Commentary

Putting Aspiration Back Into Thyroid Fine-Needle Biopsy—The Re-Emerging Role of Vacuum Assistance

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In the first large fine-needle aspiration (FNA) series reported by Zajicek,[1] aspiration with the Cameco syringe holder (Belpro Medical, Anjou, Quebec, Canada; US Food and Drug Administration registration no. 3003769953), also known as the aspiration gun, was the norm. Indeed, the first 2 words of Chapter 1 in that prescient textbook are "aspiration biopsy." Beginning with our first FNA experience in the late 1970s at the University of California, San Francisco, my colleagues and I used the aspirating gun on every case for many years. That changed with the 1986 publication by Zajdela et al,[2] whose technique is referred to as the Zajdela technique, or the French technique, or simply "needle-only" biopsy. We soon appreciated the ability of this technique to give very good cellular yield with appreciably less blood. And, as an added bonus, this technique allowed us to approach a patient with the needle concealed in the palm of our hand as opposed to holding the intimidating aspirating gun in plain sight. Before long, we used the needle-only technique almost exclusively. Despite increasing use of the needle-only technique, FNA remains the commonly used term for these biopsies, although they lacked an "A."

Since coming to Sacramento in 1982, FNA has been my main focus and the most significantly growing part of our private practice group, Outpatient Pathology Associates. Knowing that the addition of ultrasound guidance (USG) to FNA greatly improves visualization of the biopsy site and the accuracy of needle placement,[3-5] our FNA clinic acquired its first ultrasound machine in 2003. Although we have come to use exclusively the needle-only technique for our initial passes with the USG for ease of use, we still have identified 3 situations in which our ability to conveniently use aspiration optimizes diagnostic success: 1) cyst evacuation; 2) when initial needle-only sampling results in a dry tap or minimal material in cases of atrophic nodules secondary to chronicity, thyroid suppression, or thyroiditis or in rare cases of papillary carcinoma with a marked fibrous component; and 3) small or predominately cystic lymph nodes with papillary carcinoma. This commentary details those situations, including tips and traps, especially as they relate to USG.

Vacuum Assistance

In my experience, vacuum assistance is best accomplished by using a 34-inch intravenous tube extender, like that offered by Baxter (item 2C6227; "extension set with male Luer lock adapter"). These are readily available and cost less than $1 per unit in bulk. With the assistant holding the Cameco, the pathologist is free to hold the hub of the needle in 1 hand and the probe in the other hand. The needle is tethered to the assistant's Cameco by the intravenous tube extender, as indicated in Figure 1. Because manipulating the hand-held Cameco may be awkward, potentially hindering USG (Fig. 2, left), removing it gives the pathologist the ease of motion of the

familiar needle-only technique (Fig. 2, right). In addition, the pathologist's hand is no longer separated from the hub of the needle by several centimeters of metal. Both factors greatly improve USG performance with better targeting accuracy.

When the pathologist is satisfied with needle placement, he says "Now," and the assistant draws a full, continuous vacuum with the syringe holder. In cyst aspiration, vacuum is maintained throughout the entire process, even as the needle is withdrawn from the nodule. If that vacuum pressure is not maintained, then the heat


FIGURE 1. The 34-inch tube extender is shown. With the assistant holding the aspirating gun, the pathologist (on the left) has a good needle-only feel, with the tubing extender connecting the needle and the aspirator.

of the cyst fluid in the syringe holder warms the air in the syringe, producing positive pressure that will expel cyst content out of the needle onto the patient, the table, and the floor. In contrast, if the biopsy target is a solid nodule, then the pathologist says "Break" at the end of the biopsy, and the assistant disconnects the extender hub from the syringe. This step is absolutely necessary to overcome the residual vacuum in the syringe after the plunger is released, because the plunger does not return fully to its initial position. This residual vacuum, although small, is sufficient to aspirate the sample from the needle core into the hub of the needle or the intravenous tube extender. If that happens, then recovery is limited if not impossible. Always maintain vacuum in the syringe while withdrawing the needle at the end of cyst aspiration. With a solid nodule, always disconnect the intravenous tube from the syringe before removing the needle.

Vacuum Assistance: Cyst Management

The sonographic cross-section of a cyst reflects its character. Low-tension cysts are wider than they are tall, whereas tension cysts have nearly identical cross-section dimensions. An easy way to remember this is to visualize the neck vessels in ultrasound cross-section: The carotid is round, and the jugular vein is flat in patients who have normal venous tension. Tension cysts in the thyroid are often symptomatic, because patients are either uncomfortable because of the firmness of the nodule or they are in pain if there has been an acute event, such as
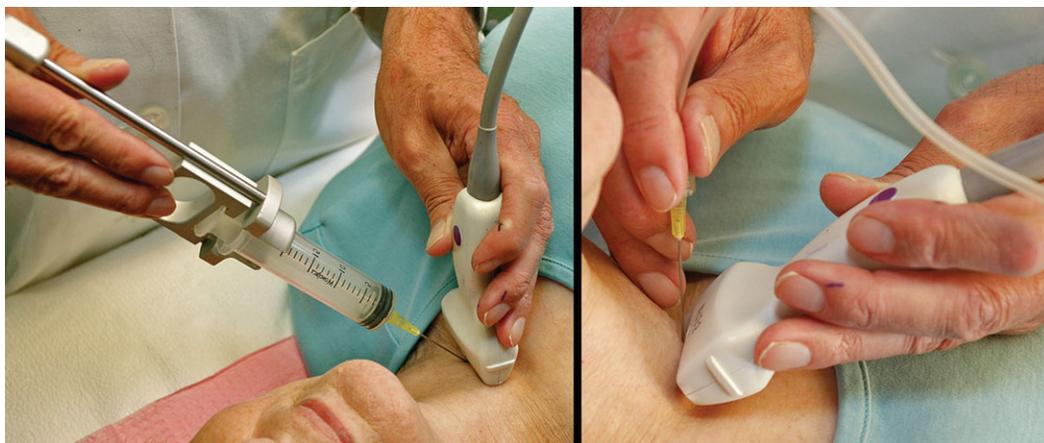
FIGURE 2. These images illustrate the aspiration gun versus the needle hub. On the *left*, the physician's hand is separated from the needle hub by the Cameco syringe holder (Belpro Medical, Anjou, Quebec, Canada). On the *right*, with the Cameco now in the assistant's hand, the pathologist had the familiar feel of the needle-only technique, because the intravenous tube extender has no mass effect.
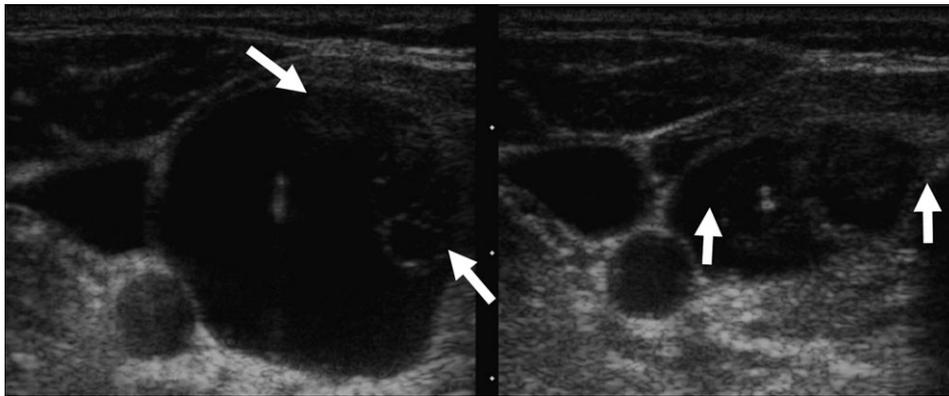
FIGURE 3. These are images of (*Left*) a tension cyst versus (*Right*) a postevacuation target. The mass in the tension cyst on the left is a flat, broad, spread out target over the anterior wall of the cyst. With the cyst contents removed, the target is more accessible with its rounded form. The bright central reflector is the needle in each image.

hemorrhage or nodular infarction. These cysts should be evacuated. Low-tension cysts that are anechoic with no vascularized, mural nodularity have a negligible cancer risk[6] and need not be evacuated unless they are symptomatic or totally overlying a posterior USG FNA target.

Cyst evacuation is greatly facilitated by vacuum assistance. In our clinic, all cysts are done under USG, because it provides greater precision. Once the pathologist places the needle into the center of the cyst using USG, the assistant applies vacuum by means of the Cameco. Because, as cyst content is removed, the needle tip will tend to drop toward the back wall of the cyst, the pathologist continually uses USG to reposition the needle tip as the evacuation progresses. If this downward motion of the needle tip is not arrested, then the needle eventually will impact the posterior wall, which will produce two problems. First, there will be incomplete cyst evacuation; and second, the needle risks lacerating the posterior wall of the cyst with possible painful acute intracystic hemorrhage. The solution is for the pathologist to maintain the needle near the anterior/posterior center of the collapsing cyst wall. Thus, as the end of the USG FNA approaches, the pathologist keeps the needle just above the posterior wall, sets down the probe, and uses this free hand to apply pressure to the collapsing cyst, maximizing cyst evacuation.

In a predominantly cystic complex nodule, it is good practice decompressing the cyst before doing the solid phase biopsy, even if the sonographic mural nodule of interest is on the anterior wall. Because most cyst content is substantially less viscous than solid phase material, even the slightest entry of the needle through the nodule into the cyst cavity will heavily dilute the solid phase material. In addition, once the large cyst component is removed, the solid phase of the nodule, which previously had been stretched out across the anterior wall by the cyst volume (Fig. 3, left), often becomes a more accessible target, now appearing larger and more oval (Fig. 3, right) as opposed to an oblong, thin target before evacuation. Now, with the cyst fluid evacuated, the pathologist can more easily complete the solid phase biopsy with a greater likelihood of success by positioning the needle, as illustrated within the solid component in Figure 3 (right). Because a mural nodule can be fibrous and/or may have an atrophic follicular component, which reduces cellular yield, as discussed below, USG FNA with vacuum assistance of such mural nodules usually produces good-quality material.

Vacuum Assistance: Benign Follicular Atrophy and Papillary Cancer With Prominent Fibrosis

Most solid thyroid nodules, whether benign or malignant, yield abundant material when properly sampled using the needle-only technique. Still, several nodules remain for which vacuum assistance is needed. Many nodules in this group demonstrate benign follicular atrophy[7] in FNA samples. The aspiration smears reveal cells with tiny nuclei about the size of erythrocytes, and a delicate, web-like cytoplasm, often with the prominent although subtle presence of numerous, bare background nuclei (Fig. 4). On the basis of consultation material observed at Outpatient Pathology Associates, smears from these nodules are often called inadequate. When the slides are examined closely, they often have abundant, but subtle, cellular
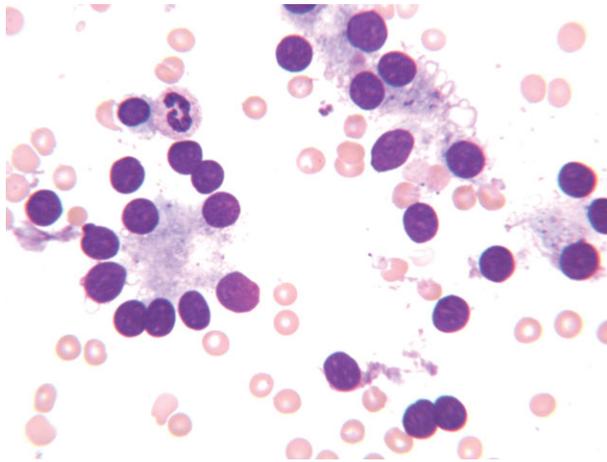
FIGURE 4. Benign follicular atrophy is shown. The nuclei are small (in the range of 1-1.5 greatest erythrocyte dimensions). The cytoplasm is delicate and web-like in areas. With diminished cytoplasm, bland, bare nuclei can be quite prominent but are easily overlooked in the background. These delicate cells are easily distorted into pseudocomplex groups by clot and smear artifact. Note the distortion-free background.
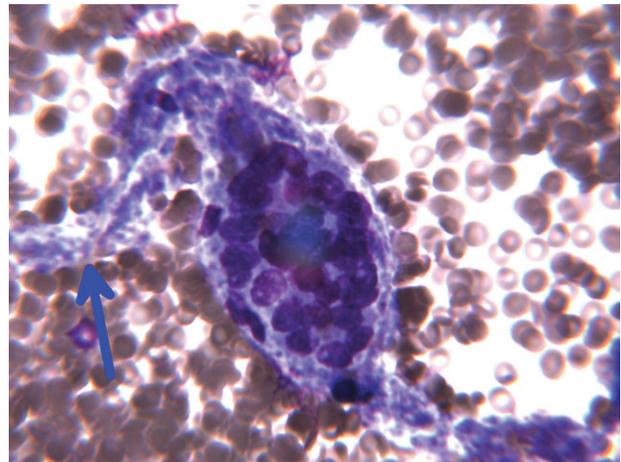
FIGURE 5. Clot-induced pseudocomplexity is observed. The original diagnosis based on clusters like these was microfollicular tumor, and surgery was recommended. On review, all complex groups were ensnared by fibrin and platelet clot (arrow). These could confidently be recognized as pseudocomplexity, because nonclotted areas had bland, atrophic follicular clusters identical to those observed in Figure 4. The review recommendation was repeat biopsy with the proper technique (not surgery).

findings that were overlooked by the initial observer. It is likely that the pathologist was looking for intact sheets of cells with well formed cytoplasm instead of the small atrophic sheet clusters and bare background nuclei. The potential for a more diagnostically dangerous outcome exists with benign follicular atrophy, because these delicate cells are easily disturbed and obscured by mechanical smear distortion and clot artifact. For example, the groups like those illustrated in Figure 5, encircled in platelet and fibrin clot, led the pathologist to an erroneous diagnosis of a microfollicular tumor with a recommendation for surgery. However, the clot-free areas actually reveal perfect bland and noncomplex sheets of benign atrophic follicular cells. Our consult diagnosis was benign thyroid nodule with a recommendation for repeat biopsy, not surgery. Unfortunately, even under optimal biopsy conditions, some of these nodules will have sparse cellularity. This represents a perfect opportunity for vacuum assistance to yield a specimen with greater cellularity, resulting in a fully diagnostic sample.

There are 2 common situations that can produce these troublesome examples of benign follicular atrophy. One group consists of patients who have been on long-term thyroid suppression or who have nodules of long duration. A second benign follicular atrophy group consists of patients with long-standing, chronic thyroiditis, who often have smoothly contoured, distinctive, hypere-

choic nodules (referred to as *white knights* on USG) that do not require biopsy,[6] although these patients may have other nodules that, based on sonographic findings, merit biopsy. In addition, in rare cases of papillary cancer with a prominent fibrous, stromal component, needle-only sampling will produce a dry tap. In these cases, a slight increase in needle resistance often is noted as the needle passes from the surrounding thyroid tissue into the targeted area. Often even with a proper needle-only technique, several these cases produce little or no visually apparent material when expelled onto the glass slide. This is a perfect situation for vacuum assistance.

Also, as a practical matter, if my first 2 needle passes yield relatively little material on simple visual inspection of the resulting smears, then I automatically switch to vacuum assistance for 2 or 3 more passes. This almost always produces sufficient material. The key to successful management of these situations is to know they exist, recognize the situation by simple visual inspection of the unstained slide, and add vacuum assistance as needed.

Vacuum Assistance: Lymph Nodes

A forthcoming edition of *Pathology Case Reviews* takes a detailed look into FNA and USG FNA of lymph nodes that is beyond the scope of this commentary. However,

vacuum assistance proves helpful in the subset of smaller lymph nodes, like those that measure ≤15 mm. If the decision has been made to sample such a lymph node, especially in a patient with a prior papillary thyroid carcinoma (PTC) or if the lymph node demonstrates roundness with a width less than twice its height, then it is disconcerting to the patient and the clinician if the biopsy is nondiagnostic. I will make 1 or 2 passes with the needle-only technique and then immediately go to vacuum assistance if I do not visualize any apparent material on those resulting smears.

Many times in a patient who had a previous PTC, the lymph node will be highly suspicious sonographically, ie, it will have a cystic complement, hypervascularity, transcapsular vascularity, microreflectors, and irregular infiltrative margins. Usually, these lymph nodes can be sampled successfully with the needle-only technique, even if the lymph node is relatively small. I use the term *microreflector* instead of *microcalcification* (MC), because that is the more sonographically appropriate terminology. I discourage the use of MC because, in my practice, well over half the time I find MC in a radiology report, it is wrong. More often than not, these microreflectors designated as MCs are colloid reflectors in a colloid nodule or, less commonly, they are short, linear, stromal reflectors in benign follicular atrophy or chronic thyroiditis. However, here is the problem: a microreflector is an observation that will always be correct, whereas MC is a conclusion based on an observation that, more often than not, in my experience, is wrong. The 2009 American Thyroid Association guidelines[4] state on page 1173 that, if microcalcifications are present, they are highly specific for PTC, although they be difficult to distinguish from colloid. My translation: microcalcifications, if present, are highly specific for PTC except when they are not. By never using MC, I never have to explain why the diagnosis is benign rather than PTC. I have observed patients who needlessly underwent rebiopsy after a benign FNA because the clinician was worried that the biopsy had missed the PTC when, in fact, the MC mentioned in the ultrasound report was a errant conclusion of the microreflectors.

A technical problem exists if the lymph node has a large cystic complement. Just as discussed above with respect to the thyroid nodule with a large cystic complement, a good approach is to use vacuum assistance and totally evacuate the cystic component so that a complex target becomes a solid target.

The diagnosis of metastatic cystic PTC has been greatly aided over the last few years with the addition of the thyroglobulin (Tg) needle wash (TgNW), which can reliably detect very low levels of Tg, approaching 0.1 ng/mL. The needle rinse has also proven helpful to identify calcitonin in suspected medullary carcinoma and parathyroid hormone in potential parathyroid nodules. For all of these ancillary tests, the same simple technique is used. One milliliter of normal saline is withdrawn from a multidose container and transferred to a small plastic specimen container with a tight-fitting lid, which is prelabeled for the patient. After the material collected by FNA of the lymph node is expressed onto slides and smeared, a portion of that 1 mL normal saline is then aspirated through the needle into the syringe until it just enters the syringe hub and is expelled back into the container. This "needle-rinse" process is repeated for each FNA pass. When the FNA is finished, the container is capped and sent to a laboratory that specializes in this test, because this is complex testing that requires experience.[8]

Figure 6 illustrates the 3 basic outcome scenarios when testing lymph nodes for metastatic PTC. If the lymph node is sonographically abnormal but not cystic, then the diagnosis is usually made with cytology, keeping in mind that some patients with solid PTC metastases may be Tg-negative. Negative Tg status does not negate positive cytology. An adequate (lymphocyte-rich) aspirate and a negative Tg is better than 99% predictive of a benign lymph node.[9] If the lymph node is complex (mixed solid and cystic), then usually both cytology and Tg will be positive. Keep in mind that, if the lymph node metastases are more cystic, then there may be fewer classic PTC sheets. However, usually, lobulated metaplastic epithelial cells are observed with dense, well defined cytoplasm, often containing septate vacuoles (Fig. 7), which, in this context, are diagnostic.[10] Finally, predominantly cystic lesions, which, even if they are evacuated and the solid phase is biopsied, may be cytologically negative, revealing only old blood and histiocytes. However, in this instance, the Tg level will be elevated, often dramatically up to the tens of thousands.

An excellent study validating this test is that of Snozek et al.[9] The objective of the test was to identify a cutoff
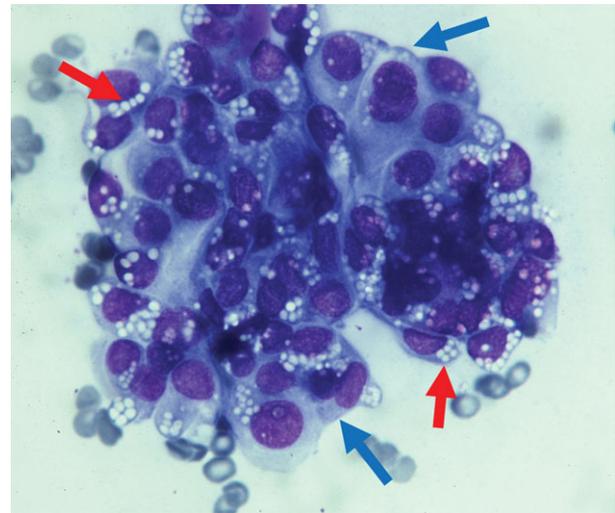
- **Thyroid PTC – Usually Solid**
- **LN Mets – Solid, Cystic, Cystic++**
    - If Solid, Cytology + (TgNW + or -)
    - If Partially Cystic, Metaplastic Cells + (TgNW +)
    - If Purely Cystic, Histiocytes (TgNW +++)

Inclusions   Metaplastic Cells   Histiocytes
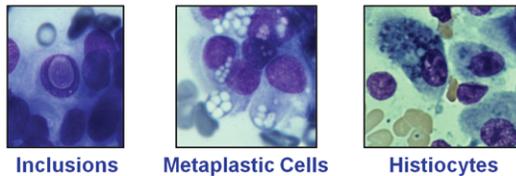
FIGURE 6. This is a pictorial summary of the 3 scenarios for papillary thyroid carcinoma (PTC) in lymph nodes described in the text. Crosses indicate the grade of positivity; −, negative; LN Mets, lymph node metastases; TgNW, thyroglobulin needle wash.

FIGURE 7. A lymph node with metastatic papillary thyroid carcinoma (PTC) and a prominent cystic component is shown. In this situation, the sheets and papillary structures of primary PTC can be absent, and the only epithelial component consists of metaplastic cells with dense, well delineated cytoplasm (blue arrows) and characteristic septate cytoplasmic vacuoles resembling small, uniform window panes generally limited to a sector or portion of the nuclear perimeter (red arrows). In contrast, histiocytic clusters have poorly defined cytoplasmic borders and variably sized vacuoles randomly arranged around nuclei.

level for Tg above which all positive cases would be identified. Those authors observed that anything greater than 1.0 ng/mL of Tg identified all true-positives. In designing any laboratory test, there is always a competition or trade-off between low false-positives and low false-negatives. In their study, the declared goal was no false-negatives, and the study achieved that. However, in so doing, the cutoff of 1.0 ng/mL produced 2 false-positive results for a false-positive rate of 4%. In analyzing data from their Figure 1A, these 2 false-negative results occurred at relatively low levels of Tg, around 3 ng/mL and 10 ng/mL.

Thus, if a lymph node is sonographically normal, then the smears reveal normal lymphocytes with no cystic or hemorrhagic changes, and if Tg comes back at a low level, such as in the midteens or less, then we would sign the case out as "benign lymphocytes without cytologic evidence of papillary carcinoma but with a Tg needle wash 9 ng/mL; see note"; and the accompanying note would then discuss the several possibilities for these low-level findings. Tg may simply be a reflection of blood contamination if the patient has high circulating Tg; or the patient may have a positive lymph node elsewhere, and the elevated Tg in the wash may represent lymph flow from that other lymph node. Early on in our experience with this test, we had a scenario like this with a Tg level in the midteens. The lymph node and several around it were surgically removed, extensively sampled, and were negative. The patient indeed had PTC somewhere in the neck, but just not in the sonographically normal lymph nodes that were sampled. Our TgNW simply detected extrinsic Tg. If the patient has

had a prior PTC, a sonographically normal lymph node, benign cytology without old blood, and a low-level Tg wash, then we simply re-examine the patient in 3 months and rebiopsy any new suspicious changes.

In closing, the Karolinska group in Sweden demonstrated that FNA was a clinically beneficial test that then spread to the United States.[11] Both in name and in practice, aspiration was always an integral part of FNA at the beginning; yet, over time, the needle-only technique became widely used, and many practitioners still use the needle-only technique with USG. However, restoring the "A" back into FNA during USG can improve the diagnostic outcome in several specific situations.

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