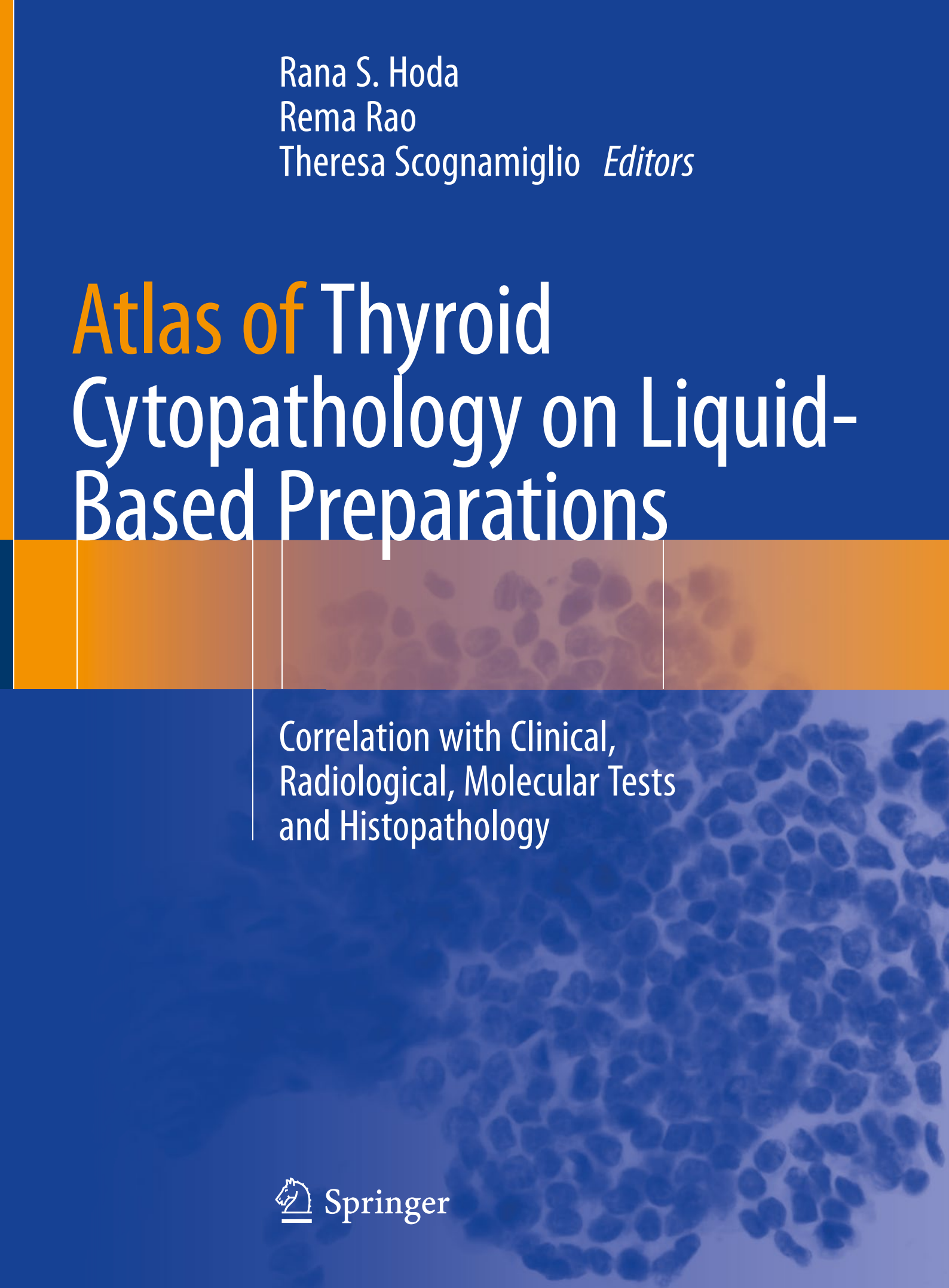
Rana S. Hoda
Rema Rao
Theresa Scognamiglio *Editors*

Atlas of Thyroid Cytopathology on Liquid-Based Preparations

Correlation with Clinical, Radiological, Molecular Tests and Histopathology



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"In memory of my loving parents, Atiqa and Shafiq Ismail; for my dear husband, Syed; my dear son, Raza; and my beloved daughter-in-law, Sehyr."

Dr. Rana S. Hoda

"To my daughter Ananya Rao, my biggest cheerleader."

Dr. Rema Rao

"To my family, friends, and colleagues; thanks for the support and encouragement."

Dr. Theresa Scognamiglio

Preface

Fine-needle aspiration (FNA) cytology of the thyroid is the initial diagnostic procedure for the evaluation of nodules and triage thereof to either observation or surgery. Traditionally, thyroid FNAs have been prepared as conventional smears (CS), but these presented certain drawbacks that led to the use of liquid-based preparations (LBP) in thyroid cytology, either as the sole preparation or in combination with CS. Although both methods are useful, the cytomorphologic differences require familiarity for accurate interpretation and avoidance of diagnostic pitfalls. This Atlas was inspired by the increasing use of LBP as a processing method for thyroid FNA.

The second edition of the *Bethesda System for Reporting Thyroid Cytopathology* (TBSRTC) has been widely accepted by pathologists and endorsed by major endocrine clinical organizations including the American Thyroid Association (ATA) for the management of thyroid nodules. It has also received global recognition. This Atlas emphasizes the use of LBP based on TBSRTC diagnostic categories.

The Atlas of Thyroid Cytopathology on Liquid-Based Preparations serves as a handy guide to diagnostic cytology on LBP. It is intended to be a ready resource to accurately diagnose thyroid lesions on LBP using key cytomorphologic features. Key cytologic differential diagnosis, gross, and histopathological correlations accompany the cytological findings.

The Atlas is lavishly illustrated with color images of various thyroid diseases that should familiarize pathologists with the differences between CS and LBP and between the two commonly used LBPs. The authors have done their best to provide clear, concise, and practical guidance pertaining to cytomorphology and the implications of thyroid FNA diagnoses for patient care in this era of precision medicine.

Rye Brook, NY, USA — Rana S. Hoda
New York, NY, USA — Rema Rao
New York, NY, USA — Theresa Scognamiglio

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Liquid-Based Preparations in Thyroid Fine Needle Aspiration

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Rana S. Hoda

Thyroid Nodules

- Estimated annual incidence of thyroid nodules in the United States is 0.1%, which translates to 300,000 new nodules every year.
- Up to 50% of the general population may have sonographically detectable thyroid nodules, although only up to 5% of these harbor a malignancy.
- The high incidence of thyroid nodules and low rate of cancer among nodules pose a clinical dilemma.
- The necessity for fine needle aspiration (FNA) is assessed by clinical and ultrasound (US) risk factors for malignant disease.

Thyroid Cancer

- Thyroid cancer is the eighth most common cancer in the United States.
- In 2019, the American Cancer Society project that there will be approximately 52,070 new cases of thyroid cancer in the United States (37,810 in women and 14,260 in men), with 2170 deaths from the disease (1150 women and 1020 men). Nearly 3 out of 4 cases are found in women. Thyroid cancer is commonly diagnosed at a younger age than most other adult cancers.
- Thyroid cancer currently makes up just 5% of newly diagnosed cancers.
- The incidence rates of thyroid cancer in both women and men increased at a rate of about 4% a year from 2005 to 2014, according to the latest available data. Thyroid cancer is the most rapidly increasing cancer in the United States, and by 2030 it will become the fourth most prevalent cancer in the United States [2].
- The rise in the detection of thyroid cancer can be attributed to the increasing use of US, which can detect small, nonpalpable thyroid nodules that were not detected in the past.

Fine Needle Aspiration of Thyroid Nodules

- FNA is the standard test for initial assessment of thyroid nodules.
- The sensitivity of FNA is 80–98% and specificity is 58–100% in the triage of patients to observation or surgery. FNA performed under US guidance is much more sensitive.
- FNA diagnoses are reported based on The Bethesda System for Reporting Thyroid Cytopathology (TBSRTC), the second edition of which was released in 2017.
- Overall, thyroid FNA shows malignancy in about 5–10% of cases; another 10–25% are indeterminate or suspicious for cancer. Findings are benign in 60–70%. Patients with nodules that are malignant or suspicious for cancer by FNA usually undergo thyroid surgery.
- Malignancy is found in more than 50% of excised thyroid nodules.
- The American Thyroid Association (ATA) recommends FNA of all thyroid nodules larger than 1 cm.
- Nodules smaller than 1 cm are aspirated if they have high-risk US features:
  - Solid or hypoechoic
  - Irregular margins
  - Height taller than width
  - Microcalcifications
  - Disrupted rim calcifications
- Molecular testing in conjunction with indeterminate cytology on FNA aids in the preoperative detection of neoplastic and malignant thyroid nodules.

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The Principal Indications for FNA in Thyroid Nodules

- Initial assessment of a newly discovered nodule
- Follow-up of benign nodules after initial assessment
- Follow-up of patients with a history of thyroid cancer for the early detection of recurrences. The current standard for follow-up consists of US and FNA every 3–6 months or as clinically indicated.

Comparison of Liquid-Based Preparations and Conventional Smears

- Traditionally, thyroid FNA has been prepared as conventional smears (CS), but liquid-based preparations (LBP) are increasingly being used, because of major technical limitations of CS (Fig. 1.1)
- The following tables list advantages and disadvantages of CS and LBP (Table 1.1), a technical comparison (Table 1.2), a morphologic comparison (Table 1.3), and cytological features of commonly encountered thyroid lesions as seen in CS and LBP (Table 1.4).

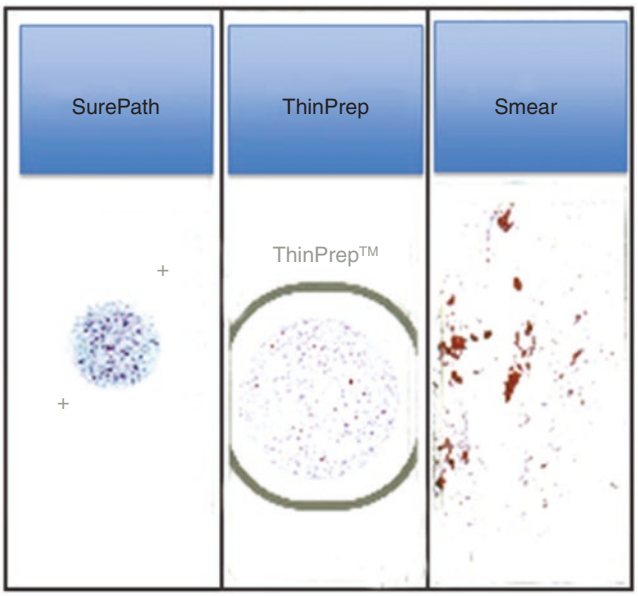

Fig. 1.1 SurePath™ (SP), ThinPrep® (TP) and conventional smear (CS). For the SP slide, the diameter of the circle is 13 mm and the specimen collection preservative medium is ethanol-based. For the TP slide, the circle where the cytologic material is deposited has a diameter of 20 mm. The specimen collection preservative medium is methanol-based. The Papanicolaou (Pap)-stained CS shows material deposited unevenly along the entire slide surface

Table 1.1 Principal advantages and disadvantages of conventional smears and liquid-based preparations

Method	Advantages	Disadvantages
Conventional smears	Inexpensive; no special preparation or staining equipment needed; simple; good cellularity; larger-sized clusters; better preserved architecture; good morphology	Multiple slides with variable cell deposition; smear-related artifacts including air-drying artifact, thick cellular areas, obscuring blood; tedious to screen
ThinPrep®[a]	Standardized and easy preparation; monolayer; less/good cellularity; better preservation; decrease in unsatisfactory specimens; uniform cell distribution; clean background; fast and easy screening; multiple slides can be prepared; additional cost is offset by improved specimen quality	Some alteration of key nuclear features and background elements; fragmentation of cell clusters; cell shrinkage; more expensive than conventional preparations
SurePath™[b]	Standardized preparation; stained on the processor; good cell yield and preservation of morphology; relative ease of screening; multiple slides can be prepared	Cells are in various planes of focus, making screening and focusing at high magnification tedious

[a]BD Diagnostics, Burlington, NC, USA
[b]Hologic, Marlborough, MA, USA

The Limitations and Artifacts of Conventional Smears

- Technical limitations of CS impair cell details and adequate assessment of cytology.
- The many limitations and artifacts result from the preparatory method and fixation:
  - *Slide labeling*: Labeling the slides (putting at least two patient identifiers) and packing them can be tedious and time-consuming.
  - *Uneven smearing of cells on slides*: The smearing of the collected sample on the slides is uneven and non-uniform (Fig. 1.2), with interindividual and intraindividual variations in smear preparation.
  - *Thick and overcrowded cellular areas*: These are fairly common and result from uneven smearing (Fig. 1.2).

Table 1.2 Technical comparison of conventional smears and liquid-based preparations

Features	Conventional smears	Liquid-based preparations
Slide Preparation	Less easy; slides are manually prepared and labeled with two patient identifiers	Easy, fully automated (TP) or semi-automated (SP)
Instrument	No special instrument required	Special instruments required
Glass slides	Ordinary glass slides	Manufacturer provides specially made & marked slides
Cost	Less expensive; no special requirements	Expensive due to costs of special instrument, solution, and slides
Specimen handling and transport	More handling, less easy transport. Air-dried slides transported in cardboard boxes; ethanol-fixed slides transported in Coplin jars	Less handling, easy transport. Specimen rinsed in tubes with proprietary collection medium, capped, and transported
Method of preparation	Time-consuming: specimen is smeared on glass slides, resulting in nonuniform and variable slides	Fast, automated, uniform, standardized preparations
Number of slides	Usually four or more	One
Cell deposition	Nonuniform deposition on the entire 5 × 2.5 cm area	Cells deposited within a well-defined marked area, a circle 20 mm (TP) or 13 mm (SP) in diameter
Fixative	Ethanol	Methanol (TP) or ethanol (SP)
Fixation and air-drying	Variable, usually 5–10 seconds; potential for air-drying artifact	Immediate, prevents air-drying artifact
Staining	Both Romanowsky & Pap stains	Only Pap stain
Obscuring elements	Present: Blood, inflammation	None or reduced
Cell distribution	Nonuniform; cell distribution in thick cellular areas with overlap	Uniform; thick cell distribution and overlap absent or reduced
Screening and time	Screening is time-consuming, tedious: many slides, cells unevenly deposited on the entire slide; obscuring elements	Screening is easy: one slide, cells deposited uniformly in a marked area with limited fields of view; obscuring elements reduced or absent
On-site evaluation	Can be performed with Romanowsky stains, such as Diff Quik (DQ) stain	Not possible

SP SurePath, *TP* ThinPrep

Table 1.3 Cytological differences between conventional smears and liquid-based preparations

Features	Conventional smears	Liquid-based preparations
Inadequate rate	Variable 15–30%	<15%
Obscuring elements	Yes	No
Background elements	Present	Reduced
Thick cellular areas	Yes	No
Colloid	Diffusely present, allows evaluation of amount	Thin colloid appears as "wrinkled tissue paper" or clumped, and thick colloid as globules; does not allow accurate quantification
Retention of large cell clusters	Yes	Inconsistent and smaller
Complex 3D fragments	Retained, easy to interpret	Fragmented, smaller, difficult to interpret due to thickness, especially in SP
Papillary structures	Easy to interpret	Difficult to interpret due to thickness, especially in SP
Follicular cells	Retain size and shape	Cells become smaller
Nuclei of papillary thyroid carcinoma (PTC)	Retain all features	Retain all features. Intranuclear pseudoinclusions (INPI) may be reduced or less apparent, especially in TP
Hürthle cells	Smooth nuclear membranes	Sometimes irregular nuclear membranes, smaller size
Aggregation of lymphocytes	No	Yes

SP SurePath, *TP* ThinPrep

Uneven staining: Because Diff Quik (DQ) staining is performed manually, overstaining is usual, due to nonuniform and thick and overcrowded cell distribution and cells partially obscured by blood (Figs. 1.2 and 1.3).

Partially obscuring blood: This artifact is fairly common and because cells cannot clearly be assessed, it usually results in overdiagnosis for fear of missing a significant lesion. In LBP, even though some blood is retained, it does not obscure cell detail (Figs. 1.4a–e and 1.5a–d).

– *Crush artifact*: Cells are fragile; if more pressure is used for smearing, the nuclei crush and smear, and cytoplasm is disrupted. This artifact is more pronounced in lesions with a lymphoid component (Fig. 1.6).

Table 1.4 Cytologic criteria for interpretation of thyroid lesions on conventional smears and liquid-based preparations

Diagnosis	Conventional smears	Liquid-based preparations
TBS II, Benign Follicular Nodule	Abundant watery colloid, honeycomb and ordered sheets and macrofollicles of small follicular cells, small uniform nuclei, clear to granular cytoplasm ± hemorrhage with hemosiderin-laden histiocytes & abundant thin colloid	All features present, single cells, bare nuclei, sheets are smaller, with smaller cells and nuclei, less watery colloid appears as "wrinkled tissue paper–like"
TBS II, Lymphocytic Thyroiditis	Mostly mature lymphocytes, few plasma cells, epithelioid histiocytes, lymphoid tangles, lymphoepithelial aggregates, sheets and clusters of Hürthle cells ± atypia, scant colloid	All features present, lymphocytes may be reduced, aggregate and may mimic follicular cells
TBS III, AUS/FLUS	Enlarged nuclei with subtle and few of the nuclear features of PTC, cells present in clusters/microfollicles. Follicular-patterned lesions may not always show PTC nuclear features if they represent FA/FC	All features present, cell clusters and microfollicles may appear tighter and cells and nuclei may appear smaller
TBS IV, Follicular Neoplasm (FN)/ Suspicious for FN	Scant or absent colloid; microfollicles or small clusters of larger (medium-sized) follicular cells arranged in honeycomb sheets with altered polarity, rounded, overlapping nuclei, ± slight nuclear irregularity, nucleoli, vascular fragments, diffuse or globules of thick colloid, usually in association with follicular cells. Nuclear clearing, grooves represent follicular-patterned PTC.	All features present, cells appear small, microfollicles may be tighter, scattered and isolated, colloid globules may be few and dispersed, or associated with follicular cells. In SP, microfollicle lumens are visualized in different planes of focus.
TBS IV, Follicular Neoplasm (FN)/ Suspicious for FN, Oncocytic Type	All features of FN as described above are present; cells are exclusively oncocytes with enlarged, monotonous nuclei, bi-nucleation, small or prominent nucleoli, dense or granular pinkish cytoplasm with distinct outline, low to high N:C, transgressing vessels or vascular fragments	All features present; oncocytes are smaller, nuclear chromatin appears more condensed, nucleoli may be more prominent
TBS V, Suspicious for Papillary Thyroid Carcinoma (PTC)	Colloid less and thick, ± dense globules "bubble gum" or thick "ropy" strands, syncytial fragments, clusters or papillary-like structures of medium- to large-sized nuclei with moderate nuclear pleomorphism, overlap, and crowding, irregular membrane, clearing and grooves (none/few INPI and absent true papillary fragments with fibrovascular core and psammoma bodies)	All features present; cells appear small; syncytial fragments, clusters or papillary-like structures are smaller with more disruption
TBS VI, Malignant, Papillary Thyroid Carcinoma (PTC)	Colloid less, hard and thick, forms dense globules "bubble gum" or "ropy" strands, large syncytial fragments, clusters, papillary fragments with fibrovascular cores of medium- to large-sized follicular cells with nuclear pleomorphism, overlap, and crowding, irregular membrane, clearing, grooves, INPI, nucleoli, ± psammoma bodies, ± cystic degeneration and histiocytes	All features present; cells appear small, ± elongated, many single cells, syncytial fragments, clusters and papillary fragments are smaller, thinner with more disruption; INPI may be reduced, smaller, and difficult to find
TBS VI, Malignant, Medullary Thyroid Carcinoma (MTC)	Cellular, small clusters of round to spindled cells with round or ovoid, slightly pleomorphic plasmacytoid (eccentrically placed) nuclei, "salt & pepper" neuroendocrine chromatin, fragments of hyaline material (amyloid); scant colloid	All features present; cells appear small, many isolated and dispersed cells, syncytial fragments; "salt & pepper" neuroendocrine chromatin retained, amyloid can be seen

FA follicular adenoma, *FC* follicular carcinoma, *FN* follicular neoplasm, *FVPTC* follicular variant of papillary thyroid carcinoma, *MTC* medullary thyroid carcinoma, *N:C* nucleus to cytoplasmic ratio, *PTC* papillary thyroid carcinoma, *SP* SurePath, *TBS* The Bethesda System

– *Nuclear features*: Evaluation of nuclear features is important in the diagnosis of thyroid lesions, particularly papillary thyroid carcinoma (PTC), so most pathologists prefer to examine specimens using the Pap stain. However, such material must be immediately fixed in alcohol to prevent air-drying artifact. Clinicians often do not appreciate how quickly the air-drying artifact occurs and do not see the impact of poor preparation. In a busy office, the clinician may unintentionally allow the slides to air-dry prior to placement in alcohol. This results in a

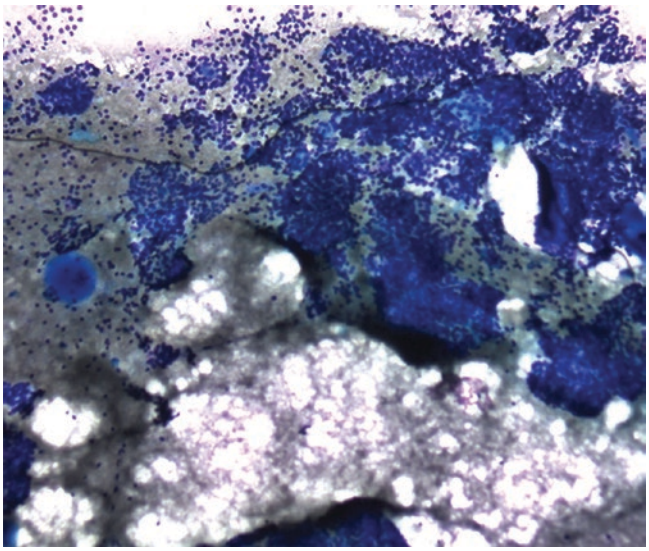

Fig. 1.2 Artifacts of CS. The smearing of the collected sample on the slides is uneven and non-uniform. Note thick and overcrowded cellular areas concentrated on the top of the slide (Diff-Quik [DQ] stain, CS)

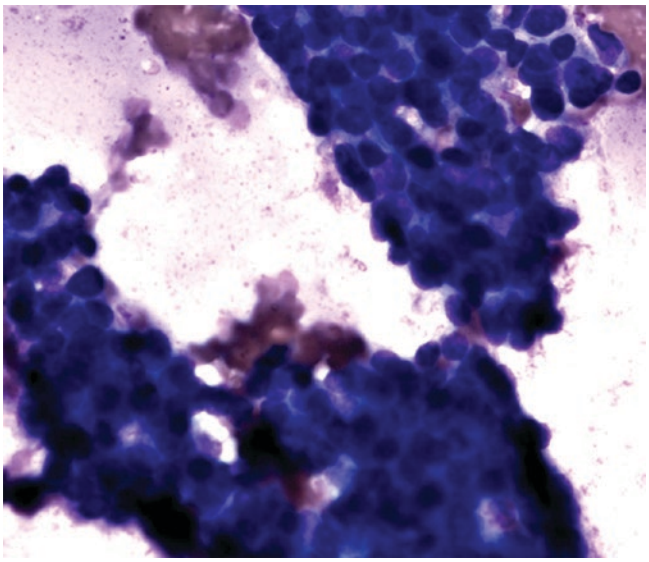

Fig. 1.3 Artifacts of CS. DQ staining in CS is uneven and overstained due to nonuniform and thick cell distribution

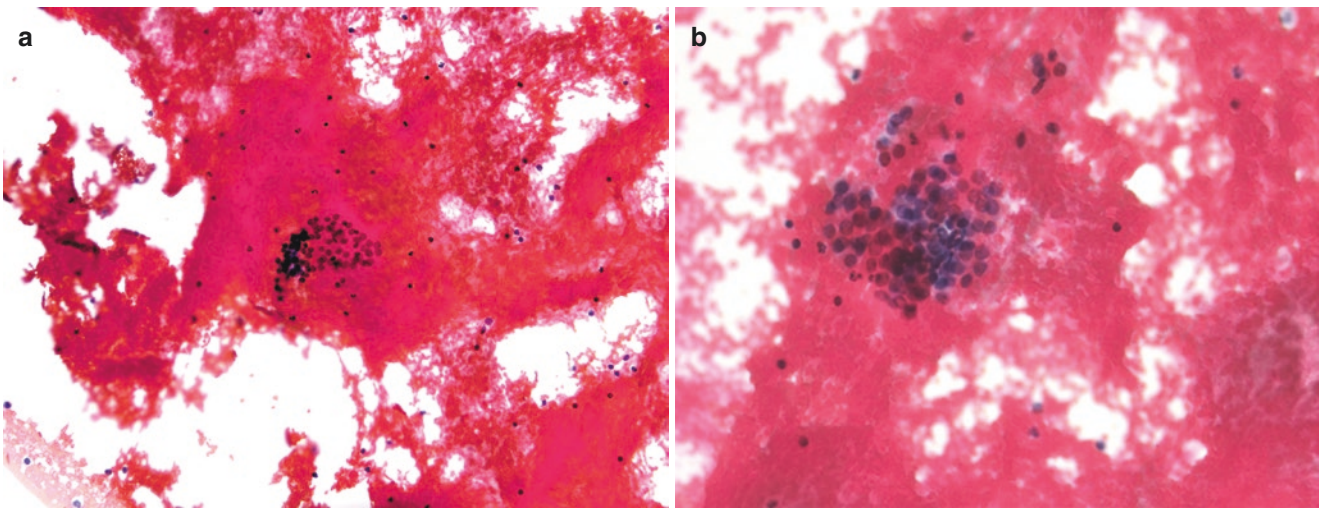

Fig. 1.4 (**a**–**e**) Artifacts of CS. (**a**, **b**) CS slide shows abundant blood, which partially obscures follicular cell detail. These are benign follicular cells. (**c**, **d**) Same case processed as TP shows background blood, but the follicular cells are not obscured. (**e**) Same case processed as a SP shows follicular cells within blood. Cell details are not obscured. Note macrophage in a different plane of focus. (**a**, **b**, Pap stain CS; **c**, **d**, Pap stain TP; e, Pap stain SP)

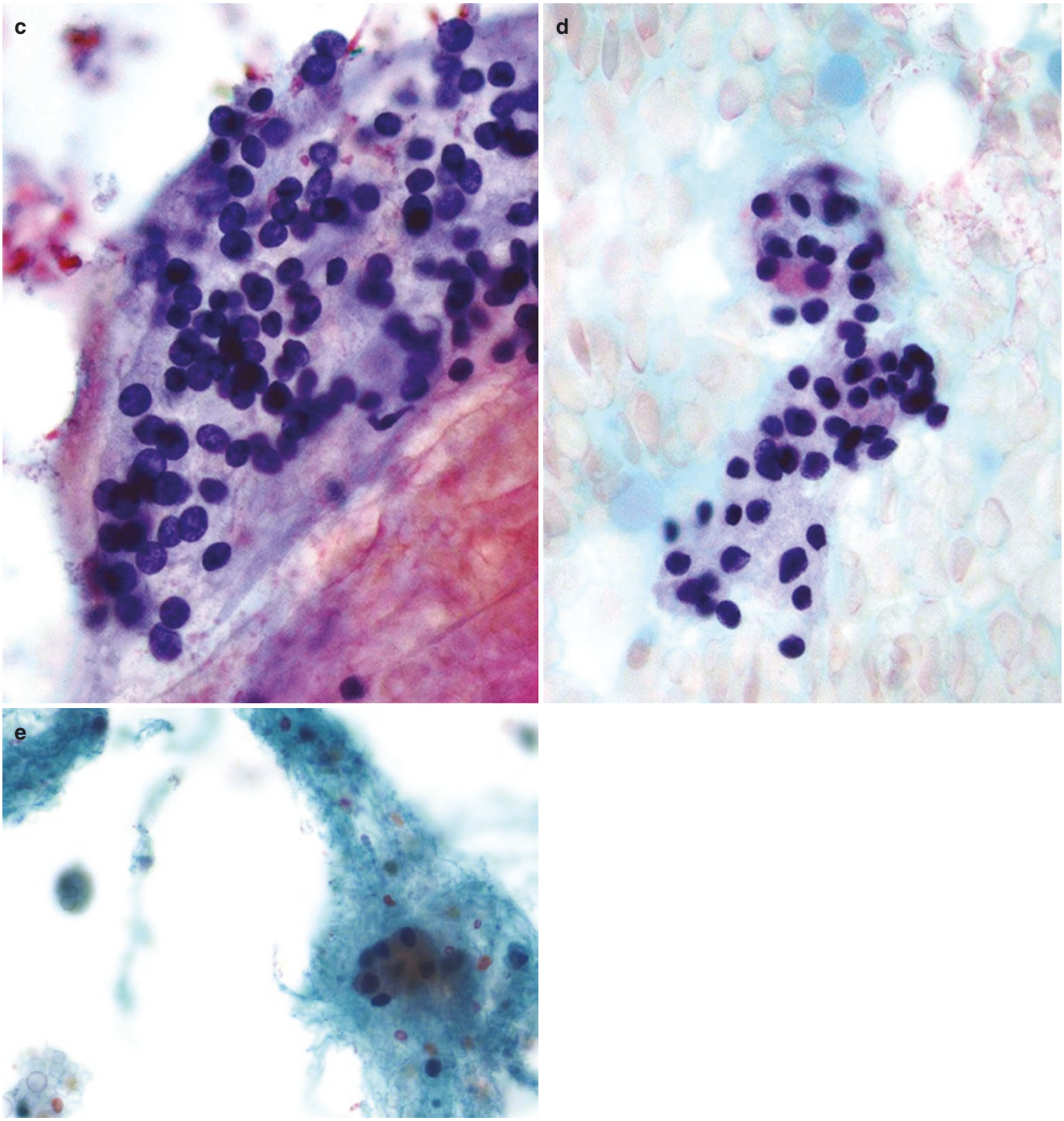

Fig. 1.4 (continued)