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Correlation between the ultrasound-guided thyroid fine needle aspiration cytology with limited molecular testing and surgical histopathology results

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Disclosure Information

I hereby declare that I have had business or personal interests in the following industrial enterprises since 1 September 2018:

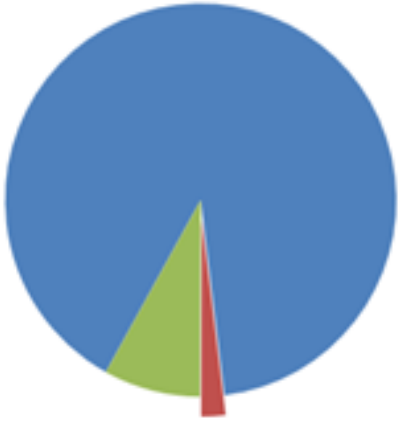
Name of the enterprise / Nature of the interest

Enterprise | Interest

No disclosure.

Thyroid Nodules

Histopathology



- Benign
- Indeterminate
- Malign

Cytology

Approximately 15-30% of cases are cytologically indeterminate.

The Bethesda System for Reporting Thyroid Cytopathology-2017

Diagnostic category	Risk of malignancy (%) NIFT-P = ca	NIFT-P # ca	Usual management
1. Nondiagnostik or unsatisfactory	5-10	5-10	Repeat FNA with ultrasound guidance
2. Benign	0-3	0-3	Clinical and sonographic follow-up
3. Atypia of undetermined significance (AUS)/ follicular lesion of undetermined significance (FLUS)	10-30	6-18	Repeat FNA, molecular testing, or lobectomy
4. Follicular neoplasm or suspicious for a follicular neoplasm (FN/SFN)	25-40	10-40	Molecular testing, or lobectomy
5. Suspicious for Malignancy (SM)	50-75	45-60	Near-total thyroidectomy or lobectomy
6. Malignant	97-99	94-96	Near-total thyroidectomy or lobectomy

OBJECTIVE

This study aims to compare the genotypic alterations for BRAF, NRAS, and KRAS mutations of FNA samples on cell block specimens in the indeterminate and malignant categories with subsequent histology of surgical specimens.

Flowchart

Total (n,%)	Abnormal cytology (n,%)	Nondiagnostic (n,%)	Benig (n,%) n
4467 (100%)	475 (10.6%)	702 (15.7%)	3290 (73.7%)

Abnormal cytology Bethesda Category (n)	III (228)	IV (100)	V (54)	VI (93)

Abnormal cytology with adequate molecular testing (n)	150							
Bethesda Category	III (n)		IV (n)		V (n)		VI (n)	
	62 cases		39 cases		16 cases		33 cases	
Molecular status/ Negative (n,%)	47 (75.8%)		24 (61.5%)		4 (25%)		11 (33.3%)	
Molecular status/ Positive (n,%)	15 (24.2%)		15 (38.5%)		12 (75%)		22 (66.7%)	
Molecular status/ Positive (n)	15 cases		15 cases		12 cases		22 cases	
	1 case	braf+kras-nras-	3 cases	braf+kras-nras-	8 cases	braf+kras-nras-	18 cases	braf+kras-nras-
	3 cases	braf+kras+nras-	1 case	braf+kras-nras+	2 cases	braf+kras+nras-	3 cases	braf-kras+nras-
	4 cases	braf-kras+nras-	6 cases	braf-kras+nras-	1 case	braf-kras-nras+	1 case	braf+kras+nras-
	7 cases	braf-kras-nras+	5 cases	braf-kras-nras+	1 case	braf-kras+nras-		

Total Thyroidectomy (n)	71											
Benign (n)	23											
Malign (n)	48											
Bethesda Category	III			IV			V			VI		
Thyroidectomy (n)	13 cases			24 cases			13 cases			21 cases		
	Benign (n,%)	Malign (n,%)		Benign (n,%)	Malign (n,%)		Benign (n,%)	Malign (n,%)		Benign (n,%)	Malign (n,%)	
	9 (69.2%)	4 (30.8%)		10 (41.7%)	14 (58.3%)		2 (15.4%)	11 (84.6%)		2 (9.5%)	19 (90.5%)	
Molecular status/ Negative (n)	8	1	30.8%	7	6	54.2%	2	2	30.8%	2	6	38.1%
Molecular status/ Positive (n)	1 nras+	1 braf+kras+ 1 kras+ 1 nras+	69.2%	1 nras+ 1 braf+ 1 kras+	2 braf+ 3 kras+ 1braf+nras+ 2 nras+	45.8%		7 braf+ 1 braf+kras+ 1 kras+	69.2%		11 braf+ 2 kras+	61.9%

MATERIALS AND METHODS

The retrospective study enrolled 150 cases diagnosed as atypical by FNA cytology

- 62 (41.3%) as AUS/FLUS,
- 39 (26.0%) as FN/SFN,
- 16 (10.7%) as SM,
- and 33 (22.0%) as malignant

on the basis of TBSRTC that had undergone molecular testing at the Department of Molecular Biology and Genetics.

MATERIALS AND METHODS

- Out of 150 cases with atypical cytologic and molecular testing, 71 had undergone a surgical procedure, and histopathology results were compared with both cytology and molecular status.
- According to the final histopathological results, cases were organized into benign and malign groups.
- The endpoint of the study was malign diagnosis on surgical follow-up.
- The NIFT-P was classified in the benign group.

RESULTS

Correlation of fine-needle aspiration cytology with surgical follow-up in the study population.

	Thyroidectomy			
	Benign	**Malign**	**Total**	
Cytology (Bethesda Category)	n (%)	n (%)	n (%)	**P**
AUS/ FLUS (III)	9 (69.2)	4 (30.8)	13 (18.3)	**0.001***
FN/SFN (IV)	10 (41.7)	14 (58.3)	24 (33.8)	
SFM (V)	2 (15.4)	11 (84.6)	13 (18.3)	
Malign (VI)	2 (9.5)	19 (90.5)	21 (29.6)	
Total	23 (32.4)	48 (67.6)	71 (100)	

*Fisher freeman halton test *p<0.05*

There were statistically significant differences in the frequency of benign and malignant histopathologic results between the cytologic categories III, IV, V, and VI (p=0.001).

RESULTS

Correlation of fine-needle aspiration cytology with surgical follow-up in the study population.

	Thyroidectomy			
	Benign	**Malign**	**Total**	
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SFM (V)	2 (15.4)	11 (84.6)	13 (18.3)	
Malign (VI)	2 (9.5)	19 (90.5)	21 (29.6)	
Total	23 (32.4)	48 (67.6)	71 (100)	

*Fisher freeman halton test *p<0.05*

As expected, the risk of malignancy was higher in samples with more severe cytologic abnormalities.

RESULTS

Correlation between results of fine-needle aspiration cytology and molecular testing.

		Cytology (Bethesda Category)				*Total*	p
		AUS/ FLUS (III)	FN/SFN (IV)	SFM (V)	Malign (VI)		
		n (%)	n (%)	n (%)	n (%)	*n (%)*	
Molecular Status	**Positive**	15 (%24,2)	15 (%38,5)	12 (%75)	22 (%66,7)	*64 (%42,7)*	[1]**0,000***
	Negative	47 (%75,8)	24 (%61,5)	4 (%25)	11 (%33,3)	*86 (%57,3)*	
BRAF	**Positive**	4 (%6,5)	4 (%10,3)	10 (%62,5)	19 (%57,6)	*37 (%24,7)*	[1]**0,000***
	Negative	58 (%93,5)	35 (%89,7)	6 (%37,5)	14 (%42,4)	*113 (%75,3)*	
KRAS	**Positive**	7 (%11,3)	6 (%15,4)	3 (%18,8)	4 (%12,1)	*20 (%13,3)*	[2]**0,772**
	Negative	55 (%88,7)	33 (%84,6)	13 (%81,3)	29 (%87,9)	*130 (%86,7)*	
NRAS	**Positive**	7 (%11,3)	6 (%15,4)	1 (%6,3)	0 (%0)	*14 (%9,3)*	[2]**0,095**
	Negative	55 (%88,7)	33 (%84,6)	15 (%93,8)	33 (%100)	*136 (%90,7)*	

[1]*Chi –square test* [2]*Fisher freeman halton test* *$p<0.05$

- Statistically significant differences existed between the overall molecular status of the categories III, IV, V, and VI (p=0.001).
- Additionally, there was a significant difference between isolated BRAF point mutations in the categories III, IV, V, and VI (p=0.001).
- The positivity of overall molecular testing and the positivity of BRAF point mutations were higher in samples with more severe cytologic categories of the Bethesda System.

RESULTS

Correlation results of surgical follow-up and the combination of cytology and molecular testing (CC-MT) in the study population.

		Thyroidectomy					
		Benign		Malign		Total	
		n	%	n	%	n%	P
Molecular Status	Negative	19	(82,5)	15	(31,3)	34 (47.9)	**0.001***
	Positive	4	(17,4)	33	**(68,8)**	37 (52.1)	
BRAF	Negative	22	(95,7)	25	(52,1)	47(66.2)	**0.001***
	Positive	1	(4,3)	23	(47,9)	24 (33.8)	
KRAS	Negative	22	(95,7)	39	(81,3)	61 (85.9)	**0.001***
	Positive	1	(4,3)	9	(18,8)	10 (14.1)	
NRAS	Negative	21	(91,3)	44	(91,7)	65 (91.5)	**0.959**
	Positive	2	(8,7)	4	(8,3)	6 (8.5)	

*Chi –square test *p<0.05*

Regarding the frequencies of overall molecular status, BRAF, and KRAS point mutations, statistically significant differences were present between the benign and malignant groups on surgical follow-up (p=0.001, p=0.001, p=0.001, respectively).

RESULTS

Correlation results of surgical follow-up and the combination of cytology and molecular testing (CC-MT) in the study population.

		Thyroidectomy					
		Benign		Malign		Total	
		n	%	n	%	n%	P
Molecular Status	Negative	19	(82,5)	15	(31,3)	34 (47.9)	**0.001***
	Positive	4	(17,4)	33	**(68,8)**	37 (52.1)	
BRAF	Negative	22	(95,7)	25	(52,1)	47(66.2)	**0.001***
	Positive	1	(4,3)	23	(47,9)	24 (33.8)	
KRAS	Negative	22	(95,7)	39	(81,3)	61 (85.9)	**0.001***
	Positive	1	(4,3)	9	(18,8)	10 (14.1)	
NRAS	Negative	21	(91,3)	44	(91,7)	65 (91.5)	**0.959**
	Positive	2	(8,7)	4	(8,3)	6 (8.5)	

*Chi –square test *p<0.05*

The frequencies of overall positive molecular status, BRAF, KRAS, and NRAS point mutations in the malign group were 68.8%, 47.9%, 18.8%, and 8.3%, respectively.

RESULTS

Positive predictive values of cytology and the combination of cytology and molecular testing (CC-MT).

	A		B		P_{A-B}
	Cytology		CC-MT		
	PPV	95% CI	PPV	95% CI	
Overall	67.6	0.555-0.782	89.2	0.746-0.970	**0.004***
AUS/ FLUS (III)	30.8	0.091-0.614	75.0	0.194-0.993	0.078
FN/SFN (IV)	58.3	0.366-0.778	72.7	0.390-0.940	0.391
SFM (V)	84.6	0.545-0.981	100	0.717-1.000	0.124
Malign (VI)	90.5	0.696-0.988	100	0.794-1.000	0.137

*Chi –square test *p<0.05*

- The addition of molecular testing to FNA cytology increased the positive predictive value (PPV) of cytology.
- PPV of cytology and CC-MT were 67.6 % and 89.2%, respectively.

RESULTS

Positive predictive values of cytology and the combination of cytology and molecular testing (CC-MT).

	A		B		P_{A-B}
	Cytology		CC-MT		
	PPV	95% CI	PPV	95% CI	
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SFM (V)	84.6	0.545-0.981	100	0.717-1.000	0.124
Malign (VI)	90.5	0.696-0.988	100	0.794-1.000	0.137

*Chi –square test *p<0.05*

- In the AUS/FLUS category, PPV of cytology and CC-MT were 30.8% and 75.0%, respectively.
- Although the p-value had marginal significance, it was probably as a result of the small number of cases.

RESULTS

Sensitivity, specificity and PPV of the CC-MT.

	Sensitivity %	Specificity%	PPV%
CC-MT (overall)	68.8	82.5	89.2
BRAF and cytology	47.9	95.7	95.8
KRAS and cytology	18.8	95.7	90.0
NRAS and cytology	8.3	91.3	66.7
AUS/ FLUS (III) CC-MT	75	88.9	75.0
FN/SFN (IV) CC-MT	57.1	70	72.7
SFM (V) CC-MT	81.8	100	100
Malign (VI) CC-MT	68.4	100	100
Indeterminate (III, IV, V) CC-MT	69.0	81.0	83.3
Indeterminate (III, IV) CC-MT	61.1	78.9	73.3

*Chi –square test *p<0.05*

In this study, the sensitivity of the CC-MT was 68.8%, specificity was 82.5%, and PPV was 89.2%.

RESULTS

Sensitivity, specificity and PPV of the CC-MT.

	Sensitivity %	Specificity%	PPV%
CC-MT (overall)	68.8	82.5	89.2
BRAF and cytology	47.9	95.7	95.8
KRAS and cytology	18.8	95.7	90.0
NRAS and cytology	8.3	91.3	66.7
AUS/ FLUS (III) CC-MT	75	88.9	75.0
FN/SFN (IV) CC-MT	57.1	70	72.7
SFM (V) CC-MT	81.8	100	100
Malign (VI) CC-MT	68.4	100	100
Indeterminate (III, IV, V) CC-MT	69.0	81.0	83.3
Indeterminate (III, IV) CC-MT	61.1	78.9	73.3

*Chi –square test *p<0.05*

When BRAF, KRAS, and NRAS were performed individually in addition to cytology, the specificities were 95.7 %, 95.7%, and 91.3%, respectively.

CONCLUSION

- Routine use of molecular analysis for all FNA samples remains controversial and is not cost-effective.
- The addition of limited molecular testing to FNA cytology may increase the PPV of cytology in indeterminate categories.
- Cell blocks can be a useful and straightforward method for molecular diagnostic studies.
- Our small panel (BRAF, KRAS, and NRAS) with high specificity and high PPV values may be used for the detection of thyroid malignancy.
- It is pertinent that the addition of limited molecular tests to indeterminate thyroid cytology may be more widely used and cost-effective in practice, particularly in developing countries.
- This approach may reduce repeated FNA, needle biopsy, or diagnostic surgery for atypical thyroid nodules.

Merci...