Review

ACTA CYTOLOGICA

Acta Cytologica 2014;58:319–329
DOI: 10.1159/000366498

Received: June 4, 2014
Accepted after revision: August 7, 2014
Published online: September 2, 2014

Thyroid Atypia of Undetermined Significance or Follicular Lesion of Undetermined Significance: An Indispensable Bethesda 2010 Diagnostic Category or Waste Garbage?

Ivana Kholová[a] Marie Ludvíková[b, c]

[a]Department of Pathology, Fimlab Laboratories, Tampere University Hospital, Tampere, Finland; [b]Institute of Biology, Faculty of Medicine in Pilsen, Charles University in Prague, Pilsen, and [c]Institute of Pathology, General University Hospital in Prague, Prague, Czech Republic

Key Words

Thyroid · Bethesda System · Atypia of undetermined significance · Follicular lesion of undetermined significance · Risk of malignancy · Cytohistological correlation · Molecular analysis · Meta-analysis

Abstract

Objective: The Bethesda System for Reporting Thyroid Cytopathology (BSRTC) was introduced in thyroid cytology in 2007 and is now generally accepted. BSRTC categories include a morphologic description and risk of malignancy as well as follow-up suggestions in each group. However, the category entitled 'atypia of undetermined significance or follicular lesion of undetermined significance' (AUS/FLUS) is problematic. This category is heterogeneous and has been overused so far. ***Study Design:*** Twenty-six studies were included in a meta-analysis. In addition to AUS/FLUS percentage, we analysed repeated AUS/FLUS percentage, cytological and histological correlations, and risk of malignancy and neoplasm for AUS/FLUS. Furthermore, stratification, inter- and intra-observer variability, and the possibility of a switch to another category and its clinical consequences were reviewed. ***Results:*** Out of a total of 81,833 cases, AUS/FLUS accounted for 10.9%, with a 34% risk of malignancy. Persistent AUS/FLUS was found in 21.6% in repeated cytology. Cytohistological correlation was analysed from 16 studies (4,964 cases), revealing 10.4% as AUS/FLUS and a 21.5% risk of malignancy. ***Conclusions:*** An AUS/FLUS category seems to be currently reasonable with clearly defined cytomorphological criteria which do not correspond unequivocally with those of the other categories. An AUS/FLUS category is justified and possible means of its improvement with immunohistochemistry, molecular analysis and imaging are discussed.

Introduction

The Bethesda System for Reporting Thyroid Cytopathology (BSRTC) was introduced into clinical practice in 2007 [1]. BSRTC is a generally accepted 6-tier system. BSRTC categories include a morphologic description, risk of malignancy and follow-up suggestions in each group (table 1). The system has standardised the reporting and management of thyroid gland fine-needle aspirations (FNA) [2]. Up to 6 years of experience has been cumulated at several centres (table 2) [3–8]. In addition, retrospective studies have also approved the feasibility of the

KARGER

E-Mail karger@karger.com
www.karger.com/acy

0001–5547/14/0584–0319$39.50/0

Correspondence to: Dr. Ivana Kholová
Department of Pathology, Fimlab Laboratories
Tampere University Hospital, PO Box 66
FI–33101 Tampere (Finland)
E-Mail ivana.kholova@sll.fimnet.fi

Table 1. The BSRTC

Diagnostic category	Risk of malignancy, %	Clinical recommendation
1. Non-diagnostic/unsatisfactory (ND/UNS)	1–4	Re-aspiration after 3-month interval
2. Benign	0–3	Follow-up
3. Atypia of undetermined significance/follicular lesion of undetermined significance (AUS/FLUS)	5–15	Re-aspiration after 3-month interval
4. Suspicious for follicular neoplasm/follicular neoplasm (SFN/FN)	15–30	Lobectomy
5. Suspicious for malignancy (except of follicular carcinoma)	60–77	Lobectomy/thyroidectomy
6. Malignant	97–99	Thyroidectomy, eventual radiation/ chemotherapy

Table 2. Summary of prospective and retrospective studies using the BSRTC: the AUS/FLUS results

First author [Ref.]	Time period	Prospective/ retrospective	Total samples, n	AUS/FLUS, n	AUS/FLUS rate, %	Malignancy rate in AUS/FLUS, %
Theoharis [3]	2008	pros	3,207	95	3.0	12 in all cases, 48 in resected cases
Kim [4]	2007–2009	pros	865	141	16.3	96.7 in resected cases
Bohacek [5]	2000–2010	pros	1,000	8	0.8	12.5 in resected cases
Bongiovanni [6]	2007–2009	pros	3,308	248	6.7	14.4 in resected cases
Ohori [7]	2007–2008	pros	2,502	513	20.5	17.1 in resected cases
Ratour [8]	2010–2011	pros	2,210	244	11	23 in resected cases
Summary of prospective studies			15,274	1,249	9.7 (0.8–20.5)	35.3 (12.5–96.7) in resected cases
Shi [9]	2004–2008	retro	8,150	174	2.1	35 in resected cases
Jo [10]	1992–2009	retro	3,080	104	3.4	17 in resected cases
Renshaw [11]	1996–2009	retro	7,089	548	7.7	25 in resected cases
Layfield [12]	2003–2007	retro	6,872	664	12.1	5 in all cases, 28 in resected cases
Marchevsky [13]	2006	retro	879	86	9.8	12.8 in all cases, 37.9 in resected cases
VanderLaan [14]	2005–2009	retro	4,691	512	10.9	27 in all cases, 46 in resected cases
VanderLaan [15]	2005–2009	retro	5,327	592	11.2	43 in resected cases
Somma [16]	2006	retro	1,737	275	15.8	26 in resected cases
Nayar [17]	2000–2006	retro	5,194	924	17.8	6 in resected cases
Faquin [18]	2005–2007	retro	n.d.	509	9–12	19 in resected cases
Wu [19]	2006–2008	retro	1,382	376	27.2	6 in resected cases
Rabaglia [20]	2008–2009	retro	765	91	11.9	13 in resected cases
Smith [21]	2007–2011	retro	179	43	24.0	48 in resected cases
Luu [22]	2004–2009	retro	7,072	222	3.1	29 in resected cases
Dincer [23]	2009–2010	retro	7,658	368	4.8	26 in resected cases
Olson [24]	2009–2011	retro	3,956	388	9.8	32 in resected cases
Al-Abbadi [25]	2010–2011	retro	205	15	7	44 in resected cases
Mondal [26]	2009–2012	retro	1,020	9	0.9	20 in resected cases
Broome [27]	2007–2010	retro	282	82	29	20 in resected cases
Tepeoglu [28]	2009–2011	retro	1,021	100	9.8	12.7 in resected cases
Summary of retrospective studies			66,559	6,082	11.4 (0.9–29)	32.7 (6–48) in resected cases
Summary of all studies			81,833	7,331	10.9 (0.8–29)	34 (6–96.7) in resected cases

n.d. = Not determined.

system (table 2) [9–28]. Furthermore, meta-analyses have shown the advantages of the system [29–32]. The new classification helps to compare national and international study results. Overall, the best function of the system is the grouping of follicular neoplasms (FN) into a separate category. However, disadvantages of the BSRTC have also been reported. They comprise mainly intra- and interpersonal variability and strict criteria for diagnostic samples. The most problematic category in the BSRTC seems to be 'atypia of undetermined significance or follicular lesion

of undetermined significance' (AUS/FLUS). In our review, we aimed to analyse the available studies, to summarize the pros and cons of the AUS/FLUS category, and to make this category more transparent and useful.

AUS/FLUS – A New Category in the Bethesda 2010 System

The AUS/FLUS category has been newly introduced in the BSRTC [2]. This category was derived from the preceding category 'indeterminate for malignancy', which was separated into suspicious for FN/FN (SFN/FN), suspicious for malignancy and AUS/FLUS categories [33]. The AUS/FLUS category is widely discussed in the literature [9, 11, 23, 29, 34]. It is a heterogeneous category including mainly sparse and compromised samples with suspicious atypical cytological or architectural features, nevertheless not sufficient to be diagnosed as FN or suspicious for malignancy [2, 34].

AUS/FLUS Heterogeneity and Categories

AUS/FLUS interpretation is appropriate in situations with a predominant or exclusive occurrence of a prominent population of microfollicles or oncocytes in paucicellular aspirates with scant colloid, in the presence of clotting and air-drying artefacts, features suggestive of papillary carcinoma, atypical cyst-lining cells, focally enlarged nuclei, atypical lymphoid infiltrate, and not otherwise specified changes [2]. The below paragraphs include the most common options in this heterogeneous category. The most important criterion for AUS/FLUS is a technically compromised sample, which is either sparse in atypical features or bloody [2]. Overall, the atypical features are not completely sufficient for another diagnostic category.

Prominent Population of Microfollicles

The sample is composed of some microfollicles, trabecular and/or crowded groups. The features are suggestive of follicular neoplasia, but not sufficient to be diagnostic. Of note, microfollicles also appear in benign FNA [2, 34]. Interestingly, Renshaw [35] found that cellular samples with mixtures of microfollicles and macrofollicles and no significant atypia have similar risks of malignancy as scant aspirates with microfollicles only and cytological atypia only.

Predominance of Oncocytes and Oncocytes Only

The samples contain predominantly or exclusively oncocytes either grouped or isolated. The paucicellularity is against the diagnosis of oncocytic FN [2, 34]. Of interest, nodular goitre composed almost only of oncocytes may be placed into both the AUS/FLUS and oncocytic FN categories. In such cases, clinical and radiological correlation is preferable to repeated FNA [34]. Oncocyte-rich cases without cellular atypia may benefit from repeated FNA [11]. Notably, oncocyte-rich carcinoma is often difficult to aspirate and aspiration results in very paucicellular smears [35].

Clotting and Air-Drying Artefacts

In poorly fixed and/or stained samples, cells look larger and the chromatin structure is compromised. Excessive blood and clots may imitate microfollicular crowding [34].

Features Suggestive of Papillary Carcinoma

Papillary carcinoma can be grouped all together in 5 BSRTC categories depending on the sample cellularity. Cystic papillary carcinoma sampled only from the cystic part and not containing epithelium is put into the nondiagnostic category. Suboptimally aspired carcinoma is placed into the AUS/FLUS category. The representative sample is either suspicious for malignancy or malignant in the BSRTC [36]. Furthermore, follicular variants of papillary carcinoma are often diagnosed as FN [37]. AUS/FLUS lesions usually contain cells with nuclear enlargement, pallor and crowding. Typical diagnostic features, such as intranuclear pseudoinclusions, grooves and nuclear contours, are not present or are minimally seen in obscured samples. In a large study of 2,210 cases, it was the most common AUS/FLUS category (36%) [8]. Knowledge of mimics of papillary carcinoma [38, 39] can reduce the AUS/FLUS rate.

Atypical Cyst-Lining Cells

Enlarged cells with grooved nuclei and small distinct nucleoli are easily recognised as benign in the context of a cystic aspirate containing colloid and macrophages; however, in isolation, it is advisable to use the AUS/FLUS category as papillary carcinoma is hard to exclude [2, 34]. Surprisingly, no cases of atypical cyst-lining cells were reported in a large study comprising 2,210 cases [8].

Focally Enlarged Nuclei

Clinical data are needed to distinguish drug and radioactive iodine-caused atypia [40, 41]. Clinicopathological

Table 3. AUS/FLUS stratification and histological follow-up

Stratification	n	Histological follow-up	Benign	Neoplastic	Malignant
Architectural [11, 21, 38]	273	131 (48)	94 (34.4)	n.d.	37 (13.5)
Papillary carcinoma features [11, 21, 22, 24, 38]	374	233 (62.3)	124 (33.2)	n.d.	108 (28.9)
Oncocytes [11, 24, 38]	224	82 (36.6)	63 (28.1)	n.d.	19 (8.5)
Technically compromised samples [17, 21]	101	41 (40.6)	22 (21.8)	11 (10.9)	8 (7.9)

Values in parentheses are percentages. n.d. = Not determined.

meetings can be useful in such cases [15, 22, 23]. Furthermore, reparative changes due to cystic degeneration or haemorrhage can also cause nuclear enlargement [2].

Atypical Lymphoid Infiltrates

Diffuse large B cell lymphomas can be easily diagnosed as suspicious for malignancy, but low-grade lymphoproliferative disorders present as samples with atypical lymphocytes that are not yet sufficient as suspicious for malignancy. Flow cytometry and immunophenotyping is required in such samples [2, 34, 42].

Not Otherwise Specified

This group includes any other atypical scenarios. In cases where the origin of atypical cells is uncertain or unknown, the lesion is allocated to this group [2].

AUS/FLUS Subclassification or Diminishment?

An AUS/FLUS subclassification according to low and high malignancy risk was suggested since atypical follicular cells can be further stratified. Nevertheless, only some authors subclassified atypical cells in their analyses and, if done, the systems of subclassification varied considerably (table 3). Six studies [11, 17, 21, 22, 24, 36] were pooled and 487 cases were histologically verified out of a total of 972 (table 3). AUS/FLUS lesions with features suggestive of papillary carcinoma showed a higher risk of malignancy (28.9%) in comparison with architectural atypia (risk of malignancy 13.5%) followed by oncocytic AUS/FLUS (8.5%) and technically compromised samples (7.9%). Only two studies in this analysis separated neoplastic lesions in output data. In agreement with the above analyses, other studies have also shown that AUS/FLUS cases with papillary carcinoma features possess a higher risk for malignancy than lesions containing oncocytes and architectural atypia [22, 43, 44]. Olson et al. [24] divided the AUS/FLUS category into 3 main subcategories, with atypical nuclear features being the most common (46%) and having a higher risk of malignancy (48% compared to 32% of all AUS/FLUS cases). In another study, AUS/FLUS samples were histologically approved as papillary carcinoma in 73.3%, including 57.1% with a follicular variant of papillary carcinoma. On the contrary, follicular carcinoma was the final histological diagnosis in only 21.1% of AUS/FLUS cases with architectural atypia [45]. The presence of oncocytes increases both the cytological diagnosis of AUS/FLUS and FNs as well as a final histological diagnosis of malignancy [46]. On the other hand, Wu et al. [19] reported AUS/FLUS cases with a lower risk of malignancy (0 vs. 7%), but with a significantly higher risk of neoplasm (89 vs. 33%). Nayar and Ivanovic [17] subclassified indeterminate lesions into morphologic groups (83%) that yielded a malignancy risk of 5% and a risk of neoplasms of 44% in 383 out of 767 surgically confirmed cases. Interestingly, cystic lesions also revealed a 5% risk of malignancy (18 surgically confirmed cases out of 77). On the contrary, scant and poorly preserved samples had a 21% malignancy risk in 29 surgically confirmed cases out of 80. It is of interest that there were no cytological atypia due to technical artefacts in 354 liquid-based samples; the majority showed papillary carcinoma-suggestive features (36%) or microfollicular architecture (23.1%) [8]. On the other hand, AUS/FLUS due to low cellularity was determined in 79% of cases in another study [7]. Similarly, suboptimal preparation led to AUS/FLUS reclassification in 58.9% of cases [47].

Stratification of the AUS/FLUS category into 'inadequate' and 'suspicious' based on different positive predictive values has been suggested [11, 36]. In the paediatric population, Smith et al. [21] suggested the necessity of cytological subclassification of the AUS/FLUS category to improve the selection of patients for subsequent surgery.

The subjectivity, inter- and intrapersonal disagreement within the AUS/FLUS category led to suggestions to group the AUS/FLUS and SFN/FN categories together [36, 45]. Pooling these categories for similar risk of malignancy was also suggested by Marchevsky et al. [13].

On the other hand, eliminating the AUS/FLUS category increases the false-negative rates of thyroid FNA. In the Shi et al. [9] study, eliminating the AUS/FLUS category revealed that 53% of the neoplastic lesions and an alarming 37% of papillary carcinomas would have been underdiagnosed as benign.

When the BSRTC was compared with a 5-tiered system, sensitivity was equally high in both (98.3 vs. 99.2%); however, specificity (54.3 vs. 22.8%) and diagnostic accuracy (76.3 vs. 56.3%) was lower in the 6-tiered BSRTC. AUS/FLUS is unique to the BSRTC and those cases increased the rate of surgery (78.8 vs. 56.4%) [6]. Nevertheless, cases of AUS/FLUS in the 5-tiered system seem to be downgraded into the benign group rather than upgraded [6, 48].

AUS/FLUS Overuse Tendency

In the majority of BSRTC studies, the AUS/FLUS category is overused and the suggested 7% threshold is rarely fulfilled. Summaries of percentages in both retrospective and prospective studies are presented in table 2; the total 81,833 cases are pooled with the average AUS/FLUS being 10.9%. The lowest AUS/FLUS percentage was even less than 1% [5, 26]. On average, AUS/FLUS was slightly higher in retrospective studies compared to prospective studies (11.4 vs. 9.7%). The highest AUS/FLUS was in the study by Broome and Solorzano [27], and two other retrospective studies also reached more than 20% [19, 21]. Only one prospective study was slightly above 20% (20.5%) [7]. Overall, the suggested 7% and lower AUS/FLUS rate was used in only half of the prospective and one third of the retrospective studies. The AUS/FLUS category is clearly generally overused.

The AUS/FLUS to malignant ratio of 1:3 is recommended based on the literature. This ratio being higher than 3 is either due to an underdiagnosis of malignancy or overdiagnosis of AUS/FLUS. Ratios lower than 1 reduce the sensitivity. Alternatively, AUS/FLUS plus suspicious for malignancy plus FN/suspicious FN to malignancy ratio is a potential performance measure; however, it has the disadvantage of being a 4-parameter calculation with 4 variables [30]. Similarly, AUS/FLUS rates <5% lower the sensitivity of the test and conversely rates >15% include poorly preserved cases otherwise diagnosed as benign or non-diagnostic [35]. Conversely, the rates for benign and malignant categories are relatively tight [49].

In an analysis of 7 pathologists, AUS/FLUS rates and AUS/FLUS plus benign rates were constant for individual pathologists (average 73.4%, rate 69.3–77.2%). Based on that fact, overuse of AUS/FLUS is due to the overstating of benign cases. Experience might shape judgment skills to make more definite diagnoses, but on the other hand the skill to recognize subtle features is needed so as not to reduce the sensitivity of the BSRTC [15].

Some prospective studies reached a 20.5% AUS/FLUS rate [7]. On the contrary, Bohacek et al. [5] reported only 0.8% of a total of 1,000 samples as being diagnosed as AUS/FLUS. However, another study with an AUS/FLUS rate of 0.9% contained 87.5% as benign samples [26]. AUS/FLUS in repeated FNA as reported in several studies is summarized in table 4. The percentage varies from 0% in the study by Jo et al. [10] to 67% in the study by Ohori et al. [7], with the average being 22%. Repeated AUS/FLUS indicates the surgical treatment [1].

A novel approach to avoid overusing the AUS/FLUS category and instead splitting the benign category has been suggested. The first group would be 'benign', as it was originally in the BSRTC, and the second group would be 'favour benign with mandatory clinical follow-up'. This mild change in classification would reduce AUS/FLUS to 2–8%, leaving only papillary carcinoma-suggestive cases and atypical microfollicles [50].

Table 4. Repeated FNA and persistent AUS/FLUS

First author [Ref.]	AUS/FLUS rate, %	Persistent AUS/FLUS in repeated FNA, %
Nayar [17]	18	27
Theoharis [3]	3	6
Ohori [7]	20.5	67
Faquin [18]	9/12	23
Jo [10]	3.4	0
VanderLaan [14]	10.9	27.9
Ratour [8]	11	19.8
Dincer [23]	4.8	0
Olson [24]	9.8	44
Mondal [26]	0.9	1
Average and range	9.39 (0.9–20.5)	21.57 (0–67)

Table 5. Cytohistological correlation of AUS/FLUS cases

First author [Ref.]	AUS/ FLUS, n	AUS/ FLUS, %	Histology, n	Histology, %	Benign	Neoplastic	Malignant	Risk of malignancy, %
Rabaglia [20]	91	11.9	32	35	28 (88)	n.d.	4 (13)	13
Smith [21]	43	n.d.	25	58	13 (52)	5 (20)	7 (28)	28
Faquin [18]	509	9/12	273	54	156 (57)	65 (24)	52 (19)	27, repeated FNA 15
Wu [19]	376	27.2	102	27.1	58 (57)	22 (21.5)	22 (21.5)	21.5
VanderLaan [14]	512	10.9	331	64.6	240 (72.5)	n.d.	91 (27.5)	27.5
Layfield [12]	673	12.1	127	18.9	87 (68.5)	8 (6.3)	36 (28.3)	28.3
Bohacek [5]	8	0.8	8	100	5 (62.5)	2 (25)	1 (12.5)	12.5
Ohori [7]	513	20.5	117	22.8	79 (70.1)	18 (12.8)	20 (17.1)	17.1
Bongiovanni [6]	248	6.7	132	53.2	113 (85.6)	n.d.	19 (14.4)	14.4
Jo [10]	101	3.4	53	52.5	29 (57)	15 (28)	9 (17)	17
Nayar [17]	924	18	430	46.5	224 (52)	181 (42)	25 (6)	6
Dincer [23]	368	4.8	88	23.9	35 (39.8)	30 (34.1)	23 (26.1)	26.1
Olson [24]	388	9.8	133	60	90 (68)	n.d.	43 (32)	32
Theoharis [3]	95	3.0	27	30.3	7 (26)	7 (26)	13 (48)	48
Tepeoglu [28]	100	9.8	79	79	64 (81)	5 (6)	10 (12.7)	12.7
Al-Abbadi [25]	15	7	9	60	0	5 (56)	4 (44.4)	44.4
Summary	4,964	10.4	1,966	39.6	1,228	363	379	21.5

Values in parentheses are percentages. n.d. = Not determined.

Inter- and Intrapersonal Variability of AUS/FLUS Evaluation

One of the shortcomings of the BSRTC seems to be the considerable intra- and interpersonal variability in respect to subjectivity of cytological evaluation. Four experts analysed 107 cases, with consensus only reached in 72 cases (67.3%). The most problematic was the AUS/FLUS category, where only 2 out of 9 cases were diagnosed in agreement among the panellists. The rate of agreement was 80% for the malignant category and 73% for the benign category [51]. Similarly, in another study, interobserver agreement was only 50% in the AUS/FLUS category, but 93.8% in the benign category and 100% in the malignant category [52]. A third study also revealed the highest intra-observer variability in the AUS/FLUS and SFN/FN categories [45]. On the contrary, the highest interobserver variability was between AUS/FLUS and 'suspicious for malignancy' [45]. Unanimous interobserver agreement was found in 63% and intra-observer agreement in 62% in the study where only 2 reviewers reclassified the cases [9]. Wu et al. [19] reported the variability among 4 pathologists to be 11, 21, 34 and 47%, respectively, with no influence of years of experience, but the pathologists with certification in cytopathology had lower AUS/FLUS rates. Another study concluded that lower AUS/FLUS rates were noticed in pathologists with cytopathology board examinations, but no correlation was found with the years of experience and the volume of workload [15]. In a review of 7 cytopathologists, AUS/FLUS varied between 3.4 and 22.4%, and annual intrapersonal rates were calculated at an average of 9.9–12.4% [15]. Interpersonal AUS/FLUS rates of 14–27% were reported by Ohori et al. [7]. Multicentre analysis showed interinstitutional variability (range 3.3–14.9%) as well as interindividual variability (range 2.5–28.6%) in AUS/FLUS ranges [12]. In summary, the BTRTC has a higher inter- and intrapersonal as well as interinstitutional variability than the 5-tier systems.

Risk of Neoplasia/Malignancy and Prognostic Role of the BSRTC

The risk of malignancy is variable and depends on the predominant morphological feature of the AUS/FLUS category [11, 43]. In various studies, it varies from 6 to 96.7% (table 2) in resected histologically proven cases. Interestingly, in retrospective studies, the risk of malignancy for AUS/FLUS was on average 32.7%, with a range of 6–48%. In prospective studies, the average was similar (35.3%), but the range higher (12.5–96.7%). To summarise, the risk of malignancy in the majority of the studies is higher than the BSRTC anticipated for the AUS/FLUS category. Alarmingly, it is more than doubled on average. The reason for this is mainly due to AUS/FLUS

overuse. In addition, heterogeneity of the AUS/FLUS category causes the variability. Table 5 analyses cytohistological correlations of 1,966 AUS/FLUS cases with a risk of malignancy of 21.5%. Importantly, the risk of neoplasia was 25.1%; however, this data was available only in 12 studies.

According to Renshaw [11], at least four different risk groups can be separated within the AUS/FLUS category. Out of them, atypia suggestive of papillary carcinoma has the highest risk of malignancy. In his study, 38% of histologically verified cases were proven to be malignant. Conversely, oncocytes containing samples were proven to be malignant only in 7% of histologically verified cases.

Surprisingly, malignancy rates among patients who went directly to surgery after a single AUS diagnosis (41%), patients having 2 successive AUS FNA diagnoses (43%) and patients with a benign aspirate after AUS (29%) were not significantly different. Based on those data, guidelines recommending repeated FNA for most cases should be re-evaluated [14]. However, the majority of studies presented opposite views. Interestingly, repeated FNA has decreased the risk of malignancy in resected cases from 27 to 15% and, similarly, the rate of benign final histological diagnosis declined from 85 to 73% [18]. In another study, malignancy rates decreased rapidly using the BSRTC, from 35 to 13%, with the same thyroidectomy rates [20]. A high AUS/FLUS rate is inversely correlated with low malignancy findings in surgically confirmed cases [10, 15, 17].

For further studies, risk of neoplasia evaluation is an important parameter, which is available only in some studies [3, 5–7, 10, 12, 14, 17–21, 23–25, 28] (table 5). Sixteen studies of 4,964 cases with 39.6% histologically approved showed a risk of neoplasia of 25.1% and a risk of malignancy of 21.5%. Up to 81% of lesions were benign on histological follow-up. It might be useful to also monitor benign neoplasms as follicular adenoma as they also require surgical excision and histological verification [23].

Age-related risk of malignancy of BSRTC categories has been recently described in a paediatric population study. Smith et al. [21] analysed the BSRTC in children and, based on cytohistological correlation, they found a higher risk of malignancy for AUS/FLUS and other undetermined categories than in adult population studies.

VanderLaan et al. [53] presumed that the aggressiveness of papillary carcinoma is increased within various BSRTC categories (III, IV, low-risk; V, VI, high-risk). However, the majority of follicular variants of papillary carcinoma are diagnosed as AUS/FLUS and SFN/FN. Follicular variants of papillary carcinoma has a favourable prognosis and the predictive role of the BSRTC was not approved [54]. Pooling the AUS/FLUS and SFN/FN categories for similar risk of malignancy was suggested by Marchevsky et al. [13].

Only limited studies were included in the stratification of AUS/FLUS morphology-related risk of neoplasm and malignancy (table 3). According to the presented analyses of the data, features suggestive for papillary carcinoma have a higher risk of malignancy than other morphological subcategories [11, 21, 22, 24, 36], as suggested also by others [43, 44].

The Potential of Diagnostic and Prognostic Improvement in AUS/FLUS Cases

The BSRTC is based on cytomorphology. This conventional diagnostic method is not able to provide an unequivocal diagnosis in all cases. In this respect, the further diagnostic strengthening of the AUS/FLUS and other indeterminate categories and clinical management seems to be exhausted. A promising means to a better reproducibility and diagnostic accuracy of the BSRTC classification consists of the implementation of additional methods and ancillary technologies focused on proteomics and molecular analysis of cells into routine FNA practice. Such procedures have not been taken into consideration in the BSRTC guidelines.

Immunohistochemistry/Immunocytochemistry

This method could be performed on cytological cell blocks or on cytological smears as well as on liquid-based specimens [55, 56]. Thyroid transcription factor-1, calcitonin and parathormone serve as markers of the histogenetic determination of cells (e.g. differentiation between thyrocytes, parafollicular and parathyroid cells) [57, 58]. DPPIV (CD26) and galectin-3 are markers of malignancy [59, 60]. The contribution of cytokeratin 19 and the HBME1 (Hector Battifora mesothelial cell 1) immunocytochemistry test to AUS/FLUS management and separating of malignant cases has also been documented [8, 56].

Molecular Analyses

Common genetic alterations detected in differentiated thyroid carcinomas are charged to the account of BRAF and RAS mutations and RET/PTC and PAX8/PPARγ rearrangement, respectively [61]. More recently, molecular detection of point mutations and rearrangements has

been successfully performed in routinely obtained fresh material as well as air-dried FNA thyroid samples, and the usefulness of these molecular analyses has repeatedly been confirmed in cytology specimens [7, 62, 63]. Numerous authors have approved the usefulness of BRAF mutation analysis in AUS/FLUS cases, particularly in the detection of papillary carcinomas with non-classic histology and prediction of the clinicopathological outcome and management of the patients [63–66]. BRAF mutation status was performed in 141 AUS/FLUS cases, with 45 mutation-positive cases; in the histologically confirmed cases, 21 were papillary carcinomas and 1 was nodular hyperplasia. On the contrary, in 11 histologically confirmed BRAF mutation-negative cases, 3 were malignant (2 papillary carcinomas, 1 follicular carcinoma) [4]. Genetic mutational markers have a very high specificity; however, their sensitivity is low because about 30–40% of differentiated thyroid cancers have no known molecular mutation detected [55, 67]. Therefore, the only way to increase the sensitivity of genomic analysis seems to be to use panels of different mutations and chromosomal rearrangements, possibly in combination with other molecular markers, like microRNA and methylation analysis, etc. [67, 68]. Panels of BRAF and RAS mutations and RET/PTC and PAX8/PPARγ chromosomal rearrangements have been successfully tested in morphologically indeterminate cytology samples. The sensitivity of these molecular tests reached 80% compared with 44% in cytomorphological examination alone [62]. Similarly, the same research group reported the results of the panel ThyroSeq for detection of 284 mutations in 12 tumour genes.

The most frequent mutations, along with BRAF and RAS, were PIK3CA, TP53, TSHR, PTEN, GNAS, CTNNB1 and RET [69]. Recently, the Afirma gene expression classifier (AGEC) was introduced. The method is based on the measurement of mRNA expression and validated to search out histologically benign nodules among those with indeterminate cytology. This test is indicated only for FNA samples with indeterminate diagnosis. AGEC evaluates the expression of 142 genes and helps to reclassify thyroid nodules with indeterminate cytology preoperatively as either benign or suspicious in order to avoid unnecessary surgery in benign cases [55, 70, 71, 72]. A multicenter clinical study recently verified the justification of the application of AGEC in routine cytological practice [73]. Although the use of genomic panels is recommended by some experts, the professional and scientific societies have adopted a conservative approach so far [55, 74, 75].

Recently, an aberrant microRNA profile has been described in thyroid cancers as compared to normal thyroid tissue [76]. miR-222, miR-221 and miR-146b have in particular been upregulated in papillary thyroid carcinomas. Significant differences in miR-221, miR-222 and miR181b levels have been documented between FNA samples of papillary carcinomas and benign thyroid nodules [76]. Several associations of distinct miRNA levels with clinicopathological features were described. Little is known about the miRNA profile in thyroid follicular tumours with overlap between malignant, benign and non-neoplastic nodules [77, 78]. Better understanding of microRNA expression in benign and non-neoplastic thyroid nodules seems to be a basic prerequisite for further application of microRNA analysis in indeterminate FNA.

Core-Needle Biopsy

Core-needle biopsy was shown to improve AUS/FLUS non-diagnostic and suspicious categories in comparison with repeated FNA [79, 80]; however, its use is controversial due to the risk of haemorrhage. Core-needle biopsies enable the straightforward use of immunohistochemistry.

Imaging Methods

Correlation of cytopathology with various imaging methods was suggested as a possible diagnostic improvement [15, 22, 23]. Preoperative positron emission tomography-computed tomography or ultrasonography of cytologically indeterminate thyroid nodules could provide reliable information and help in thyroidectomy decision making [81–84]. Other promising methods such as tissue high-resolution proton magnetic resonance and ^{99m}Tc-methoxyisobutylisonitrile thyroid scintigraphy in thyroid FNA were recently introduced [85, 86].

Validity of the AUS/FLUS Category and the Future

The above-discussed difficulties and limitations of the AUS/FLUS category enhance the importance of the continuous training in cytopathology with the utilisation of representative educational source materials, the paramount importance of cytohistological correlations, and strengthening of technical skills in FNA sampling, handling and the processing of samples [15, 28, 49, 87]. The importance of clinical, radiological and cytopathological correlation in the management of patients has been suggested as a possible future direction by many [15, 22, 23, 49], although this is already done in many centres. Fur-

thermore, group consensus reviews can help reduce the AUS/FLUS rates [87]. VanderLaan et al. [15] stressed that familiarity with the BSRTC can reduce AUS/FLUS rates.

Conclusions

The AUS/FLUS category seems to be currently reasonable with clearly defined cytomorphological criteria, which do not correspond unequivocally with those of the other categories. The AUS/FLUS category is definitely not merely a 'waste basket' for cases that we are not able to further diagnose cytologically. Efforts should be made to reduce the number of cases classified as AUS/FLUS in the future. The AUS/FLUS category has been overused so far and the risk of malignancy is higher than anticipated. Promising directions consist both in lowering the amount of poorly representative samples with artefacts, and in the implementation of new sophisticated methods facilitating and specifying the cytological diagnosis in not entirely representative samples.

Acknowledgements

This study was supported by the Charles University Research Fund (programme PRVOUK, project No. P36). I.K. was supported by the Emil Aaltonen Foundation and EVO grants of Pirkanmaa Hospital District.

References

1 Cibas ES, Ali SZ: The Bethesda System for Reporting Thyroid Cytopathology. Am J Clin Pathol 2009;132:658–665.

2 Ali ZS, Cibas ES: The Bethesda System for Reporting Thyroid Cytopathology. New York, Springer, 2010.

3 Theoharis CG, Schofield KM, Hammers L, Udelsman R, Chhieng DC: The Bethesda thyroid fine-needle aspiration classification system: year 1 at an academic institution. Thyroid 2009;19:1215–1223.

4 Kim SK, Hwang TS, Yoo YB, Han HS, Kim DL, Song KH, Lim SD, Kim WS, Paik NS: Surgical results of thyroid nodules according to a management guideline based on the BRAF V600E mutation status. J Clin Endocrinol Metab 2011;96:658–664.

5 Bohacek L, Milas M, Mitchell J, Siperstein A, Berber E: Diagnostic accuracy of surgeon-performed ultrasound-guided fine-needle aspiration of thyroid nodules. Ann Surg Oncol 2012;19:45–51.

6 Bongiovanni M, Crippa S, Baloch Z, Piana S, Spitale A, Pagni F, Mazzucchelli L, Di Bella C, Faquin W: Comparison of 5-tiered and 6-tiered diagnostic systems for the reporting of thyroid cytopathology: a multi-institutional study. Cancer Cytopathol 2012;120:117–125.

7 Ohori NP, Nikiforova MN, Schoedel KE, LeBeau SO, Hodak SP, Seethala RR, Carty SE, Ogilvie JB, Yip L, Nikiforov YE: Contribution of molecular testing to thyroid fine-needle aspiration cytology of 'follicular lesion of undetermined significance/atypia of undetermined significance'. Cancer Cytopathol 2010;118:17–23.

8 Ratour J, Polivka M, Dahan H, Hamzi L, Kania R, Dumuis ML, Cohen R, Laloi-Michelin M, Cochand-Priollet B: Diagnosis of follicular lesions of undetermined significance in fine-needle aspirations of thyroid nodules. J Thyr Res 2013;2013:250347.

9 Shi Y, Ding X, Klein M, Sugrue C, Matano S, Edelman M, Wasserman P: Thyroid fine-needle aspiration with atypia of undetermined significance: a necessary or optional category? Cancer Cytopathol 2009;117:298–304.

10 Jo VY, Stelow EB, Dustin SM, Hanley KZ: Malignancy risk for fine-needle aspiration of thyroid lesions according to the Bethesda System for Reporting Thyroid Cytopathology. Am J Clin Pathol 2010;134:450–456.

11 Renshaw AA: Should 'atypical follicular cells' in thyroid fine-needle aspirates be subclassified? Cancer Cytopathol 2010;118:186–189.

12 Layfield LJ, Morton MJ, Cramer HM, Hirschowitz S: Implications of the proposed thyroid fine-needle aspiration category of 'follicular lesion of undetermined significance': a five-year multi-institutional analysis. Diagn Cytopathol 2009;37:710–714.

13 Marchevsky AM, Walts AE, Bose S, Gupta R, Fan X, Frishberg D, Scharre K, Zhai J: Evidence-based evaluation of the risks of malignancy predicted by thyroid fine-needle aspiration biopsies. Diagn Cytopathol 2010;38:252–259.

14 VanderLaan PA, Marqusee E, Krane JF: Clinical outcome for atypia of undetermined significance in thyroid fine-needle aspirations: should repeated FNA be the preferred initial approach? Am J Clin Pathol 2011;135:770–775.

15 VanderLaan PA, Krane JF, Cibas ES: The frequency of 'atypia of undetermined significance' interpretations for thyroid fine-needle aspirations is negatively correlated with histologically proven malignant outcomes. Acta Cytol 2011;55:512–517.

16 Somma J, Schlecht NF, Fink D, Khader SN, Smith RV, Cajigas A: Thyroid fine needle aspiration cytology: follicular lesions and the gray zone. Acta Cytol 2010;54:123–131.

17 Nayar R, Ivanovic M: The indeterminate thyroid fine-needle aspiration: experience from an academic center using terminology similar to that proposed in the 2007 National Cancer Institute Thyroid Fine Needle Aspiration State of the Science Conference. Cancer Cytopathol 2009;117:195–202.

18 Faquin WC, Baloch ZW: Fine-needle aspiration of follicular patterned lesions of the thyroid: diagnosis, management, and follow-up according to national cancer institute (NCI) recommendations. Diagn Cytopathol 2010;38:731–739.

19 Wu HH, Rose C, Elsheik TM: The Bethesda System for Reporting Thyroid Cytopathology: an experience of 1,382 cases in a community practice setting with the implication for risk of neoplasm and risk of malignancy. Diagn Cytopathol 2012;40:399–403.

20 Rabaglia JL, Kabbani W, Wallace L, Holt S, Watumull L, Pruitt J, Snyder WH, Nwariaku FE: Effect of the Bethesda System for Reporting Thyroid Cytopathology on thyroidectomy rates and malignancy risk in cytologically indeterminate lesions. Surgery 2010;148:1267–1273.

21 Smith M, Pantanowitz L, Khalbuss WE, Benkovich VA, Monaco SE: Indeterminate pediatric thyroid fine needle aspirations: a study of 68 cases. Acta Cytol 2013;57:341- 348.

22 Luu MH, Fischer AH, Stockl TJ, Pisharodi L, Owens CL: Atypical follicular cells with equivocal features of papillary carcinoma is not a low-risk cytologic diagnosis. Acta Cytol 2011;55:526–530.

23 Dincer N, Balci S, Yazgan A, Guney G, Ersoy R, Cakir B, Guler G: Follow-up of atypia and follicular lesions of undetermined significance in thyroid fine needle aspiration cytology. Cytopathology 2013;24:385–390.
24 Olson MT, Clark DP, Erozan YS, Ali SZ: Spectrum of risk of malignancy in subcategories of atypia of undetermined significance. Acta Cytol 2011;55:518–525.
25 Al-Abbadi MA, Shareef SQ, Ali JA, Yousef MM: Application of the Bethesda System for Reporting Thyroid Cytopathology in the Eastern Province of Saudi Arabia: phase I pilot retrospective analysis. Acta Cytol 2013;57:481–488.
26 Mondal SK, Sinha S, Basak B, Roy DN, Sinha SK: The Bethesda system for reporting thyroid fine needle aspirates: a cytologic study with histologic follow-up. J Cytol 2013;30:94–99.
27 Broome JT, Solorzano CC: The impact of atypia/follicular lesion of undetermined significance on the rate of malignancy in thyroid fine-needle aspiration: evaluation of the Bethesda System for Reporting Thyroid Cytopathology. Surgery 2011;150:1234–1241.
28 Tepeoglu M, Bilezikci B, Bayraktar SG: A histological assessment of the Bethesda System for Reporting Thyroid Cytopathology (2010) abnormal categories: a series of 219 consecutive cases. Cytopathology 2014;25:39–44.
29 Bongiovanni M, Spitale A, Faquin WC, Mazzucchelli L, Baloch ZW: The Bethesda System for Reporting Thyroid Cytopathology: a meta-analysis. Acta Cytol 2012;56:333–339.
30 Krane JF, VanderLaan PA, Faquin CF, Renshaw AA: The atypia of undetermined significance/follicular lesion of undetermined significance: malignant ratio. Cancer Cytopathol 2012;120:111–116.
31 Olson MT, Boonyaarunnate T, Atlinboga AA, Ali SZ: 'Suspicious for papillary thyroid carcinoma' before and after The Bethesda System for Reporting Thyroid Cytopathology: impact of standardized terminology. Acta Cytol 2014;58:15–22.
32 Boonyaarunnate T, Olson MT, Ali SZ: 'Suspicious for a follicular neoplasm' before and after the Bethesda System for Reporting Thyroid Cytopathology: impact of standardized terminology. Acta Cytol 2013;57:455–463.
33 Baloch ZW, LiVolsi VA, Asa SL, Rosai J, Merino MJ, Randolph G, Vielh P, DeMay RM, Sidawy MK, Frable WJ: Diagnostic terminology and morphologic criteria for cytologic diagnosis of thyroid lesions: a synopsis of the National Cancer Institute Thyroid Fine-Needle Aspiration State of Science Conference. Diagn Cytopathol 2008;36:425–437.
34 Bongiovanni M, Krane JF, Cibas ES, Faquin WC: The atypical thyroid fine-needle aspiration: past, present, and future. Cancer Cytopathol 2012;120:73–86.
35 Renshaw AA: Subclassification of atypical cells of undetermined significance in direct smears of fine-needle aspirations of the thyroid: distinct patterns and associated risk of malignancy. Cancer Cytopathol 2011;119:322–327.
36 Singh RS, Wang HH: Eliminating the 'atypia of undetermined significance/follicular lesion of undetermined significance' category from the Bethesda System for Reporting Thyroid Cytopathology. Am J Clin Pathol 2011;136:896–902.
37 Kurian EM, Dawlett M, Wang J, Gong Y, Guo M: The triage efficacy of fine needle aspiration biopsy for follicular variant of papillary thyroid carcinoma using the Bethesda reporting guidelines. Diagn Cytopathol 2012;40:E69–E73.
38 Pusztaszeri MP, Sauder KJ, Cibas ES, Faquin WC: Fine-needle aspiration of primary Langerhans cell histiocytosis of the thyroid gland, a potential mimic of papillary thyroid carcinoma. Acta Cytol 2013;57:406–412.
39 Taweevisit M, Sampatanukul P, Thorner PS: Ectopic thymoma can mimic benign and malignant thyroid lesions on fine needle aspiration cytology: a case report and literature review. Acta Cytol 2013;57:213–220.
40 Granter SR, Cibas ES: Cytologic findings in thyroid nodules after 131I treatment of hyperthyroidism. Am J Clin Pathol 1997;107:20–25.
41 Smejkal V, Smejkalova E, Rosa M, Zeman V, Smetana K: Cytologic changes simulating malignancy in thyrotoxic goiters treated with carbimazole. Acta Cytol 1985;29:173–178.
42 Boonyaarunnate T, Olson MT, Ali SZ: Impact of flow cytometry in thyroid cytology. Acta Cytol 2013;57:562–566.
43 VanderLaan PA, Marqusee E, Krane JF: Usefulness of diagnostic qualifiers for thyroid fine-needle aspirations with atypia of undetermined significance. Am J Clin Pathol 2011;136:572–577.
44 Onder S, Firat P, Ates D: The Bethesda System for Reporting Thyroid Cytopathology: an institutional experience of the outcome of indeterminate categories. Cytopathology 2014;25:177–184.
45 Walts AE, Bose S, Fan X, Frishberg D, Scharre K, de Peralta-Venturina M, Zhai J, Marchevsky AM: A simplified Bethesda System for Reporting Thyroid Cytopathology using only four categories improves intra- and inter-observer diagnostic agreement and provides non-overlapping estimates of malignancy risks. Diagn Cytopathol 2012;40:E62–E68.
46 Yazgan A, Balci S, Dincer N, Kiyak G, Tuzun D, Ersoy R, Cakir B, Guler G: Hürthle cell presence alters the distribution and outcome of categories in the Bethesda System for Reporting Thyroid Cytopathology. Cytopathology 2014;25:185–189.
47 Song JY, Chu YC, Kim L, Park IS, Han JY, Kim JM: Reclassifying formerly indeterminate thyroid FNAs using the Bethesda system reduces the number of inconclusive cases. Acta Cytol 2012;56:122–129.
48 Mihai R, Parker AJ, Roskell D, Sadler GP: One in four patients with follicular thyroid cytology (THY3) has a thyroid carcinoma. Thyroid 2009;19:33–37.
49 Ohori P, Schoedel KE: Variability of atypia of undetermined significance/follicular lesion of undetermined significance diagnosis in the Bethesda System for Reporting Thyroid Cytopathology: sources and recommendations. Acta Cytol 2011;55:492–498.
50 Renshaw AA, Krane JF: Can changing the terminology for benign aspirates reduce the atypia of undetermined significance/follicular lesion of undetermined significance rate in thyroid fine-needle aspirates? Cancer Cytopathol 2013;121:175–178.
51 Cochand-Priollet B, Schmitt FC, Tötsch M, Vielh P: The Bethesda terminology for reporting thyroid cytopathology: from theory to practice in Europe. Acta Cytol 2011;55:507–511.
52 Bhasin TS, Mannan R, Manjari M, Mehra M, Sekhon AKG, Chandey M, Sharma S, Kaur P: Reproducibility of 'The Bethesda System for reporting Thyroid Cytopathology': a multicenter study with review of the literature. J Clin Diagn Res 2013;7:1051–1054.
53 VanderLaan PA, Marqusee E, Krane JF: Features associated with locoregional spread of papillary carcinoma correlate with diagnostic category in the Bethesda System for Reporting Thyroid Cytopathology. Cancer Cytopathol 2012;120:245–253.
54 Kleiman DA, Beninato T, Soni A, Shou Y, Zarnegar R, Fahey TJ: Does Bethesda category predict aggressive features in malignant thyroid nodules? Ann Surg Oncol 2013;20:3484–3490.
55 Xing M, Haugen BR, Schlumberger M: Progress in molecular-based management of differentiated thyroid cancer. Lancet 2013;381:1058–1069.
56 Cochand-Priollet B, Dahan H, Laloi-Michelin M, Polivka M, Saada M, Herman P, Guillausseau PJ, Hamzi L, Poté N, Sarfati E, Wassef M, Combe H, Raulic-Raimond D, Chedin P, Medeau V, Casanova D, Kania R: Immunocytochemistry with cytokeratin 19 and anti-human mesothelial cell antibody (HBME1) increases the diagnostic accuracy of thyroid fine-needle aspirations: preliminary report of 150 liquid-based fine-needle aspirations with histological control. Thyroid 2011;21:1067–1073.
57 Absher KJ, Truong LD, Khurana KK, Ramzy I: Parathyroid cytology: avoiding diagnostic pitfalls. Head Neck 2002;24:157–164.
58 Bhanot P, Yang J, Schnadig VJ, Logroño R: Role of FNA cytology and immunochemistry in the diagnosis and management of medullary thyroid carcinoma: report of six cases and review of the literature. Diagn Cytopathol 2007;35:285–292.

59 Bartolazzi A, Orlandi F, Saggiorato E, Volante M, Arecco F, Rossetto R, Palestini N, Ghigo E, Papotti M, Bussolati G, Martegani MP, Pantellini F, Carpi A, Giovagnoli MR, Monti S, Toscano V, Sciacchitano S, Pennelli GM, Mian C, Pelizzo MR, Rugge M, Troncone G, Palombini L, Chiappetta G, Botti G, Vecchione A, Bellocco R: Galectin-3-expression analysis in the surgical selection of follicular thyroid nodules with indeterminate fine-needle aspiration cytology: a prospective multicentre study. Lancet Oncol 2008;9:543–549.

60 Kholova I, Ryska A, Ludvikova M, Cap J, Pecen L: Dipeptidyl peptidase IV expression in thyroid cytology: retrospective histologically confirmed study. Cytopathology 2003;14:27–31.

61 Bhaijee F, Nikiforov YE: Molecular analysis of thyroid tumors. Endocr Pathol 2011;22:126–133.

62 Nikiforov YE, Steward DL, Robinson-Smith TM, Haugen BR, Klopper JP, Zhu Z, Fagin JA, Falciglia M, Weber K, Nikiforova MN: Molecular testing for mutations in improving the fine-needle aspiration diagnosis of thyroid nodules. J Clin Endocrinol Metab 2009;94:2092–2098.

63 Adeniran AJ, Hui P, Chhieng DC, Prasad ML, Schofield K, Theoharis C: BRAF mutation testing of thyroid fine-needle aspiration specimens enhances the predictability of malignancy in thyroid follicular lesions of undetermined significance. Acta Cytol 2011;55:570–575.

64 Colanta A, Lin O, Tafe L, Ghossein R, Nafa K, Mitchell T, Ladanyi M, Arcila M: BRAF mutation analysis of fine-needle aspiration biopsies of papillary thyroid carcinoma: impact on diagnosis and prognosis. Acta Cytol 2011;55:563–569.

65 Di Benedetto G, Fabozzi A, Rinaldo C, Rinaldi CR: BRAF test and cytological diagnosis with a single fine needle cytology sample. Acta Cytol 2013;57:337–340.

66 Chung SY, Lee JS, Lee H, Patk SH, Kim SJ, Ryu HS: Cytomorphological factors and BRAF mutation predicting risk of lymph node metastasis in preoperative liquid-based fine needle aspirations of papillary thyroid carcinoma. Acta Cytol 2013;57:252–258.

67 Ferraz C, Eszlinger M, Paschke R: Current state and future perspective of molecular diagnosis of fine-needle aspiration biopsy of thyroid nodules. J Clin Endocrinol Metab 2011;96:2016–2026.

68 Zhang B, Liu S, Zhang Z, Wei J, Qu Y, Wu K, Yang Q, Hou P, Shi B: Analysis of BRAFV600E mutation and DNA methylation improves the diagnostics of thyroid fine needle aspiration biopsies. Diagn Pathol 2014;9:45.

69 Nikiforova MN, Wald AI, Roy S, Durso MB, Nikiforov YE: Targeted next-generation sequencing panel (ThyroSeq) for detection of mutations in thyroid cancer. J Clin Endocrinol Metab 2013;98:E1852–E1860.

70 Li H, Robinson KA, Anton B, Saldanha IJ, Ladenson PW: Cost-effectiveness of a novel molecular test for cytologically indeterminate thyroid nodules. J Clin Endocrinol Metab 2011;96:E1719–E1726.

71 Alexander EK, Kennedy GC, Baloch ZW, Cibas ES, Chudova D, Diggans J, Friedman L, Kloos RT, LiVolsi VA, Mandel SJ, Raab SS, Rosai J, Steward DL, Walsh PS, Wilde JI, Zeiger MA, Lanman RB, Haugen BR: Preoperative diagnosis of benign thyroid nodules with indeterminate cytology. N Engl J Med 2012;367:705–715.

72 Ward LS, Kloos RT: Molecular markers in the diagnosis of thyroid nodules. Arq Bras Endocrinol Metab 2013;57:89–97.

73 Alexander EK, Schorr M, Klopper J, Kim C, Sipos J, Nabhan F, Parker C, Steward DL, Mandel SJ, Haugen BR: Multicenter clinical experience with the Afirma gene expression classifier. J Clin Endocrinol Metab 2014;99:119–125.

74 Ali SZ, Fish SA, Lanman R, Randolph GW, Sosa JA: Use of the Afirma® gene expression classifier for preoperative identification of benign thyroid nodules with indeterminate fine-needle aspiration cytopathology. PLoS Curr 2013;11:5.

75 Hodak SP, Rosenthal DS, American Thyroid Association Clinical Affairs Committee: Information for clinicians: commercially available molecular diagnosis testing in the evaluation of thyroid nodule fine-needle aspiration specimens. Thyroid 2013;23:131–134.

76 Nikoforova MN, Tseng GC, Steward D, Diorio D, Nikoforov YE: MicroRNA expression profiling of thyroid tumors: biological significance and diagnostic utility. J Clin Endocrinol Metab 2008;93:1600–1608.

77 Stokowy T, Wojtas B, Fujarewicz K, Jarzab B, Eszlinger M, Paschke R: MicroRNAs with the potential to distinguish follicular thyroid carcinomas from benign follicular thyroid tumors: results of a meta-analysis. Horm Metab Res 2014;46:171–180.

78 Shen R, Liyanarachchi S, Li W, Wakely PE Jr, Saji M, Huang J, Nagy R, Farell T, Ringel MD, de la Chapelle A, Kloos RT, He H: MicroRNA signature in thyroid fine-needle aspiration cytology applied to 'atypia of undetermined significance' cases. Thyroid 2012;22:9–16.

79 Na DG, Kim J, Sung JY, Baek JH, Jung KC, Lee H, Yoo H: Core-needle biopsy is more useful than repeat fine-needle aspiration in thyroid nodules read as nondiagnostic or atypia of undetermined significance by the Bethesda System for Reporting Thyroid Cytopathology. Thyroid 2012;22:468–474.

80 Hakala T, Kholová I, Sand J, Saaristo R, Kellokumpu-Kehtinen P: A core-needle biopsy provides more malignancy-specific results than fine-needle aspiration biopsy in thyroid nodules suspicious for malignancy. J Clin Pathol 2013;66:1046–1050.

81 Muñoz Pérez N, Villar del Moral JM, Muros Fuentes MA, López de la Torre M, Arcelus Martínez JI, Becerra Massare P, Esteva Martínez D, Cañadas Garre M, Coll Del Rey E, Bueno Laraño P, Ferrón Orihuela JA: Could 18F-FDG-PET/CT avoid unnecessary thyreoidectomies in patients with cytological diagnosis of follicular neoplasm? Langenbecks Arch Surg 2013;398:709–716.

82 Kim BS, Ryu HS, Kang KH: The value of preoperative PET-CT in papillary thyroid cancer. J Int Med Res 2013;4:445–456.

83 Lee KH, Shin JH, Lyun Y, Hahn SY: Atypia of undetermined significance in thyroid fine-needle aspiration cytology: prediction of malignancy by US and comparison of methods for further management. Ann Surg Oncol 2014;21:2326–2331.

84 Moon HJ, Kim EK, Kwak LY: Malignancy risk stratification in thyroid nodules with benign results on cytology: combination of thyroid imaging reporting and data system and Bethesda system. Ann Surg Oncol 2014;21:1898–1903.

85 Jordan KW, Adkins CB, Cheng LL, Faquin WC: Application of magnetic-resonance-spectroscopy-based metabolomics to the fine-needle aspiration diagnosis of papillary thyroid carcinoma. Acta Cytol 2011;55:584–589.

86 Heinzel A, Muller D, Behrendt FF, Giovanella L, Mottaghy FM, Verburg FA: Thyroid nodules with indeterminate cytology: molecular imaging with ^{99m}Tc-methoxyisobutylisonitrile (MIBI) is more cost-effective than the Afirma® gene expression classifier. Eur J Nucl Med Mol Imaging 2014;41:1497–1500.

87 Jing X, Knoepp SM, Roh MH, Hookim K, Placido J, Davenport R, Rasche R, Michael CW: Group consensus review minimizes the diagnosis of 'follicular lesion of undetermined significance' and improves cytohistologic concordance. Diagn Cytopathol 2012;40:1037–1042.