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37

38 **RUNNING TITLE:** TBSRTC: Frequently Asked Questions

39

40 **KEYWORDS:** thyroid nodule; fine-needle aspiration; diagnostic category; papillary thyroid
41 carcinoma; follicular thyroid carcinoma; indeterminate cytology; medullary thyroid carcinoma;

42 NIFTP

43 **ABSTRACT**

44 **Background:** The recent update of the Bethesda system for reporting thyroid cytology (TBSRTC)
45 is a very important development in the evaluation of thyroid nodules. Clinical experience and
46 scientific literature both show that practitioners performing thyroid FNA are accustomed to
47 basing the clinical management of patients on reports using TBSRTC. Specifically, clinicians are
48 familiar with the per cent risk of malignancy (ROM) corresponding to each TBSRTC diagnostic
49 category (DC), as well as with the respective recommendation for clinical management.
50 However, most clinicians are much less familiar with the specific considerations that lie
51 between a specific DC, on the one end, and the respective ROM and associated management
52 recommendation, on the other end.

53 **Summary:** A deeper understanding of the system can enlighten the clinician's thinking about
54 the specific nodule under examination and can guide the decision-making process in a more
55 meaningful way. Such an understanding can only be developed via close, two-way
56 communication between cytopathologists and clinicians. Through this type of interaction in our
57 tertiary medical center, we identified a set of recurring issues of particular importance for
58 clinical practice, which we report here in the form of 16 Frequently Asked Questions (FAQ)
59 posed by the clinician to the cytopathologist.

60 **Conclusions:** For each FAQ, we provide an answer based on the literature, our experience, the
61 new version of TBSRTC and the new World Health Organization classification of tumors of
62 endocrine organs.

63

64 INTRODUCTION

65 Thyroid fine-needle aspiration cytology (FNAC) is the most accurate and cost-effective
66 tool in the initial management of patients with thyroid nodules, and its diagnostic yield can be
67 increased when it is associated with ultrasound (US) examination and, in case of indeterminate
68 cytological diagnosis, with molecular genetic testing. Although it is not perfect, thyroid FNA has
69 reduced the number of surgeries performed by better distinguishing nodules that require
70 surgery from those that do not (1-6). A major landmark was the creation of a uniform system
71 for reporting thyroid cytopathology after a 2007 conference in Bethesda, MD, hence named
72 “the Bethesda system for reporting thyroid cytopathology” (TBSRTC) (7). TBSRTC consists of 6
73 diagnostic categories (DCs): non-diagnostic/unsatisfactory (ND/UNS); benign (B); atypia of
74 undetermined significance or follicular lesion of undetermined significance (AUS/FLUS);
75 follicular neoplasm/suspicious for follicular neoplasm (FN/SFN); suspicious for malignancy (SM);
76 and malignant (M). Each DC is associated with a specific ROM and a respective clinical
77 management recommendation. This has contributed to making TBSRTC very popular across the
78 world, as witnessed by the high number of publications using it (8-11). TBSRTC has also
79 contributed to facilitating the communication between the cytopathologists and the clinicians
80 who perform FNA or manage patients according to FNAC results. By increasing the quality and
81 reproducibility of thyroid cytology, TBSRTC has become highly popular also in the clinical
82 community, as shown by its endorsement by the American Thyroid Association (ATA) as part of
83 the revised 2015 ATA guidelines for the management of thyroid nodules in adults (12).

84 Recently, the second edition of TBSRTC was published (13); the update was made
85 necessary by mainly two reasons. Firstly, recent advances in the molecular diagnosis of thyroid
86 nodules made it important to specify their place in the post-FNA management algorithm for
87 each specific DC. Secondly, the non-invasive encapsulated follicular variant of papillary thyroid
88 carcinoma (FV-PTC) was renamed as non-invasive follicular thyroid neoplasm with papillary-like
89 nuclear features (NIFTP), and it was recognized by the new World Health Organization (WHO)
90 classification of tumors of endocrine organs as a lesion whose malignant potential is much
91 lower than that of conventional papillary thyroid carcinoma (PTC) (14). As a consequence, the
92 recalculated ROM ranges also needed to take into account whether NIFTP is considered as a
93 carcinoma or not (15).

94 The update of TBSRTC is thus a very important and welcome development. Indeed,
95 clinical experience shows that practitioners performing thyroid FNA are accustomed to basing
96 the clinical management of the patients on reports using TBSRTC. Specifically, clinicians are
97 familiar with: (i) the per cent risk of malignancy associated with each TBSRTC diagnostic
98 category, and (ii) the respective recommendation for clinical management (the options in the
99 original version were: observe, repeat FNA or refer for surgery). However, most clinicians are
100 much less familiar with the specific considerations and details that lie between a specific DC, on
101 the one end, and the respective ROM and associated management recommendation, on the
102 other end. This is unfortunate, because a deeper understanding of the system can enlighten the
103 clinician's thinking about the specific nodule under examination and can guide the decision-
104 making process in a more meaningful way. Such an understanding can only be developed via
105 close, two-way communication between the cytopathologist and the clinician. Based on this

106 type of interaction in our thyroid clinic, as well as on an informal survey among our
107 endocrinology colleagues dealing routinely with thyroid patients in our tertiary medical center,
108 we identified a set of recurring issues of particular importance for clinical practice, which we
109 report here in the form of Frequently Asked Questions (FAQ) posed by the clinician to the
110 cytopathologist. For each FAQ, we provide an answer based on the literature, our experience,
111 the new version of TBSRTC and the new WHO classification of tumors of endocrine organs (13,
112 16).

113

114 **FAQ 1: What are the most important modifications in the updated version of TBSRTC?**

115 The most important modification in the updated version of TBSRTC concerns the ROM.
116 First, the ROM ranges have been updated according to the most recent literature data.
117 Moreover, for each DC, two different ROM ranges are indicated: one by considering NIFTP as
118 carcinoma and the other by considering NIFTP as a non-malignant or pre-malignant lesion.

119 The general schema of 6 DCs is maintained, as well as the designation of each individual
120 DC. The updated version of TBSRTC includes some explanations that were necessary to avoid
121 subjective interpretations possible in the previous classification. In particular, the AUS/FLUS DC
122 should not be split, meaning that it should not be used to identify separately cases with
123 cytological (mostly nuclear) atypia – i.e., AUS – and cases with architectural (mostly
124 microfollicular) atypia – i.e., FLUS. The terms AUS and FLUS are to be considered synonymous
125 and used together as AUS/FLUS. The same applies to the terms FN and SFN (FN/SNF). The
126 cytopathologist has the option of adding a descriptive comment to this DC (as to all other DCs),

127 which may be useful to better predict the histological diagnosis of the lesion in question. This is
128 particularly important after the reclassification of the non-invasive encapsulated FV-PTC as
129 NIFTP. In our institution, in case of cytological features suggestive of NIFTP, the following
130 comments are added to the diagnosis as a note: “The presence of rare atypical nuclear features
131 in this follicular-patterned lesion suggests the possibility of a FV-PTC or NIFTP”.

132 The advent of NIFTP made necessary also an adjustment in the FN/SFN DC. In the
133 updated version, cases with slight nuclear atypia are also included in this DC, and they can
134 correspond to NIFTPs found on histology. Conversely, because the M DC must retain a high
135 positive predictive value for cancer, it should comprise only cases with multiple typical nuclear
136 features of PTC; these can include nuclear enlargement, nuclear membrane irregularities,
137 frequent nuclear grooves, abnormal chromatin clearing and/or nuclear inclusions. Cases of
138 NIFTP typically have less well-developed nuclear atypia and almost never have nuclear
139 inclusions. Psammoma bodies are rare in FNAC specimens but are very helpful when present as
140 they are not found in NIFTP. Papillary arrangement also, by definition, is absent in NIFTP. Given
141 that papillary architecture excludes NIFTP, it is important to be aware that nodules can still be
142 classified in the M DC as a cytological diagnosis of PTC even if they do not display abundant
143 papillary structures, because the latter are not always present and thus not necessary for
144 diagnosis; in such cases, the diagnosis is usually supported by the presence of abundant and
145 convincing nuclear atypia.

146

147 **FAQ 2: What are the reasons for a ND/UNS classification? Does it depend primarily on the**
148 **nodule, the FNA operator or the cytopathologist? And what are the implications?**

149 A ND/UNS classification normally does not depend on the cytopathologist, because she or he
150 needs to follow specific predefined criteria to evaluate the quality and adequacy of the sample
151 (Cf. FAQ 5-7 for more details). In that sense, it is unlikely that a more “defensive”
152 cytopathologist will triage borderline and/or difficult cases into the ND/UNS DC (but rather into
153 the AUF/FLUS DC; Cf. FAQ 7-9).

154 There are some rare types of nodules that can be associated with a high risk of ND/UNS results,
155 such as solitary fibrous tumors, schwannomas, fibrotic Hashimoto’s disease or Riedel’s
156 thyroiditis. In these cases, the target lesion contains very few, if any, follicular cells.

157 In the majority of cases then, the reason for ND/UNS DC rests with the FNA operator, and it has
158 to do with poor technique in sampling, slide preparation or fixation (Cf. FAQ 5 and 7 for more
159 details on specific quality issues). According to the Bethesda guidelines, no more than 10% of
160 specimens should be classified as ND/UNS. However, the percentage of nodules classified as
161 ND/UNS in real life varies widely in the literature, ranging from 1-2% to as high as 45-50% (17).
162 The higher end of this spectrum is way beyond the acceptable 10% threshold and thus clearly
163 reflects poor practice. At this higher end, the ROM could also be significantly impacted,
164 especially if there is a systematic bias, associated with the underlying reason for the high
165 percentage of ND/UNS specimens, notably marginal specimens due to poor sampling or
166 preparation techniques. Therefore, in order to keep the rate of ND/UNS reports as low as
167 possible, or at least within acceptable limits (10%), specimen quality is of paramount

168 importance. This is why it is imperative that non-cytopathologist operators who perform
169 thyroid FNA (most commonly endocrinologists or radiologists) receive dedicated training on
170 quality issues related to FNA technique and sample preparation (18). Those who do not meet
171 the 10% benchmark should be made aware (e.g., by their cytopathologist, or by their clinical
172 supervisor if still in training) and further structured training to reach this goal should be
173 expected.

174 Poor specimen quality is a main cause of false-negative diagnoses; this can occur when the
175 material is either not representative or so scant or poorly preserved that neoplastic cells cannot
176 be identified (18). In addition, poor specimen quality is also implicated in false-positive
177 diagnoses, when the cytopathologist attempts to force a diagnosis in cases with marginal
178 material (18). Thus, high rates of ND/UNS samples cause increased cost and morbidity
179 associated not only with repeat testing but also with unnecessary surgery; indeed, a substantial
180 number of patients with ND/UNS results, especially after repeat FNA, will be addressed for
181 surgery (Cf. FAQ 3), and it is well-known that after thyroid surgery about 2% of patients suffer
182 from permanent laryngeal nerve damage and about 2% suffer from post-operative
183 hypoparathyroidism.

184

185 **FAQ 3: When a nodule yields a ND/UNS result, is it more or less likely to be malignant?**

186 The malignancy risk associated with a non-diagnostic category was not clearly stated in
187 the original TBSRTC publication (7). According to a large meta-analysis, the malignancy risk of
188 this category, calculated among resected cases, was 9-32%, which is higher than that of a

189 benign diagnosis (9, 12). However, resected cases are a selected group of the total population
190 of the ND/UNS nodules, often operated because of worrisome US features; a reasonable
191 extrapolation of the overall malignancy risk in this category is 5-10%, as stated in the new
192 version of the TBSRTC (17). This is the reason why close follow up or even surgery is suggested
193 for the 30% of all ND/UNS cases that are re-aspirated and that yield a second ND/UNS result,
194 associated or not with suspicious US features. In case of one or more ND/UNS FNAC results, one
195 can consider performing the FNA under US guidance followed by rapid on-site evaluation
196 (ROSE); another option is core biopsy, as recommended by other reporting systems for such
197 non-diagnostic cases (19, 20).

198

199 **FAQ 4: When a nodule yields a ND/UNS result, can the biopsy be repeated rapidly, or does a**
200 **3-6 month waiting period apply as for AUS/FLUS results?**

201 For the cytopathologist, the 3-6 month waiting period before repeating the FNA after a
202 ND/UNS result is justified by the presence of reparative and regenerative changes, which, if
203 sampled during the second FNA, can lead to a false-positive cytological diagnosis. On the other
204 hand, from the clinician's perspective, one can just repeat the biopsy without delay, and
205 perform a delayed third biopsy in case of a AUS/FLUS result on the second FNAC. This strategy
206 will allow to reassure many patients immediately and to avoid 3 months of possible worry or
207 even distress. Two studies have actually suggested that a 3 month waiting period is not
208 necessary for initially non-diagnostic aspirates (21, 22); the same might be true for initially
209 atypical aspirates (AUS/FLUS), but this particular question has not yet been addressed with

210 sufficiently high numbers of cases (21). The ATA 2015 guidelines state that a waiting period is
211 probably not necessary (12).

212

213 **FAQ 5: When a nodule aspirated under US guidance yields a few isolated normal (non-**
214 **atypical) follicular cells, why it is classified as ND/UNS and not as benign?**

215 The widespread use of FNA coupled with US allows the operator to be certain that the
216 aspirated material indeed comes from the intended target lesion. However, even if the FNA
217 practitioner is sure about which lesion has been sampled, this is not sufficient for the
218 cytopathologist to establish a diagnosis of benignity based only on a few normal, non-atypical
219 follicular cells. One of the major achievements of TBSRTC was that it addressed not only DCs
220 but also quality issues, procedures, and standardization of reporting terminology. One of these
221 topics concerns the specimen's adequacy. The assessment of pre-analytical issues, such as
222 specimen adequacy, according to specific criteria, is the basis to ensure a high-quality result
223 with a low false-negative rate, as well as to ensure that any downstream molecular test is
224 applied on the appropriate target cell population.

225 In general, there is a minimum requirement of 6 groups of follicular cells, which should
226 contain at least 10 thyrocytes each. These follicular cell groups should be well-preserved, well-
227 stained and not covered by blood cells that obscure their features (7). Of note, the
228 cytopathologist cannot combine cells present in two or more ND/UNS results to try to meet the
229 above criteria. The problem with isolated thyrocytes, even when they are present in a well-
230 prepared and well-stained specimen coming from a nodule properly sampled under US

231 guidance, is that they do not permit the cytopathologist to appreciate the architectural
232 arrangement of the underlying lesion. It is thus impossible to establish whether the lesion is
233 macrofollicular or microfollicular.

234 The clinician should also be aware of some exceptions to the above criteria. Some FNA
235 aspirates may be diagnosed as benign even without the presence of 6 groups of follicular cells
236 with at least 10 thyrocytes each. This concerns aspirates from: (1) colloid nodules, which are
237 extremely dilated follicles filled with colloid, producing a specimen composed entirely of colloid
238 material; (2) nodules with inflammation (typically in the context of autoimmune, infectious, or
239 chronic inflammatory thyroid disease), where in the presence of abundant colloid and
240 abundant inflammatory cells, a few follicular cells are sufficient to diagnose the nodule as
241 benign; and (3) cystic nodules, where the typical cystic content (macrophages,
242 hemosiderophages, red blood cells, fibrin and colloid) should be classified in the ND/UNS DC;
243 nevertheless, in such cases, the clinician can treat the nodule as benign based on a
244 clinicopathological correlation with non-suspicious US imaging compatible with a pure cyst
245 (often aspirated for volume reduction and/or symptomatic relief of compressive symptoms)
246 (17).

247 Lastly, FNAC of developmental thyroid cysts can yield only cystic fluid, macrophages and
248 rare epithelial cells (mostly squamous) with a benign appearance. In such cases, a diagnosis of
249 benignity consistent with a developmental cyst such as a thyroglossal duct cyst can be rendered
250 cytologically; a clinicopathological correlation should be encouraged.

251

252 **FAQ 6: Why is there still a residual risk of malignancy associated with a benign classification?**

253 Indeed, even if the FNA is performed under US guidance, and thus the clinician is sure
254 about having sampled the correct target nodule, the ROM is not equal to zero. The reported
255 ROM range taken from TBSRTC is 1-3%, while risk estimates reported in the literature vary
256 between 1-10% and can be as high as 22% in nodules larger than 3 cm (23). One possible
257 explanation concerns cases with suboptimal preparation and staining that are incorrectly
258 diagnosed as benign even though they should have been classified as ND/UNS. In this respect, it
259 is also important to note that although 6 clusters composed of 10 thyrocytes each qualify a
260 specimen as adequate for diagnosis, more abundant material generally facilitates a more
261 secure diagnosis and thereby contributes to minimize the ROM in this category. When samples
262 are properly prepared and stained, discrepancies arise mostly due to errors in the
263 interpretation of the cytological features, especially in the category of FV-PTC, where nuclear
264 changes are subtle; if such features are not properly recognized, then a false-negative diagnosis
265 may be rendered. Finally, a rare caveat is the macrofollicular variant of FTC (24, 25); these
266 tumors show capsular and/or vascular invasion, yet the FNA yields primarily macrofollicles, and
267 thus the lesion is classed as ND/UNS and not as FN/SFN, which is the case with the common FTC
268 variant, where microfollicles are predominant. These caveats justify the management
269 recommendation to perform at least one US follow-up examination of patients with a benign
270 FNAC diagnosis.

271

272 **FAQ 7: Are there underlying clinical conditions that favor classification of nodules as**
273 **AUS/FLUS? If so, might informing the cytopathologist change the diagnosis?**

274 Some lesions are classified under the AUS/FLUS category because the specimen is
275 qualitatively compromised. A badly smeared, fixed or stained preparation is thus classified as
276 AUS/FLUS because of technical reasons that do not depend on the nature of the lesion itself or
277 any associated clinical conditions. For example, FNA on patients treated with anticoagulants
278 can yield bloody aspirates. In this case, smears can be covered by blood that obscures the
279 characteristics of the follicular cells and prevents their correct interpretation. In such a
280 scenario, awareness of the anticoagulation treatment will not change the classification, as the
281 issue is technical. In contrast, when the sample shows cytological atypia, it is of paramount
282 importance that the cytopathologist has been informed of the patient's clinical conditions in
283 order to correlate them correctly with the cytological findings. For example, antithyroid
284 medications (thionamides) could be responsible for the presence of atypical thyrocytes with a
285 so-called "flaming cytoplasm"; if such treatment is not disclosed by the clinician, the cytology
286 might be inappropriately reported as atypical (AUS/FLUS or even FN/SFN).

287 Other important information to disclose to the cytopathologist is prior external beam
288 radiation therapy or radioactive iodine therapy. Both can result in cellular enlargement and
289 nuclear atypia that can lead to classification in the AUS/FLUS or SM DC (26).

290 Clinically evident cases of thyroiditis are occasionally subjected to FNA for diagnostic or
291 research purposes. In cases of florid or sclerosing thyroiditis without a clearly identified nodule
292 on US, slightly atypical nuclei (clearing of the chromatin, increased nuclear size, grooves) in an

293 otherwise benign-appearing aspirate can be correctly interpreted as related to thyroiditis, thus
294 classified as benign and avoiding repeat FNA or further interventions.

295 Because it is widely fibrotic, sclerosing thyroiditis may yield too few cells upon FNA; in
296 such cases, the scanty cellularity can be considered worrisome in case of presence of some
297 atypical cells suggesting PTC. Indeed, slightly atypical nuclei with the same characteristics as in
298 sclerosing thyroiditis can be observed in cases of PTC with desmoid-type fibromatosis, a rare
299 PTC variant that presents with a well-defined nodule containing a hyperechoic zone on US
300 consistent with sclerosis/fibrosis. Thus, the clinical context, including the US characteristics of
301 the lesion, is critical to guide the interpretation of the cytological findings (27).

302 These examples illustrate how the communication by the clinician of relevant clinical
303 information to the cytopathologist is essential in order to correctly interpret atypical cytological
304 findings. The clinicopathological correlation can facilitate a correct interpretation of the
305 observed atypia and thus guide the further clinical management of the patient. An exhaustive,
306 yet user-friendly requisition form can greatly help to ensure that the clinician does not omit any
307 important clinical information that the cytopathologist could need (Table 1).

308

309 **FAQ 8: For a AUS/FLUS nodule, is it clinically relevant to explain the specific subcategory, the**
310 **reason for the classification and the type of cancer possibly associated?**

311 The AUS/FLUS DC comprises several scenarios with different associated ROM (28). In
312 the new version of TBSRTC the generic term AUS/FLUS is maintained, but it is suggested to add

313 a note describing the pattern of the lesion among the most common patterns that have been
314 identified in a large literature review (13). These patterns include nuclear atypia (i.e., the
315 presence of features associated with PTC); architectural atypia [(i.e., the presence of
316 microfollicles suggesting follicular adenoma vs. follicular thyroid carcinoma (FTC)); oncocytic
317 features (i.e., the presence of Hürthle cells with eosinophilic granular cytoplasm and prominent
318 nucleoli); and “not otherwise specified” (NOS) in case the atypia observed cannot be classified
319 in any of the aforementioned patterns. Among these four patterns, the malignancy risk
320 decreases progressively from nuclear atypia (highest) to NOS (lowest). Knowledge of the
321 precise ROM associated with the specific qualifier of a AUS/FLUS lesion can be very useful for
322 the clinician who is charged to discuss repetition of the FNA with the patient and/or to propose
323 alternatives. One such alternative can be molecular genetic testing, as also suggested by the
324 ATA 2015 guidelines and the ETA 2017 guidelines (Figure 1) (12, 29). For example, without
325 knowing the qualifier of the AUS/FLUS diagnosis, one might propose a molecular test for a
326 AUS/FLUS case diagnosed as such because of quality issues (Figure 2), which would be
327 inappropriate. Moreover, in an effort to propose a personalized cytology, and in view of the
328 paucity of material frequently observed in AUS/FLUS cases, the cytopathologist together with
329 the clinician (as is the practice in our center) can also select the most appropriate molecular
330 markers, such as mutational analysis of the BRAF V600E point mutation and PET/PTC
331 rearrangements in cases with nuclear atypia, or the BRAF K601E, RAS point mutations and
332 PAX8/PPAR gamma rearrangement in cases with architectural atypia. The 2017 ETA guidelines
333 provide a detailed discussion of the potential and limitations of molecular genetic testing (29).

334

335 **FAQ 9: When will a predominantly microfollicular lesion be classified as AUS/FLUS and when**
336 **as FN/SFN?**

337 Predominance of microfollicles can be observed in case of a paucicellular aspirate or in
338 case of a highly cellular aspirate. In the first situation, the appropriate diagnosis would be
339 “AUS/FLUS, architectural atypia”. The cytopathologist is reluctant to induce a diagnostic
340 lobectomy in these cases and prefers to have the patient undergo a repeat FNA in the hope of
341 obtaining more material that will allow to reach a more accurate diagnosis. In the second
342 situation, a highly cellular aspirate with predominance of microfollicles, the appropriate
343 diagnosis would be FN/SFN. What is still not clearly defined is the minimum amount of
344 microfollicles necessary for a FN/SFN diagnosis. Also, it is important to remember that slight
345 nuclear atypia is now included in the FN/SFN DC; in fact, in presence of a microfollicular pattern
346 with nuclear atypia, it is also possible that the lesion is a NIFTP (which can only be diagnosed on
347 surgical pathology), as already mentioned in FAQ1.

348

349 **FAQ 10: Can a FN/SFN nodule be a PTC?**

350 In the FN/SFN DC (10-40% ROM) are usually classified lesions that contain a
351 predominant or exclusive population of microfollicles. When such lesions are subjected to
352 diagnostic surgery (normally lobectomy), the main histological correlates of these aspirates are
353 benign proliferations, namely hyperplastic nodules/follicular adenomas, and in a lower
354 proportion malignant lesions, namely FTC (9, 13). Some malignant cases corresponded in the
355 past to FV-PTC. This variant is characterized by a microfollicular structure and subtle nuclear

356 changes in the sense of PTC, namely nuclear clearing and grooves, with few or no nuclear
357 inclusions; these subtle nuclear changes can often pass unnoticed, leading to a FN/SFN
358 diagnosis of these lesions (30, 31). With the modification in the nomenclature and the
359 introduction of NIFTP as a lesion of low malignant potential, fewer PTC cases will be found in
360 the FN/SFN diagnostic category, thus reducing the lower end of the ROM of the FN/SFN DC (32,
361 33). Notwithstanding this improvement in the diagnostic classification, some invasive FV-PTC
362 still will be diagnosed in the FN/SFN DC, because the presence or absence of capsular or
363 vascular invasion cannot be assessed on cytological material.

364

365 **FAQ 11: Can a SM nodule be other than PTC?**

366 In the majority of cases, a SM nodule turns out to be PTC upon histological examination.
367 In this DC are classified cases that contain atypical nuclear features suspicious for PTC (either
368 the classical or the follicular variant), but that are not sufficient for a conclusive diagnosis of
369 PTC. However, the degree of suspicion is higher than that of the cytological atypia component
370 in the AUS/FLUS DC (Cf. FAQ 8); as a consequence, surgery is indicated (Cf. FAQ 16). Nuclear
371 atypia, in particular nuclear pseudoinclusions, are not seen exclusively in PTC, but sometimes
372 also in medullary thyroid carcinoma (MTC), along with salt-and-pepper chromatin, granular
373 cytoplasm and absence of colloid. MTC is actually the second most frequent histological
374 diagnosis in case of SM cytological findings (when all the above characteristic of MTC are not
375 present). Other types of tumors that can be suspected on cytology and confirmed on histology
376 include trabecular adenoma, poorly differentiated thyroid carcinoma (PDTC), anaplastic thyroid

377 carcinoma (ATC), lymphoma, sarcoma and metastases of extra-thyroidal primary tumors. A
378 good percentage of NIFTP also fall in this DC, which is why the ROM of an SM classification
379 decreases substantially when NIFTP is not considered a cancerous lesion (7, 13, 32, 33).

380

381 **FAQ 12: What is the major cytological difference between the SM and M DCs?**

382 The main difference between the SM and M DCs is that in the former the cytological
383 criteria for malignancy are not completely met, yet the level of suspicion is high. Histologically
384 proven FTCs are typically not found in the SM or M DCs. This is because the criteria for
385 malignancy in follicular lesions are histological, requiring examination of the tumor's capsule
386 and of the vessels in the capsule; therefore, these tumors cannot be diagnosed purely on
387 cytological grounds. Except for FTC, which, as mentioned, is typically not classified in the SM
388 DC, all other types of thyroid carcinoma may be classified in this DC based on a preoperative
389 FNAC if the cytological criteria present are not sufficient to warrant a confident diagnosis of
390 malignancy. Among epithelial tumors, MTC, PDTC or ATC can be classified in the SM DC, but the
391 most frequent type is by far PTC. For PTC, SM designation is usually reached in cases with
392 limited material and/or when some of the following features are missing: pseudoinclusions,
393 psammoma bodies, papillary structures, nuclear membrane irregularity and nuclear grooves. In
394 such a scenario, when a microfollicular pattern is present, there is a highly probability that the
395 lesion is FV-PTC, but the cytopathologist cannot be totally certain.

396

397 **FAQ 13: In which TBSRTC DC would a NIFTP be classified?**

398 Even though a diagnosis of NIFTP can only be made on surgical pathology, it is
399 interesting to consider the spectrum of possible presurgical cytological diagnoses associated
400 with these lesions. It has been shown that histologically proven NIFTP had been classified
401 preoperatively in mainly three DCs: AUS/FLUS, FN/SFN and SM, with frequencies that were
402 variable among different centers (32, 33). Like for any other lesion, a lesion later shown to be a
403 NIFTP may be classified preoperatively in the ND/UNS category, when the material is
404 insufficient. Beyond that, the precise DC into which a specific lesion later proven to be a NIFTP
405 may be classified on presurgical cytology depends on various factors, including the degree of
406 nuclear atypia, the extent of microfollicular architecture, the quality of the specimen and, last
407 but not least, the experience of the cytopathologist. A pathology-proven NIFTP should normally
408 not have been classified as a benign lesion on cytology, because the presence of atypia and/or
409 microfollicles warrants classification in a DC with higher ROM. It should also typically not have
410 been classified as a malignant lesion, because papillary structures are absent, the degree of
411 nuclear atypia is milder and the presence or absence of capsular and vascular invasion cannot
412 be assessed on cytological material. Nevertheless, the risk of the M DC also decreased slightly
413 after the introduction of NIFTP (from 97-99% to 94-96%) (15), indicating that a small number of
414 nodules ultimately shown to be NIFTP do end up in the M DC based on FNAC.

415 From a pre-surgical point of view, given that NIFTP is considered a lesion with a low
416 malignant potential, the most important consequence of renaming non-invasive encapsulated
417 FV-PTC into NIFTP is that it resulted in a decrease of the ROM of the aforementioned DCs

418 (AUS/FLUS, FN/SFN and SM). Among multicentric studies, the corresponding reduction of the
419 ROM varied greatly (33). For this reason, the new Bethesda version provides a range for the
420 ROM taking into account the new nomenclature (Table 2). Because the introduction of NIFTP is
421 quite recent and not yet ubiquitously accepted, the new Bethesda version cites two ROM
422 ranges for each DC, a higher one for when NIFTP is considered a cancerous lesion (not shown)
423 and a lower one when it is considered a lesion with low malignant potential (Table 2).
424 Admittedly, if one subscribes to the NIFTP concept, then only the respective lower ROM ranges
425 are relevant.

426

427 **FAQ 14: Which signs raise suspicion of MTC, and in which DC is an MTC likely to be classified?**

428 Depending on the suspicious features present in each particular case, MTC is usually
429 diagnosed in the SM or M DC. The most striking cytological features suggestive of MTC are the
430 absence of colloid and the presence of a salt-and-pepper chromatin and of a granular
431 eosinophilic cytoplasm. Presence of nuclear pseudoinclusions does not exclude a diagnosis of
432 MTC, as MTC can indeed also present with abundant nuclear pseudoinclusions (Figure 3). MTC
433 is in fact considered a great mimicker, as it can assume the most disparate cytological features,
434 such as spindle cells or oncocytic cells; this can occasionally lead to classification in the SM DC
435 as suspicious for PTC, or in the M DC as PTC or even as sarcoma or metastatic disease. In cases
436 where MTC is suspected based on clinical features or based on cytological findings of ROSE at
437 the time of FNA sampling, then collection of material for cell block can allow for
438 immunocytochemical staining for calcitonin, confirming the diagnosis if cytomorphology alone

439 does not allow for a definitive diagnosis. Measurement of calcitonin (which should be high in
440 MTC) and possibly also thyroglobulin (which should be low or undetectable) in the needle
441 washout is also very helpful in such cases.

442

443 **FAQ 15: When a suspicious lymph node is aspirated in the context of a co-existing thyroid**
444 **nodule, how relevant is it for the cytopathologist that the nodule also be aspirated?**

445 Strictly speaking, it is not necessary, because in general the cytological diagnosis of the
446 lymph node is independent from that of the thyroid nodule. In rare cases when there are some
447 atypical cells in the FNAC of the lymph node that are suspicious for PTC, an ancillary study, such
448 as immunostaining for thyroglobulin or TTF-1, if positive, can confirm the presence of
449 metastatic PTC. Finally, measuring thyroglobulin in the needle washout of the FNA sample can
450 confirm metastatic disease when there is paucity or lack of tumor cells in the specimen and an
451 immunohistochemical staining cannot be performed. Because this is obviously not known
452 beforehand, routine measurement of thyroglobulin in the aspirate (or at least conservation of
453 an appropriate sample for later measurement if necessary) should be strongly considered.

454 As a general point, if a thyroid nodule is suspicious and warrants FNA, it is overall logical
455 to biopsy it at the same time as the suspicious lymph node, because otherwise, if the lymph
456 node FNA is negative, then the question about the nature of the thyroid nodule would remain
457 and the patient would need to return for FNA of the thyroid nodule. On the other hand, if a
458 lymph node is highly suspicious on US and the thyroid contains multiple nodules of which none
459 is highly suspicious, it may be reasonable to perform FNA only on the lymph node, which will be

460 sufficient to guide further management if the result confirms metastasis of thyroid carcinoma,
461 given that total thyroidectomy with compartment-based lymph node dissection is indicated in
462 such cases.

463

464 **FAQ 16: In which cases is a frozen section useful to guide surgical management?**

465 Given the high risk of malignancy in SM cases, surgery is normally warranted with a
466 diagnostic and therapeutic intent. If there is a dilemma between total thyroidectomy and initial
467 diagnostic lobectomy (if indicated with completion surgery in case of malignancy confirmed on
468 histology), then preoperative confirmation of malignancy may also be achieved by molecular
469 genetic testing, in particular by detecting alterations associated with PTC with very high positive
470 predictive value, such as a BRAF V600E mutation or a RET/PTC rearrangement. Alternatively, or
471 for cases where molecular genetic testing results do not confirm malignancy, a frozen section
472 analysis during diagnostic lobectomy may provide perioperative confirmation of malignancy in
473 some cases. This depends largely upon the recognition of typical features of classical PTC,
474 notably papillary structures and severe nuclear atypia. There are two main limitations: The first
475 is that the quality of the specimen obtained during a frozen section is lower than that obtained
476 during routine histopathological examination. Thus, among lesions classified in the SM DC that
477 are finally proven to be classical PTC cases on histology, not all could be confirmed as such on
478 frozen section analysis. The second limitation is that the single frozen section obtained may not
479 be representative of the lesion as a whole. Therefore, follicular patterned lesions are
480 inappropriate candidates for frozen section analysis, because even in cases of invasive FV-PTC

481 or FTC, the likelihood of detecting capsular or vascular invasion in a single frozen section is
482 exceedingly low.

483

484 **CONCLUSIONS**

485 Although there are no formal studies on this topic, close communication between the
486 cytopathologist and the clinician can help to optimize the diagnostic accuracy of thyroid FNAC.
487 In our experience, good ways to interact constructively and to develop a deeper mutual
488 understanding of the intricacies and challenges of each other's discipline include joint US-FNA
489 clinics with ROSE for selected nodules; multidisciplinary tumor boards; clinicopathological
490 discussions of cases in the cytopathology unit while studying the slides of typical and atypical
491 cases under a multi-observer microscope; as well as dedicated combined workshops and
492 practical courses. We hope that the present overview will serve as an additional resource to
493 this end.

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Table 1. Outline of the thyroid FNA requisition form used in our center to transmit clinical information to the cytopathologist.

Patient's name Date of birth Unique identifier in the hospital	
Nodule's dimensions and volume length (cm) x width (cm) x thickness (cm) volume (ml)
Nodule's localization	right lobe / left lobe / isthmus superior / middle / inferior
Nodule's US characteristics	microcalcifications: Y / N central vascularization: Y / N irregular margins: Y / N hypoechoogenicity: Y / N irregular or incomplete halo: Y / N taller than wide: Y / N cystic component: Y (.....%) / N
Suspicious lymph node(s)	Y / N
Previous FNA	Y / N If yes: Date:..... Result:.....
Thyroid autoimmunity	none / Hashimoto's / Graves'
TSH level mIU/L
Thyroid medications	none / antithyroid drugs / levothyroxine
Previous external beam radiotherapy	Y / N
Previous radioiodine treatment	Y / N
Family history of thyroid carcinoma	Y / N
Personal history of thyroid carcinoma	Y / N
Non-thyroidal primary malignancy	Y (specify.....)/ N

Table 2. The updated risk of malignancy ranges and management recommendations proposed by the new version of TBSRTC.

Bethesda DC*		% ROM* (NIFTP ≠ cancer)	Management recommendation
ND/UNS	Non diagnostic, unsatisfactory	5 - 10	Repeat FNA with ultrasound guidance
B	Benign	0 - 3	Clinical and ultrasonographic follow-up
AUS/FLUS	Atypia of undetermined significance or follicular lesion of undetermined significance	6 - 18	Repeat FNA, molecular testing or lobectomy
FN/SFN	Follicular neoplasm or suspicious for a follicular neoplasm	10 - 40	Molecular testing, lobectomy
SM	Suspicious for malignancy	45 - 60	Near-total thyroidectomy or lobectomy
M	Malignant	94 - 96	Near-total thyroidectomy or lobectomy

*DC: diagnostic category; ROM: risk of malignancy. Adapted from Cibas ES, Ali SZ 2017 The 2017 Bethesda System for Reporting Thyroid Cytopathology. Thyroid 27:1341-1346.

FIGURE LEGENDS

Figure 1: A classical variant of PTC initially classified as AUS/FLUS and then diagnosed as M (PTC) via molecular genetic testing. A 25-year-old female with a 2.3 cm nodule in the left thyroid lobe underwent US-guided FNAC. **A.** Few groups of thyrocytes were present on the slide (liquid based cytology, Papanicolaou staining, 600x) and presented focal atypia, namely rare grooves (arrows). The result rendered was AUS/FLUS. According to TBSRTC, she should undergo repeat FNAC, but she refused. In the context of cellular atypia without architectural atypia in a specimen that was not highly cellular, targeted molecular genetic testing was performed for BRAF hotspot mutations and RET/PTC translocations. **B.** Pyrosequencing demonstrated a c.1799T>A (p.V600E) BRAF mutation, diagnostic for PTC. The patient underwent surgery and histopathology confirmed the diagnosis of a PTC, classical variant.

Figure 2: A case of NIFTP classified in the Bethesda AUS/FLUS and SM DCs. A 52-year-old female with a 1.8 cm nodule located in the isthmus underwent US-guided FNAC. **A.** The specimen was highly cellular but badly fixed and stained. Thyrocytes were enlarged, stained reddish and chromatin details were not well visible. Some probable grooves (arrows) were identified and a possible nuclear pseudoinclusion (arrowhead) was also suspected (smear, Papanicolaou staining, 400x). The poor quality of the specimen did not allow establishing a definitive cytological diagnosis, and the case was rendered as AUS/FLUS. **B.** The patient underwent a repeat US-guided FNAC 6 months later with a SM diagnosis (suspicious for PTC): the smears were hypercellular with abundant microfollicular structures, abundant grooves (arrows) and what were thought to be nuclear pseudoinclusions (arrowhead). The patient underwent diagnostic lobectomy (without frozen section) (smear, Papanicolaou staining, 200x).

C. The histological specimen was consistent with NIFTP; in contrast to the initial cytological suspicion (A), no nuclear pseudoinclusions were identified, only chromatin clearing was present (hematoxylin and eosin staining, 200x).

Figure 3: A case of MTC correctly classified in the M DC and confirmed as a neuroendocrine tumor using immunocytochemistry. **A.** An aspirate from a 75-year-old man showing plasmocytoid, polygonal cells and nuclei with granular chromatin. Some nuclear pseudoinclusions were present (arrows); pseudoinclusions are not exclusively present in PTC, but also in MTC (Papanicolaou staining, 400x). **B.** Based on the immunocytochemical confirmation of the neuroendocrine nature of the lesion (Chromogranin staining) the final diagnosis was: positive for malignant cells consistent with MTC (Papanicolaou staining, 600x).

Figure 1

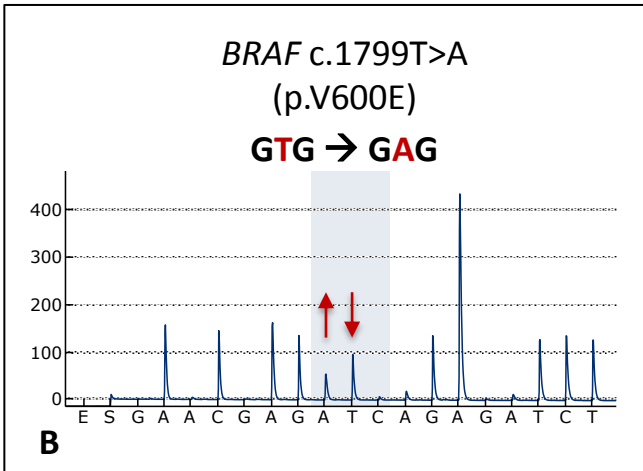
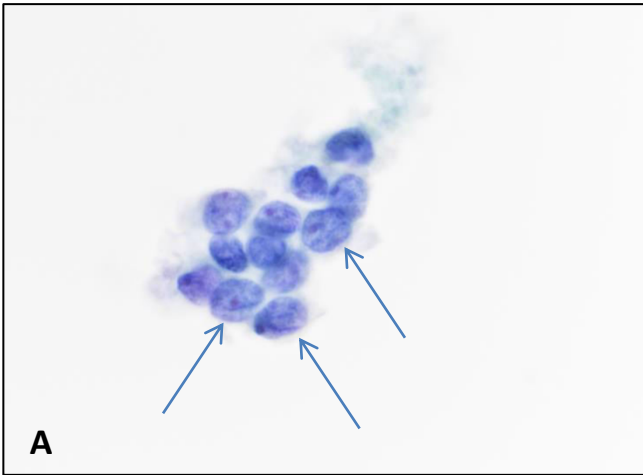


Figure 2

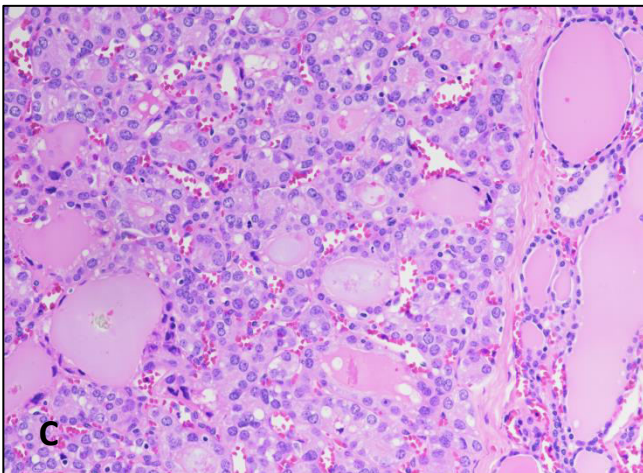
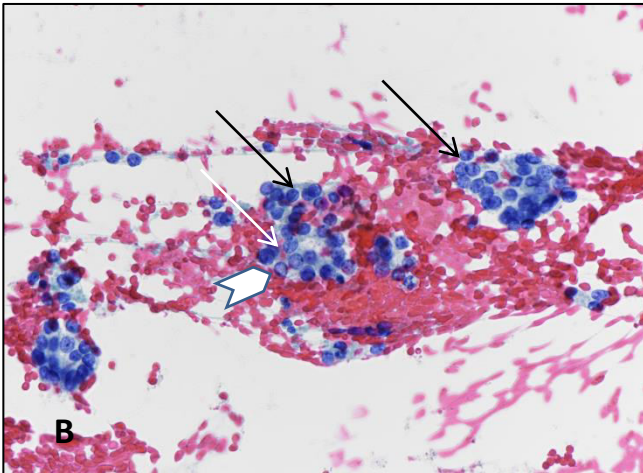
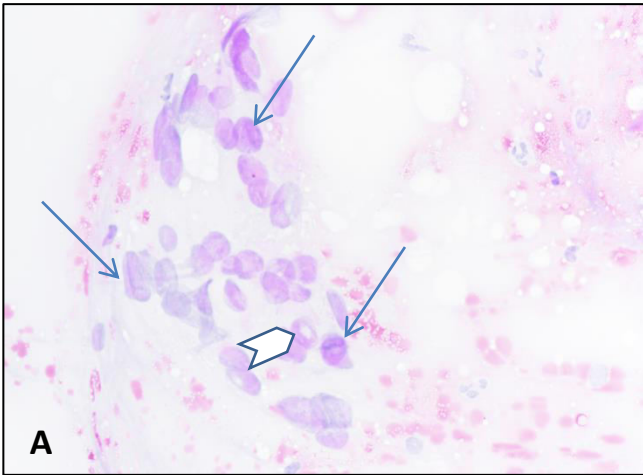


Figure 3

