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SFE-AFCE-SFMN 2022 consensus on the management of thyroid nodules

SFE-AFCE-SFMN 2022 Consensus on the management of thyroid nodules : Role of molecular tests for cytologically indeterminate thyroid nodules

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ABSTRACT

The SFE-AFCE-SFMN 2022 consensus deals with the management of thyroid nodules, a condition that is a frequent reason for consultation in endocrinology. In more than 90% of cases, patients are euthyroid, with benign non-progressive nodules that do not warrant specific treatment. The clinician's objective is to detect malignant thyroid nodules at risk of recurrence and death, toxic nodules responsible for hyperthyroidism or compressive nodules warranting treatment.

The diagnosis and treatment of thyroid nodules requires close collaboration between endocrinologists, nuclear medicine physicians and surgeons, but also involves other specialists. Therefore, this consensus statement was established jointly by 3 societies: the French Society of Endocrinology (SFE), French Association of Endocrine Surgery (AFCE) and French Society of Nuclear Medicine (SFMN); the various working groups included experts from other specialties (pathologists, radiologists, pediatricians, biologists, etc.). Because of the emerging role of molecular fine-needle cytology diagnostics, the French Endocrine Society convened a panel of experts to review the evidence for the diagnostic value of molecular tests performed on cytologically indeterminate thyroid nodules.

1. General introduction

Fine needle aspiration cytology (FNAC) is currently the best tool to evaluate the malignant or benign nature of a thyroid nodule preoperatively. Approximately 20% to 30% of cases are classified as cytologically indeterminate, corresponding to categories III and IV of the Bethesda classification. The challenge is to improve the diagnosis of nodules with "indeterminate" cytology in order to avoid unnecessary surgery for nodules that will turn out to be benign in 70–80% of cases. Conversely, in the case of malignancy of the cytologically indeterminate nodule, the operation may sometimes appear inappropriate *a-posteriori*, if it consisted in a lobectomy alone. For these three categories, the clinician needs other tools to refine management, and molecular biology has emerged as a powerful tool. The acceleration of knowledge about thyroid tumorigenesis, thanks in particular to the advent of high-throughput "Next-Generation" sequencing (NGS), has enabled the development of molecular tools. Thus, in the United States, several molecular tests have been marketed for about 10 years, at high prices ($3000 to $6000), and have been implemented in the management of nodules with indeterminate cytology.

Theoretically, for a molecular test to reclassify a cytologically indeterminate nodule as benign or malignant, it would be necessary to be able to identify a molecular abnormality within each cancer and for this molecular abnormality to be specific to malignancy, or to determine gene expression profiles specific to benignity or

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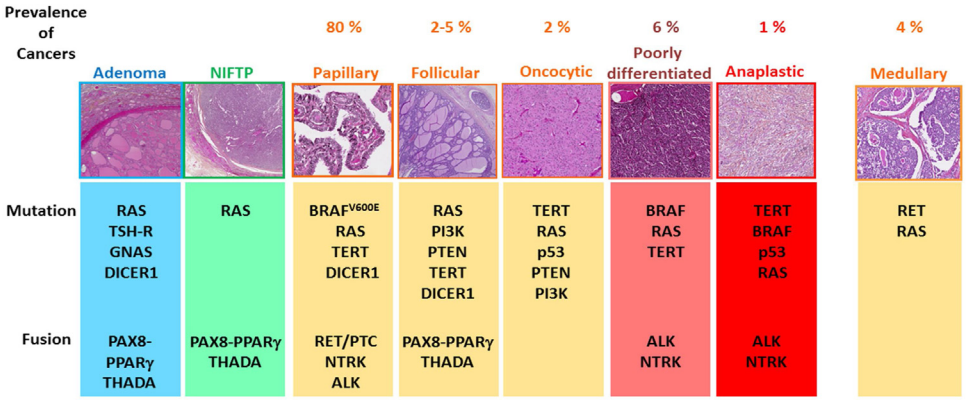

Fig. 1. Main molecular alterations described in thyroid tumors. NIFTP: Non-invasive Follicular Thyroid Neoplasm.

malignancy. However, the presence of molecular abnormalities in both benign and malignant tumors means that it may never be possible to achieve these ideal theoretical performances.

We will detail here the known molecular alterations within the different types of thyroid tumor, the general methodological principles of the various molecular tests used in the context of a cytologically indeterminate thyroid nodule (7-gene panel, ThyroSeq, Afirma™ GSC + Xpression Atlas, ThyGeNEXT/ThyraMIR®), and their performances as reported in the literature.

2. Known molecular alterations

Most of the molecular alterations identified in thyroid tumors concern the effectors of two signaling pathways involved in tumorigenesis, the MAPK (Mitogen-Activated Protein Kinase) pathway and the PI3 K (phosphoinositide 3-kinase) pathway [1,2]. An important concept is the mutually exclusive nature of the alteration that initiates thyroid tumorigenesis: i.e., there is only one initiating mutation or fusion within a signaling pathway within a given cancer. During the course of tumor progression, particularly in the case of dedifferentiation, other molecular alterations may appear. Some molecular alterations are specific to cancers (BRAFV600E mutations, TERT (TElomerase Reverse Transcriptase) promoter mutation), while others (RAS mutations, PAX8/PPAR fusion) are also found in benign tumors and NIFTP (non-invasive follicular thyroid neoplasm with papillary like nuclear features). The factors explaining why these molecular anomalies do not systematically lead to malignant transformation are not known.

The main mutations and gene fusions identified in the different types of thyroid tumor are summarized in Fig. 1 [3–6].

3. Methodological aspects: general principles

3.1. Screening for mutations, fusions and analysis of gene expression, including microRNAs

Three types of molecular alteration are screened for in the cytological sample: 1) sequence variants (point mutations, small insertions/deletions); 2) chromosomal rearrangements resulting in fusion transcripts; and 3) changes in gene or microRNA expression. Mutations are preferably identified by DNA sequencing (PCR and targeted sequencing, high-throughput DNA sequencing). Fusion transcripts and gene expression are studied on RNA (by RNA sequencing). Among the high-throughput sequencing approaches, a distinction should be made between those using targeted panels that enable the efficient identification of actionable mutations or fusions, and global approaches, such as whole-genome or whole-exome sequencing or transcriptome, which are less commonly used.

3.2. Samples used for molecular analysis

The quality of the molecular results obtained depends on the quality and quantity of the sample analyzed. From a practical point of view, a dedicated sample or 2 needle rinses of passages carried out for cytology, are transferred into a conservation solution, limiting nucleic acid degradation (in particular, very fragile RNA), and sent to the molecular biology laboratory for processing. Analysis is also possible using smears [7] or cytoblocks. The amount of suspicious/tumor cells in the sample, although not always assessable, is also an important criterion for interpretation of results.

4. Performance of the main molecular tests

The various molecular tests detailed below are classed as 'rule-out' and 'rule-in' tests.

The performance of the various published and/or marketed molecular tests is detailed here, in terms of:

- sensitivity (Se: probability of "positive" test in case of cancer);
- specificity (Sp: probability of "negative" test in case of a benign nodule);
- positive predictive value (PPV: ability of the test to predict malignancy if positive);
- negative predictive value (NPV: ability of the test to rule out malignancy if negative).

In contrast to Se and Sp, PPV and NPV depend on the prevalence of malignancy in the sample of nodules studied, explaining, at least in part, the variability in performance reported for the same molecular test from one publication to another. Extrapolation of the PPVs and NPVs of the tests presented here to another sample must therefore take this into account. For example, if the cancer prevalence in the first study was higher than that in the second and the sensitivities and specificities do not change, the PPV of the first study will be higher than that of the second, and the NPV lower.

The lower bounds of the 95% confidence intervals for the various performance parameters of molecular tests presented in the Tables below should be taken into account when interpreting the overall performance of the test. These bounds reflect the possible statistical error in the performance calculations of the test, depending on the sample size. As a very practical example, if the calculated sensitivity of a test in a study is 94%, this means that only 6 out of 100 tests are false negatives (the molecular test was negative, whereas the nodule is a cancer: so, 6 unrecognized cancers for 100 nodules

Table 1
Performance of the 7-gene panels for Bethesda III and IV nodules.

1st author, Year	Nodules N	Samples	Nodules treated with surgery	Malignancy prevalence	Se	Sp	NPV	PPV
Bardet 2021 [9]	121	Fine needle aspirate, dedicated pass	121	13.2%	50%[a]	95%[a]	93%[a]	61.5%[a]
Bellevicine 2020 [10]	895	Fine needle aspirate, dedicated pass	113 + 24 with 2nd puncture Bethesda 2	42/137 = 31%	67%[a]	69%[a]	82%[a]	49%[a]
Eszlinger 2017 [11]	280	Air-dried fine needle aspiration slide	225	42/225 = 19%	36%	88%	86%	41%
Bongiovanni 2015 [13]	32 (Bethesda IV)	Air-dried fine needle aspiration slide	32	6/32 = 19%	67%	92%	92%	67%
Eszlinger 2015 [12]	163	Air-dried fine needle aspiration slide	163	45/163 = 28%	49%	92%	82%	71%
Eszlinger 2014 [14]	141	Air-dried fine needle aspiration slide	141	22/141 = 16%	18%	86%	85%	19%
Nikiforov 2011 [15]	900	Fine needle aspirate, rinsed needle	461	93/461 = 20%	59%[a]	98%[a]	90%[a]	87%[a]

[a] In these studies, performance was recalculated on Bethesda III and IV nodules only.

Table 2
ThyroSeq® performance for Bethesda III and IV nodules.

1st author, Year	Nodules (*n*)	Samples	Nodules treated with surgery (*n*)	Malignancy/ NIFTP prevalence (*n*, %)	Se % (95%CI)	Sp % (95%CI)	NPV % (95%CI)	PPV % (95%CI)
Steward 2019 ThyroSeqV3 [28]	247	Fine needle aspirate	247	68 (28%)	94 (86–98)	82 (75–87)	97 (93–99)	66 (56–75)
Jug 2020 ThyroSeqV3 [27]	91	ND	28	10 (11%)	100 (ND)	85[a] (ND)	100 (ND)	39 (ND)
Jug 2020 ThyroSeqV2 [27]	94	ND	20	5 (5%)	80 (ND)	97[a] (ND)	ND	ND
Livhits 2021 ThyroSeqV3 [26]	163	Fine needle aspirate	60	32 (20%)	97 (84–100)	85[b] (77–91)	99[b] (95–100)	63 (48–77)
Desai 2021 ThyroSeqV3 [25]	415	ND	127	70 (17%)	93 (84–98)	90 (86–93)	98 (96–99)	67 (59–75)
Marcadis 2019 ThyroSeqV2 [24]	273	Fine needle aspirate	273	108 (40%)	85 (–)	62 (–)	86 (–)	59 (–)
Taye 2018 ThyroSeqV2 [22]	156	-	63	13 (21%)	83 (–)	43 (–)	91 (–)	27 (14–44)
Valderrabano 2017 [19]	190	-	102	20 (20%)[c]	70 (46–88)[c]	77 (66–85)[c]	91 (82–97)[c]	42 (25–61)[c]

ND: not determined; 95%CI: 95% confidence interval.
[a] If the calculation is done on operated nodules only, specificity is 22% with ThyroSeqV3 and 79% with ThyroSeqV2.
[b] If the calculation is done on operated nodules only, specificity is 36% and NPV 91%.
[c] Assuming that the nodules with negative molecular test that are not operated on are all benign.

tested). However, if the lower limit of the 95% confidence interval for sensitivity is 86%, this means that, for this same test, if another sample had been tested, there could be 14 false negatives out of 100 tests (i.e., 14 unrecognized cancers out of 100 nodules tested). Finally, it should also be noted that in some studies, the performance of the tests is calculated by assuming that all non-operated nodules with a negative molecular test are histologically benign, introducing an additional possible margin of error.

4.1. 7-gene panel

The 7-gene panel is a molecular test for the identification of targeted point mutations (BRAF (p.V600E and p.K601E), NRAS (codon 61), HRAS (codon 61), KRAS (codon 12 and codon 13)) and fusion transcripts (RET/PTC1, RET/PTC3 and PAX8/PPARγ), developed in 2009 by Nikiforov et al. [8].

Its main advantage lies in its ease of implementation. The test is performed on the fine-needle aspirate or from the cytology slide, and can be easily developed in a laboratory at a low cost compared to commercial tests based on NGS techniques.

Performance is variable and depends on the type of molecular alteration detected (specific to thyroid cancer or not). While BRAF mutations or RET/PTC-1 rearrangements are highly specific, RAS mutations are frequently found in follicular adenomas or NIFTP. The overall assessment of the performance of this test should therefore be interpreted with caution.

Table 1 shows the performance of the panel in various published studies [9–15]. It consistently has low sensitivity and therefore a high proportion of false negatives. The overall high specificity probably varies according to the type of mutations identified, but PPV is still low due to the low prevalence of malignancy in the Bethesda III and IV categories.

The studies span a long period of time, during which the TNM histological classification of thyroid cancers and the Bethesda cytological classification of FNAC have been updated. Studies that did not specify the Bethesda cytology classification were excluded.

Panels including a smaller number of genes have also been published (Mancini et al. [16], Moses et al. [17]).

The 7-gene panel has been evaluated in some studies [9–12,15] in the Bethesda V category, in which the prevalence of malignancy is higher than in the Bethesda III and IV category. Sensitivity and specificity were comparable to the data for Bethesda III and IV. On the other hand, PPV was high (90-100%), while NPV ranged between 9% and 72%.

4.2. Next Generation Sequencing

4.2.1. ThyroSeq®

ThyroSeq® is a molecular test based on high-throughput NGS technology, with successive versions [18–32], enabling screening for an increasing number of mutations and gene fusions.

Various studies analyzed the performance of ThyroSeqV2® and/or V3® in Bethesda III and IV nodules; a summary is given in Table 2 [19,22,24–28].

There are sparse data on the performance of ThyroSeq® in Bethesda V nodules. In the study by Stewart et al. [28], only 10 patients with Bethesda V cytology were included out of 257 nodules with ThyroSeq® analyzed.

4.2.2. Afirma™ GSC+Xpression Atlas®

Afirma™ is a molecular test. The first version, "Gene Expression Classifier" (Alexander et al. [15]), developed in 2011, was replaced in 2017 by the Afirma™ "Genomic Sequencing Classifier" (GSC) + "eXpression Atlas" (XA)® (Patel 2018 [33]). Centralized cytology review and 2 dedicated needle passes are required.

The initial test was based on the analysis of the expression level of 167 genes to classify indeterminate thyroid FNAC as "benign'

Table 3
Performance of the Afirma™ GSC+Xpression Atlas test for Bethesda III and IV nodules.

1st author, Year	Nodules N	Samples	Nodules treated with surgery	Malignancy/ NIFTP prevalence (n, %)	Se[a]	Sp[a]	NPV[a]	PPV[a]
Polavarapu et al., 2021 [35]	124	Fine needle aspirate, dedicated pass	49	39%	94%[a]	17%[a]	83%[a]	41%[a]
Yang et al., 2022 [36]	51	Fine needle aspirate, dedicated pass	21	43%	100%[a]	58%[a]	100%[a]	64%[a]
Geng et al., 2021 [37]	133	Fine needle aspirate, dedicated pass	46	37% (NIFTP considered non-malignant)	100%	42%	100%	61%
Zhang et al., 2021 [38]	137 (only BIII)	Fine needle aspirate, dedicated pass	43	21%	100%	41.2%	100%	31%
Gortakowski et al., 2021 [39]	92	Fine needle aspirate, dedicated pass	13	61.5%	100% [63–100]	73.7 [48.8–?]	97% [84.2–99.9]	61.5% [31.6–86.1]
Livhits et al., 2021 [26]	201	Fine needle aspirate, dedicated pass	70	44%	100% [88.8–100]	30.8% [17–47.6]	100% [73.5–100]	53.5% [39.9–66.7]
San Martin et al., 2020 [40]	121	Fine needle aspirate, dedicated pass	42	76.2%	90.6%[a]	50%[a]	62.5%[a]	85.3%[a]
Angell et al., 2019 [41]	114	Fine needle aspirate, dedicated pass	37	46%	94%[a]	20%[a]	90%[a]	50%[a]
Endo et al., 2019 [42]	164	Fine needle aspirate, dedicated pass	27	66%	100% [77.8–100]	17% [2–48]	100% [16–100]	60% [39–79]
Harrell et al., 2019 [43]	139	Fine needle aspirate, dedicated pass	45	64%	97% [82–100]	44% [20–70]	88%	76%
Patel et al., 2018 [33]	190	Fine needle aspirate, dedicated pass	190	23.7%	91.1 (79–98)	68.3 (60–76)	96.1 (90–99)	47.1 (36–58)

[a] In these studies, performance was recalculated on nodules with histological confirmation only.

Table 4
Performance of ThyGeNEXT/ThyraMIR and ThyGenX in Bethesda III and IV nodules.

1st author, Year	Nodules (*n*)	Samples	Nodules treated with surgery (*n*)	Malignancy/ NIFTP prevalence (*n*, %)	Se % (95%CI)	Sp % (95%CI)	NPV % (95%CI)	PPV % (95%CI)
Lupo 2020 [44]	178	Cytology slides	178	54 (30%)	95 [86–89][a]	90 [84–95][a]	97 [91–99][a]	39 [32–46][a]
Labourier 2015 [45]	109	Fine needle aspirate	109	35 (32%)	89 [73–97]	85 [75–92]	94 [85–98]	74 [58––6]

[a] performance adjusted for prevalence of malignancy.

or "suspicious'. It had an excellent NPV but was limited by low specificity (about 7-50%) and insufficient PPV [34].

In the current version of the test, screening for $BRAF^{V600E}$ mutations and the *RET::PTC* fusion transcript is performed prior to gene expression analysis. In the absence of one these two alterations, mRNA sequencing of 10,196 genes is performed. The risk of malignancy in the "benign" category is estimated by the supplier to be 4%, while the risk of malignancy in the "doubtful" category is approximately 50%.

The results of the various studies that evaluated the new version of the Afirma™ test [26,35–43] are presented in Table 3. They exclusively concern nodules classified cytologically as Bethesda III or IV. In the majority of these studies, NIFTPs are classified along with malignant lesions, as they require surgical management.

4.3. *ThyGeNEXT/ThyraMIR®*

The ThyGeNEXT/ThyraMIR combines a high-throughput sequencing panel for mutations in 10 genes and 38 different fusions with quantification of the expression of a panel of 10 microRNAs. Test results are "negative", "positive" or "doubtful".

Only one retrospective series reported the performance (Se, Sp, PPV, NPV) of the ThyGeNEXT/ThyraMIR, in 197 nodules classified as Bethesda III, IV and V, in patients who were all operated on [44]. Performance for Bethesda III and IV nodules is reported in Table 4. Performance for Bethesda V nodules was not analyzed due to the small number of patients in this category ($n = 19$). The performance of the previous version of the test, ThyGenX, was reported in 2015 [45] in a retrospective series of 109 Bethesda III or IV nodules in patients who were all operated on (Table 4).

The comparative performance of the main marketed molecular tests is summarized in Fig. 2.

5. Medico-economic aspects

To date, none of these molecular tests are registered in the French national health insurance nomenclature and therefore are not covered, which limits their distribution in France.

The cost of commercial tests (ThyGeNEXT/ThyraMIR®, Afirma™ GSC + Xpression Atlas®, ThyroSeq) is around $3,000 to $6,000 in the United States.

6. Future outlook and recommendations

The encouraging performance of the various marketed molecular tests reported in the literature and their potential clinical impact should lead to funding of studies testing these strategies in France.

The literature data show that molecular analyses limited to the sequencing of a limited number of genes do not have sufficient performance.

Recommendation 4.1. Given the cost of commercial tests, we advocate developing less expensive academic tests in France, in particular by high-throughput NGS, screening for mutations and gene fusions reportedly involved in thyroid tumorigenesis. Grade B, expert opinion

In the interests of cost optimization, it is worth developing a "pan-tumor" panel of the most common molecular targets for

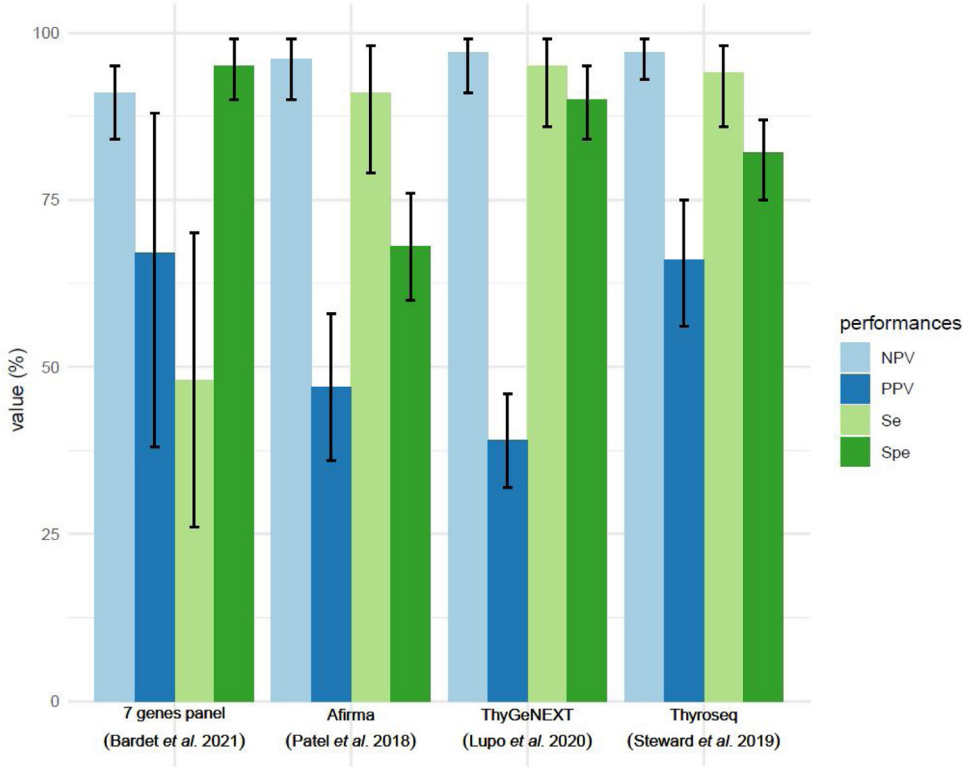

Fig. 2. Summary of the performance of the main molecular tests.

cancers frequently managed in adults. However, these panels will need to be evaluated against the histologic gold-standard before they can be used as predictive tests. Similarly, an evaluation of the medico-economic and quality-of-life benefit associated with molecular tests for management of cytologically indeterminate nodules should be carried out in France.

Recommendation 4.2. We suggest that, considering the very good sensitivity and NPV, molecular testing should have a place in the management of cytologically indeterminate Bethesda III or IV nodules, with the aim of avoiding diagnostic surgery. Grade B, expert opinion

Given the high prevalence of malignancy in Bethesda V nodules (suspicious for malignancy), the performance of the molecular tests reported above is not sufficient to avoid diagnostic surgery. In the future, some molecular tests with high specificity and PPV could guide the extent of thyroid surgery for Bethesda V nodules, especially if a possible prognostic impact is confirmed.

Finally, it should be emphasized that the interpretation of molecular tests depends on the molecular alteration identified, as some are specific to or highly predictive of malignancy ($BRAF^{V600E}$, TERT promoter mutations) whereas others may be present in both benign and malignant lesions.

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Disclosure of interest

The authors declare that they have no competing interest.

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