from the CNB wash may actually have been displaced when the sheath of the needle is retracted forward to cover the tissue slot in the needle at the time of the biopsy itself. Although these instruments are well fitted with respect to the scale of the human eye, there may be enough space between the needle and sheath to dislodge cells on a smaller scale. This theory may also explain why the rate of recovery was poor for the specimens that were predominantly fibrous, as there were no cells to dislodge along the needle tract, there being only fibrous material instead.

Other cases with poor recovery were attributable to sampling error. The quality of the cells evaluated in the cytospin arm of this project may have resulted in better scores, *i.e.* better nuclear and cytoplasmic detail, if evaluated using a platform other than the cytospin method. The cytospin approach used in this project is dependent on human efficiency during processing to prevent problems that influence detail such as air drying artifacts. In contrast, the promise of a microfluidic approach, where cells are contained within a fluidic environment and hence will not air dry, may represent an alternative method for the confirmation that targeted cells are present and their recovery for molecular analysis possible (15).

The washes did not alter the quality of the post-wash CNB morphology. The eight cases with a low proportion of tumor present in the CNB after washing with PBS were still considered either diagnostic for tumor, or suspicious for tumor. The six cases with a low proportion of tumor present in the CNB after washing with Cell Stripper were still considered diagnostic for tumor or suspicious for tumor. For these cases, the corresponding CNB specimens dedicated for immediate FFPE with the intention of serving as control material had a similarly low proportion of tumor present in the CNB. We interpret these findings to indicate that the recovered cells from the washes did not deplete the CNB tissue of tumor cells. These recovered cells may have loosened or dissociated from the periphery of the tissue core and would have otherwise been lost if processed by regular FFPE.

An enrichment step for the washed tumor cells was not included in this experiment. This may have affected the cellularity of recovered washed cells towards the higher end. It is possible that cells may be lost through the cytospin technique during the centrifugation step in this experiment. Enrichment approaches will need to be incorporated in future studies in order to increase the diagnostic accuracy of any assays being performed on recovered cells.

We believe this approach can provide a boon for molecular testing of image-guided and superficially sampled lesions. A recent study suggested that CNB is underutilized in the U.S.A., resulting in a negative impact on the diagnosis and treatment of certain cancer patients (16). An image-guided CNB can be taken to establish an initial diagnosis of a lesion, or can be performed along the time frame of the patient's treatment and recovery, specifically to document recurrence or progression (17, 18). Even if the primary tumor had previously been sequenced, the recovered cells from a washed CNB may provide the opportunity to perform additional molecular diagnostic testing. Although the numbers of cells recovered is small, we are currently testing methods that would ensure enough material is present for molecular sequencing, in particular, conditional reprogramming of these cells and unbiased amplification of the targeted cell DNA (19, 20). We believe these approaches, along with the sequencing performed on these cells, will one day be considered integral to providing insight into the chronological diversification or emergence of certain clones, the mutational reason for acquired resistance to therapy, and a molecular snapshot from which other surveillance methods (*e.g.* circulating tumor DNA or circulating tumor cells) can be gauged.

In conclusion, we demonstrated that the washing of a CNB can recover cells for ancillary testing. We found that an enzymatic approach to washing CNBs did more harm than good. Because the number of cells recovered in the cytospins were comparable between the CNBs washed with PBS and those washed with Cell Stripper, we hypothesize that cells that are dissociated from the tissue are recovered due to the washing procedure or mechanical trauma associated with the spring-loaded return of the sheath to cover the needle, and not necessarily the actual type of wash solution. By traditional processing methods, these cells are lost because no existing protocol is utilized to recover them, and hence potentially diagnostic material is wasted. In this era where pathologists are being expected to do more with less tissue, washing of a CNB may represent an approach whereby diagnostic cells can be recovered and saved for molecular testing without affecting the integrity of the tissue. In addition, it would allow pathologists to continue performing diagnoses based on traditional methods, such as morphology and immunohistochemistry, the mainstay of anatomic pathology and a critical factor in arriving at a correct diagnosis (21).

Acknowledgements

The Authors wish to thank John Nyquist, MS, CMI for the illustration of Figure 1.

References

1 Gupta S, Wallace MJ, Cardella JF, Kundu S, Miller DL and Rose SC: Quality improvement guidelines for percutaneous needle biopsy. J Vasc Interv Radiol *21*: 969-975, 2010.

2 Breuning W, Fontanarosa J, Tipton K, Treadwell JR, Launders J and Schoelles K: Systematic review: comparative effectiveness of core-needle and open surgical biopsy to diagnose breast lesions. Ann Intern Med *152(4)*: 238-246, 2010.

3 Hoadley KA, Yau C, Wolf DM, Chemiack AD, Tamborero D, Ng S, Leiserson MD, Niu B, McLellan MD, Uzunaangelov V, Zhang J, Kandoth C, Akbani R, Shen H, Omberg L, Chu A, Margolin AA, Van't Veer LJ, Lopez-Bigas N, Laird PW, Raphael BJ, Ding L, Robertson AG, Byers LA, Mills GB, Weinstein JN, Van Waes C, Chen Z, Collisson EA, Cancer Genome Atlas Research Network, Benz CC, Perou CM and Stuart JM: Multiplatform analysis of 12 cancer types reveals molecular classification within and across tissues of origin. Cell *158(4)*: 929-944, 2014.

4 Liu S, Wang H, Zhang L, Tang C, Jones L, Hua Y, Liying B, Wang A, Liu Z,Lou F, Zhang D, Sun H, Dong H, Zhang G,Dong D, Guo Z, Guo B, Yan H, Yan C, Wang L, Su Z, Li Y, Huang XF, Chen SY and Zhou T: Rapid detection of genetic mutations in individual breast cancer patients by next-generation DNA sequencing. Hum Genetics *9*: 2, 2015.

5 Lindeman NI, Cagle PT, Beasley MB, Chitale DA, Dacic S, Giaccone G, Jenkins RB, Kwiatkowski DJ, Saldivar JS, Squire J, Thunnissen E and Ladanyi M: Molecular testing guideline for selection of lung cancer patients for EGFR and ALK tyrosine kinase inhibitors. J Thorac Oncol *8(7)*: 823-859, 2013.

6 Martinez de Lecea MG and Rossbach M. Translational genomics in personalized medicine – scientific challenges *en route* to clinical practice. The HUGO J *6*: 2, 2012.

7 Ofiara LM, Navasakulpong A, Ezer N and Gonzalez AV: The importance of a satisfactory biopsy for the diagnosis of lung cancer in the era of personalized treatment. Current Oncol *19*: S16-S23, 2012.

8 Dogan S, Becker JC, Rekhtman N, Tang LH, Nafa K, Ladanyi M and Klimstra DS: Use of touch imprint cytology as a simple method to enrich tumor cells for molecular analysis. Cancer Cytopathol *121(7)*: 354-360, 2013.

9 Masood S, Feng D, Tutuncuoglu O, Fischer G, Bakhshandeh M, Bertholf RL and Wolfson D: Diagnostic value of imprint cytology during image-guided core biopsy in improving breast heatlh care. Ann Clin Lab Sci *41(1)*: 8-13, 2011.

10 Moghadamfalahi M, Podoll M, Frey AB and Alatassi H. Impact of immediate evaluation of touch imprint cytology from computed tomography guided core needle biopsies of mass lesions: Single institution experience. CytoJournal *11*: 15, 2014.

11 Rekhtman N, Kazi S, Yao J, Dogan S, Yannes A, Lin O, Silk M, Silk T and Durack JC: Depletion of core needle biopsy cellularity and DNA content as a result of vigorous touch preparations. Arch Pathol Lab Med *139*: 907-912, 2015.

12 Wauters CA, Sanders-Eras CT, Koolstra BW and Strobbe LJ: Modified core wash cytology procedure for the immediate diagnosis of core needle biopsies of breast lesions. Cancer *117(5)*: 333-337, 2009.
13 Zhou J, Belov L, Huang PY, Shin JS, Solomon MJ, Chapuis PH, Bokey L, Chan C, Clarke SJ and Christopherson RI: Surface antigen profiling of colorectal cancer using antibody microarrays with fluorescence multiplexing. J Immunol Methods *355*: 40-51, 2010.
14 Tong LC, Rudomina D, Rekhtman N and Lin O: Impact of touch preparations on core needle biopsies. Cancer Cytopathol *122(11)*: 851-854, 2014.
15 Mojica, WD Oh, KW, Lee H, H, Furlani E and Sands AM: Maximizing derivable information from cytologic specimens for pathologic and molecular diagnostics. J Am Soc Cyotpathol *4*: 141-147, 2015.
16 Eberth JM, Xu Y, Smith GL, Shen Y, Jiang J, Buchholz TA, Hunt KK, Black DM, Giordano SH, Whitman GJ, Yang W, Shen C, Elting L and Smith BD: Surgeon influence on use of needle biopsy in patients with breast cancer: A national Medicare study. J Clin Oncol *32*: 2206-2216, 2014.
17 Lindstrom LS, Karlsson E, Wilking UM, Johansson U, Hartman J, Lidbrink EK, Hatschek T, Skoog L and Bergh J: Clinically used breast cancer markers such as estrogen receptor, progesterone receptor and human epidermal growth factor receptor 2 are unstable throughout tumor progression. J Clin Oncol *30(21)*: 2601-2608, 2012.
18 Jones SJM, Laskin J, Li YY, Griffith OL, An J, Bilenky M, Butterfield YS, Cezard T, Chuah E, Corbett R, Fejes AP, Griffith M, Yee J, Martin M, Mayo M, Melnyk N, Morin RD, Pugh TJ, Severson T, Shah SP, Sutcliffe M, Tam A, Terry J, Thiessen N, Thomson T, Varhol R, Zeng T, Zhao Y, Moore RA, Huntsman DG, Birol I, Hirst M, Holt RA and Marra MA: Evolution of an adenocarcinoma in response to selection by targeted kinase inhibitors. Genome Bio *11*: R82, 2010.
19 Liu X, Ory V, Chapman S, Yuan H, Albanese C, Kallakury B, Timofeeva OA, Nealon C, Dakic A, Simic V, Haddad BR, Rhim JS, Dritschilo A, Riegel A, McBride A and Schlegel R: ROCK inhibitor and feeder cells induce the conditional reprogramming of epithelial cells. Am J Pathol *180(2)*: 599-607, 2012.
20 Zong C, Lu S, Chapman AR and Xie XS: Genome-wide detection of single-nucleotide and copy-number variations of a single human cell. Science *338*: 1622-1626, 2012.
21 Mukhopadhyay S: Utility of small biopsies for diagnosis of lung nodules: doing more with less. Mod Pathol *25*: S43-S47, 2012.

Received August 3, 2015
Revised September 11, 2015
Accepted September 16, 2015