With NGS, new hope for managing thyroid nodules

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April 2013—Faced with assessing one of the hundreds of thousands of patients who present with thyroid nodules each year, clinicians know that the initial diagnostic steps are straightforward. With fairly good reliability, using ultrasound examination, fine-needle aspiration, and cytologic examination, they can determine in about 70 to 80 percent of cases whether the nodule is benign or malignant. And good treatment options exist for those in the latter category.

It's the 20 to 30 percent of patients with "indeterminate cytology"—not definitively benign and not definitively malignant—who preoccupy Yuri Nikiforov, MD, PhD, vice chair and director of the Division of Molecular and Genomic Pathology at the University of Pittsburgh. As Dr. Nikiforov describes in an article published Feb. 19 online in *Clinical Cancer Research*, "New Strategies in Diagnosing Cancer in Thyroid Nodules: Impact of Molecular Markers," by pinpointing new molecular markers, researchers at UPMC expect to make a profound impact on the management of patients with thyroid nodules.

Some mutational markers are already available to clinicians through academic pathology laboratories and commercial companies. "What is emerging and is really even more exciting is the use of next-generation DNA sequencing panels based on multiple known and new markers," Dr. Nikiforov says. His institution, which is now conducting clinical validation of panels in the molecular lab, expects within two months to launch what he believes to be the first CLIA-validated laboratory-developed molecular test for thyroid nodules based on NGS. Soon, he predicts, academic pathology labs and eventually commercial companies will adopt and use the panel for diagnostic purposes.

"This is a huge advance in how we can risk-stratify patients with thyroid cancer," he says. The panel is still not ideal and needs to be improved further, he adds, but with NGS, he is confident that improvements won't be far behind.

There is a significant need for additional markers in thyroid nodules, says Dr. Nikiforov, who has been researching thyroid cancer for two decades and molecular markers for 15 years. "The biggest challenge is that thyroid nodules are extremely common and particularly that they increase as people age. Some population studies show by the age of 50 about five to 10 percent of all individuals have palpable thyroid nodules, and ultrasound screening tends to find 50 percent of people have such nodules by age 60."

"So this is a very, very common condition. The cancer frequency in the nodules is low; less than five percent of the nodules are malignant. Therefore, the real challenge is to be sure we can separate benign from malignant nodules without performing a diagnostic surgery."

Dr. Yuri Nikiforov at UPMC: “The more mutations we know, the more we can test for, and the more accurate the tests will be.”

Thyroid cancer incidence has been growing steadily in the U.S. and many other countries over the past four decades, and it is now the fastest growing cancer type in women in the United States. Why the incidence of thyroid cancer is rising remains unclear, although Dr. Nikiforov and colleagues have just submitted a paper for publication that discusses molecular genetic evidence pointing to probable chemical or dietary factors as a reason for the increase.

The introduction of fine-needle aspiration in the 1970s and 1980s has significantly reduced the number of unnecessary thyroid surgeries, Dr. Nikiforov notes.

“Approximately 75 percent of all FNAs can be reliably diagnosed through cytological examination as either benign (65 to 70 percent) or malignant (about five percent). So about 25 percent are indeterminate. And this is a large-scale problem.” In the U.S. about 150,000 FNA biopsies per year are indeterminate.

At the University of Pittsburgh, Dr. Nikiforov started using a limited panel of known molecular markers in 2007. Their big advantage, he says of the markers, is in significantly improving the algorithm for clinically managing patients with thyroid cancer. Even with the panel of seven markers, though, all patients with nodules that have indeterminate cytology cannot be triaged reliably. “Using NGS, we can input many more mutations and do it cheaply, so we are not limited any longer to the few most common mutations. The more mutations we know, the more we can test for, and the more accurate the tests will be,” Dr. Nikiforov says.

The new research initiatives and expanding knowledge have a twofold impact on clinical practice, he explains. One is the ability to find when nodules are benign and not have to subject those patients to unnecessary surgery. Second, many patients who have nodules with indeterminate cytology undergo lobectomy—removal of one of the two lobes of the thyroid—because the diagnosis is not clear, and 70 percent of those cases are benign. But if the diagnosis is cancer, “those patients have to come back for the second surgery to complete removal of the thyroid.”

No one likes that. “The surgeons hate it because you operate twice, and for the second surgery there is a higher probability of significant surgical complications such as recurrent laryngeal nerve injury and permanent hypoparathyroidism. The ideal approach is an up-front total thyroidectomy.” So the molecular markers are extremely helpful, he says, because if mutations are found, there is more than a 90 percent chance it is cancer,

and those patients can avoid the surgical complications and expense of a two-step surgery and have a total thyroidectomy as the initial surgical approach.

In 2009 guidelines issued by the American Thyroid Association, the mutational markers *BRAF, RAS, RET/PTC*, and *PAX8/PPARgamma* were recommended for patients with indeterminate cytology on FNA to help guide management. “In our center, these markers are readily available, and I know in some other medical centers they are getting accepted as routine. Several commercial laboratories offer this panel too.” An ATA task force, of which Dr. Nikiforov is a member, is working now on the revision of the guidelines. “Use of the molecular markers is being carefully considered, and it is my hope that the revised guidelines will endorse even more strongly the use of these new diagnostic tools.”

Under the Bethesda National Cancer Institute reporting system for thyroid nodules, the indeterminate cytology encompasses three diagnostic subgroups. “One is this FLUS/AUS, with the lowest cancer risk, on average 15 percent. The second is FN/SFN [follicular or oncocytic neoplasm or suspicious for follicular neoplasm], and the last is SMC [suspicious for malignant cells]. Under the microscope, you can issue one of those three diagnoses, and each has different risks,” Dr. Nikiforov says.

“We know if they go for surgery, eight out of 10 patients with FLUS/AUS cytology will have a benign nodule, so surgery was not needed for those patients. These are the lowest-risk nodules, and yet because we cannot successfully diagnose cancer initially, most of those patients do go for surgery. Where we see a very big impact of mutational markers is on this group, because if these nodules are negative for all mutations, the risk of cancer is only six percent. It’s so low they don’t need to be operated on. They can be followed up on with periodic ultrasound examination. On the other hand, if a mutation is found, then the risk is 85 percent verified, and those patients should go for surgery.”

This recommendation was a fairly recent development, Dr. Nikiforov notes. “A new management algorithm was first proposed in 2011 based on a study we published in the *Journal of Clinical Endocrinology & Metabolism*, which allowed us to conclude that patients with FLUS/AUS and negative for all mutations can go without surgery.” So far, he says, that study is the largest to date on molecular markers and subsequent management of patients. It was a multidisciplinary effort, he says, led by a team of endocrinologists, endocrine surgeons, radiologists, cytopathologists, and molecular pathologists.

Right now, when patients come in and have FLUS/AUS cytology, most go for surgery. “It’s kind of like ‘one size fits all,’ but only 10 to 20 percent of these nodules are cancer. Using this new diagnostic tool, we can individualize the approach, recommending some for surgery, some for followup with ultrasound, and so on.”

For those patients diagnosed with thyroid cancer, an accepted management is that they have a complete thyroidectomy and radioactive iodine treatment. But radioactive iodine is a radiation treatment and has some risks. The majority of thyroid cancers are low-grade cancers, Dr. Nikiforov points out. “They’re very unlikely to recur and kill patients. Nonetheless, we treat all of them with aggressive therapy. Therefore, one other need for mutational markers is we must use them to separate, to identify only those thyroid cancers that are really aggressive and need to be further treated after surgical removal. For those that are not aggressive, we need an individualized approach—and that can be done with *BRAF* mutations plus additional markers we are developing.”

BRAF mutation correlates with a higher chance of recurrence and tumor-related death, he notes. “Treatment standards are now in development, but some patients with positive *BRAF* mutation found in the needle before surgery may benefit from more extensive surgery to be sure the tumor is removed completely. Those cancers that don’t have this mutation may not need radioactive iodine therapy.”

What remains to be explored? “The point mutations of the *BRAF* and three *RAS* genes and *RET/PTC1, RET/PTC3*, and *PAX8/PPARgamma* rearrangements are known to occur in approximately 70 to 75 percent of all thyroid cancers, which means there are still about 25 percent of cancers that don’t have mutations that can be detected by molecular markers. So this is a current limitation of this seven-marker panel.”

However, another 10 to 15 percent of cancers have less common known mutations. For example, *TSHR* mutations, *BRAF* rearrangements, and additional types of *RET/PTC* rearrangements are known to occur in well-differentiated thyroid cancers, and *TP53, PIK3CA*, and *CTNNB1* mutations are known to occur in poorly differentiated and anaplastic carcinomas. “We know about those. But the problem is they occur in only about one to two percent of thyroid cancers.” For that reason, until recently it’s been difficult economically to test more than several markers, Dr. Nikiforov says. “It would become very expensive if every nodule were tested for all of these markers using the routine laboratory assays.” With the introduction of next-gen sequencing, this problem is resolved. “We have prepared a large panel to be run using NGS that will include not only the seven main mutations but all rare mutations that nevertheless occur in another 10 to 15 percent of cancers. So this addition will increase the sensitivity and specificity of cancer detection.”

Still remaining are about 10 to 15 percent of cancers that don’t have any known mutations. “Although most of them are very low-grade, indolent cancer, we still need to find markers for these tumors,” he says. “If you find those, then you create an ideal molecular test that will be able to accurately stratify all nodules with indeterminate cytology into either definitively benign or malignant.” Could such a test be developed? Dr. Nikiforov is optimistic. His laboratory is actively involved in whole-genome and whole-transcriptome sequencing of thyroid tumors with no known mutations, and The Cancer Genome Atlas (TCGA) is also sequencing 500 thyroid papillary cancers. “We expect that effort may also lead to the discovery of new mutational markers. In fact, we have already discovered several novel chromosomal rearrangements and point mutations in thyroid cancer that can be used as additional diagnostic markers.”

While some European publications have supported use of similar panels for diagnosis or as a marker for more aggressive carcinoma, the markers are not widely used in Europe, Dr. Nikiforov says. He believes the U.S. is in the vanguard in this effort.

“We currently routinely use seven mutational markers that identify 70 to 75 percent of tumors. Next, the NGS panel that we expect to introduce within two months will cover about 85 to 90 percent of cancers, including virtually all aggressive cancers. And my goal is to eliminate the uncertainty of indeterminate cytology from our care for patients with thyroid cancer.” At UPMC, first cytologic evaluation will be performed, then molecular tools applied to the 30 percent indeterminate to separate them into two groups, either benign or malignant, with high accuracy. “And that will tell our clinicians exactly how to manage those patients. Not only that, we will be able to predict how aggressive the patient’s cancer is, and how to treat it in a very individualized manner.”

Over the long term, Dr. Nikiforov believes virtually all mutations can be identified and put onto a molecular marker panel to test for thyroid cancer. “Now that we have the ability to use NGS to sequence the whole genome, we have a reasonable hope this will happen—and happen soon.”

Anne Paxton is a writer in Seattle.