



## The Detection of Circulating Tumour DNA in Thyroid Disease

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Thyroid nodules are extremely common, with five per cent of the population having palpable nodules and up to 50 per cent of the population having nodules that can be detected on ultrasounds. Nodules are up to five times more common in women.

Despite the common occurrence of nodules, only five to 10 per cent of them harbour cancer. The standard evaluation of patients with thyroid nodules includes an ultrasound and needle biopsy. If the ultrasound characteristics are not worrisome and the needle biopsy is benign, nodules can be observed, as the chance of malignancy is low. Conversely, if the needle biopsy is conclusive for malignancy then surgery is indicated, as the nodule is almost certainly cancerous.

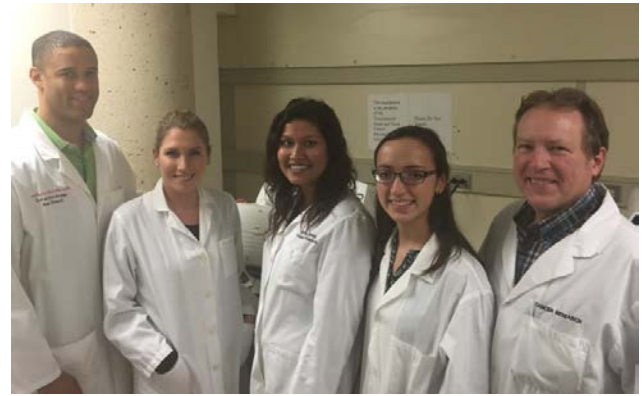
Although the standard work-up clearly directs patient care in this situation, approximately 50 per cent of the time needle biopsies and ultrasound results can be indeterminate and provide only a relative risk of malignancy.

Most commonly, approximately 25 per cent of needle biopsies are reported as follicular lesions of undetermined significance (FLUS) or follicular neoplasms that have 10 per cent and 30 per cent chances, respectively, of being malignant.

Typically, patients with these fine needle biopsy results undergo diagnostic surgery, with only a small proportion found to have cancer. Although thyroid surgery is generally safe, there are still risks including bleeding, scarring, pain, nerve injury, voice alteration and calcium issues. Thus, many patients undergo unnecessary surgery and suffer side effects that could potentially be avoided if improved diagnostics were available to better differentiate benign from cancerous nodules.

In addition to diagnostic challenges, patients that are conclusively diagnosed with thyroid cancer on final pathology need to be monitored for recurrence. This is typically done with ultrasounds as well as thyroglobulin levels (a protein secreted in thyroid tissue); however, neither is perfectly accurate for picking up recurrences early.

Thyroglobulin specifically can be undetectable in the blood stream even in the 10 per cent of patients who still have their



Members of the Thyroid Cancer Translational Research Team (from left): Anthony Nichols, MD; Morgan Black, MSc; Nicole Pinto, PhD candidate; Kara Ruicci, MD/PhD candidate; and John Barrett, PhD.

thyroid in place. Ideally, a non-invasive blood test could assist with diagnosis as well as patient follow-up.

### **The background on our research project:**

All cells contain DNA (genetic material) that can potentially be released into the blood stream when cells die. Cancer cells harbour genetic changes (mutations) that can enter the blood stream and be detected, as this mutant DNA has a different sequence than the DNA from normal tissue. This DNA enters the bloodstream at a higher rate than in normal tissue, as a significant subset of cells die in the rapidly growing tumour as part of the tumour outgrows its blood supply (tumour necrosis).

In many cancer types, scientists have been able to identify this mutant DNA, which is known as circulating tumour DNA (ctDNA). Interestingly, ctDNA appears to be highly correlated with disease status, with ctDNA levels dropping after successful therapy and rising at the time of disease relapse.

There are preliminary studies of the detection of ctDNA in thyroid cancer hinting at its potential as a diagnostic and therapeutic tool. Most of these studies have been done using a real-time polymerase chain reaction (PCR) technique to amplify and detect DNA.

We're aiming to refine the detection of ctDNA in the evaluation of thyroid nodules and to work toward integrating this technology into clinical care. Our initial research,



presented below, uses the real-time PCR technique; however, we're transitioning to a genetic sequencing machine called the ion Torrent, which we believe will give more accurate and reproducible results.

### Progress to date:

- A graduate student and a medical student have been specifically recruited for this project. Two research associates recruit patients in the clinic and facilitate blood-sample collection. A pathologist and a pathology resident are coordinating the retrieval of the thyroid tumour specimens.
- Ethics approval for this study has been obtained from Western University. More than 120 patients with thyroid nodules scheduled to undergo surgery have given their consent, and pre-operative blood samples have been obtained. Ten patients have declined the post-operative blood draws (as thyroid patients often have multiple post-operative blood tests).

A total of 76 patients have had both pre- and post-operative blood work. We've obtained the final pathology results from all patients as well as tumour samples from the primary thyroid tumours whether benign or malignant.

- The molecular testing completed thus far includes testing pre-operative blood samples from the first 62 patients for the BRAF mutation by real-time PCR. Pre- and post-operative blood samples as well as formalin-fixed samples from the index thyroid nodule were also tested in 38 patients.

We've compared the pre- and post-operative blood work and discovered that the ctDNA levels became undetectable post-operatively in all but one case in which complete tumour removal wasn't possible - suggesting its utility as a tumour marker. In addition, we found that the presence of ctDNA was associated with more aggressive tumours (i.e. larger tumours with extension outside the thyroid).

Surprisingly, we detected the mutant BRAF in the blood of two patients who had no cancer in their thyroid. This could be happening because the mutation is not exclusive to thyroid cancer (i.e. some patients have other

cancers that are giving this positive result), or because a small subset of benign nodules (five to 10 per cent) harbour this mutation and represent pre-malignant lesions.

### Future directions:

The Department of Otolaryngology has purchased an ion Torrent sequencer, which can test for a variety of mutations (not just BRAF). The advantage of this system is that it can test multiple genes all in the same run, and it reads each gene many times (approximately 20,000) so we can quantify the relative amount of mutated DNA to normal DNA in a quantitative "digital" fashion.

We believe this technique will be markedly more accurate than the real-time PCR. We've already purchased and received a custom-designed "thyroid panel" to carry this out. Our graduate student Dr. Krupal Patel will be running this assay over the months to come.

In addition, we've leveraged our samples and obtained an industry grant from Thermo Fisher Scientific to also test our samples on their proprietary digital PCR platform. This will provide us with another technique to compare our ctDNA levels to determine which methodology is the most cost-effective, sensitive and specific.

### Impact and significance:

Although our preliminary results do not clearly distinguish between benign and cancerous tumours, the addition of more genes may resolve this shortcoming.

Our results to date indicate that this technique may be highly valuable for identifying patients with aggressive disease as well as for monitoring for relapse after surgery. If validated, this technology can help us to offer highly personalized care for patients suffering with thyroid disease.

Thank you for your generosity in supporting the London Health Sciences Centre (LHSC) research project *The Detection of Circulating Tumour DNA in Thyroid Disease*. We're pleased to provide this impact report updating you on the research progress made possible through your generosity!

Note: This year, the TFC donated \$32,883.55 to this project, funds that were raised over several years by the *Touch of Spring* Fashion Show in London ON.