Cytologic Features of Small-Cell Carcinoma on ThinPrep®

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The use of ThinPrep® (TP) technology for fine-needle aspiration (FNA) cytology has become widely accepted. However, some literature suggests that small-cell carcinoma may present a diagnostic pitfall due to morphologic alterations. In this study, we retrospectively compared 14 FNA of small-cell carcinoma prepared using TP with corresponding conventional smears (CS). We also examined the TP appearance of 23 other small round-cell lesions in order to determine if differential diagnostic features were preserved. TP and CS were evaluated semiquantitatively for background, architecture, chromatin quality, nuclear molding, nuclear smearing, nucleolar prominence, amount of cytoplasm, nuclear size, and single-cell necrosis. The data were analyzed using the McNemar χ^2 test.

TP slides of small-cell carcinoma showed a cleaner background than CS ($P < 0.005$). Although some degree of nuclear molding was preserved, it was decreased in amount ($P < 0.025$) and subtler in quality. Similarly, nuclear smearing was present but decreased in amount ($P < 0.05$), and less prominent qualitatively. The amount of discernible cytoplasm was greater on TP ($P < 0.005$). No significant differences were found for any of the other parameters studied. The presence of nuclear molding was the single most useful feature in differentiating small-cell carcinoma from other small round-cell tumors on TP.

Small-cell carcinoma may be diagnosed with confidence by FNA using TP. However, pathologists should be aware of certain morphologic alterations in order to avoid diagnostic pitfalls. Diagn. Cytopathol. 2003;29:8–12.

Key Words: ThinPrep®; cytology; small-cell carcinoma; fine-needle aspiration

The use of ThinPrep® (TP) methodology (Cytyc, Malborough, MA) for the processing of fine-needle aspirates has gained wide acceptance, and several studies found good correlation between TP and conventional smear (CS) diagnoses.[1–7] Advantages of TP over conventional smears include uniform thickness, elimination of air-drying artifacts, better cellular preservation, and elimination of obscuring blood and exudate.[1] The TP technique is especially useful in settings where a pathologist or cytotechnologist is not available to prepare high-quality smears at the time of aspiration. Although the cytomorphologic findings are generally similar on TP and CS for many pathologic entities, a number of important differences have been described.[1,4,8,9] In a previous study on the effects of TP processing on fine-needle aspiration (FNA) morphology, we observed differences between the appearances of small-cell carcinoma on TP compared to CS.[1] The number of cases, however, was too small for statistical analysis. In the current study, we sought to perform a more rigorous analysis of the cytomorphologic differences of FNA of small-cell carcinoma on TP and CS, using a larger number of cases. We also examined TP of FNA of several other small round-cell lesions in order to evaluate whether critical diagnostic distinctions could be made.

Materials and Methods

Fine-needle aspirates of 14 cases of small-cell carcinoma prepared by both CS and TP were retrieved from the files of the University of Michigan Department of Pathology. Nine were paratracheal lymph node aspirates performed by Wang needle aspiration during bronchoscopy. One was a CT-guided aspirate of a liver mass in a patient with pulmonary small-cell carcinoma. Three were superficial lymph node aspirates in patients with pulmonary small-cell carcinoma (one cervical lymph node, one axillary lymph node, and one supraclavicular lymph node). One was an aspirate of an adrenal mass in a patient with pulmonary small-cell carcinoma. Also retrieved were TP of 23 other small round-cell tumors, including FNA of 6 lymphomas, 4 reactive lymph nodes, 3 rhabdomyosarcomas, 2 Merkel-cell carcinomas, 1 immature teratoma, 1 Ewing's sarcoma, 1 intra-abdominal desmoplastic round-cell tumor, 1 neuroblastoma, and 4 cases of medulloblastoma in cerebrospinal fluid. TP smears of all FNA were prepared from needle rinsings obtained following preparation of CS. On average, 3–4 passes through the lesion were performed. All TP and CS were stained using the Papanicolaou technique. Only cases judged to be of sufficient cellularity were included in the study.

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Table I. Scoring System for Diagnostic Parameters

	Score		
	1	*2*	*3*
Background	Clean	Blood/exudate	
Architecture	Mostly single cells, few clusters	Even mix of single cells and clusters	Mostly clusters, few single cells
Chromatin	Finely granular	Other	
Nuclear molding	Absent	Focal	Diffuse
Nuclear smearing	Absent	Elongation only	Complete chromatin blurring
Nucleoli	Absent or barely discernible	Evident but inconspicuous	Prominent/macronucleoli
Cytoplasm	Absent or barely discernible	Thin rim or eccentric collection	
Nuclear size	2–3 times mature lymphocyte	>3 times mature lymphocyte	
Single-cell necrosis	Absent	Present	

Papanicolaou-stained TP and CS were evaluated by both authors on a double-headed microscope, using a semiquantitative scoring system for the following parameters: blood/exudate, architecture, chromatin quality, nuclear molding, nuclear smearing, nucleolar prominence, amount of cytoplasm, nuclear size, and presence of necrosis (Table I). The background was assessed for blood and inflammatory cells, and was categorized as clean (free or nearly free of blood /exudates) or as containing significant blood/exudates (enough blood/exudate to be considered partially obscuring). Additionally, the presence of background necrosis (either amorphous necrotic material or apoptotic bodies) was recorded. Nuclear chromatin was judged as either finely granular (i.e., typical, stippled, salt and pepper, neuroendocrine chromatin pattern) or other. Nuclear molding was judged to be absent, focal (present but only after a careful search), or diffuse (widespread, easily found). Nuclear smearing was recorded as absent, elongation only (nucleus elongated and stretched, but without complete loss of chromatinic detail), or complete chromatinic blurring (loss of chromatin detail and rather stringy appearance). Nucleoli were described as absent, present but inconspicuous, or prominent. Cytoplasm was described as absent, thin-rimmed, or eccentric collection. Nuclear size was recorded as 2–3 times or larger than the size of a resting lymphocyte (Table I). Cytologic examination was conducted in a blinded fashion. Differences between slide preparation methods (TP vs. CS) were assessed using the McNemar χ^2 test. The study was conducted in accordance with the guidelines of the University of Michigan Medical School Institutional Review Board for Human Subject Research.

Results

TP of small-cell carcinoma showed a cleaner background than corresponding CS ($P < 0.005$). On low-power examination, both CS and TP showed a mixture of single cells and clusters. However, small-cell carcinoma appeared on CS as large and small cohesive sheets in a background of numerous discohesive cells with many doublets, triplets, and short cords (Fig. C-1A). On TP, small-cell carcinoma appeared as loosely cohesive small clusters in a background of numerous single cells (Fig. C-1B). Few doublets and short cords could be identified at high power. Nuclear molding was subtler on TP ($P < 0.025$). Focal, subtle molding was appreciated on TP (Fig. C-2A) compared to the diffuse, tight molding seen on CS (Fig. C-2B). Nuclear smearing was seen in all TP and CS, but was decreased in amount on TP ($P < 0.05$). In addition, qualitative differences in nuclear smearing were appreciated on TP. While on CS the smeared chromatin appeared as long strands of blue material spread across large areas (Fig. C-3A), smeared nuclear material on TP was more focal and appeared as elongated nuclei (Fig. C-3B) or tangles of threadlike material (Fig. C-3C). The amount of discernible cytoplasm was significantly greater on TP ($P < 0.005$). On CS, the cytoplasm was usually absent or only faintly discernible (Fig. C-4A), while on TP a thin rim or eccentric collection of pale cytoplasm was frequently visible (Fig. C-4B). Single-cell necrosis was absent in four TP but seen in all CS; this difference was not statistically significant. Qualitatively, single-cell necrosis on TP appeared as scattered apoptotic bodies contained with small droplets of necrotic material (Fig. C-5). Nucleoli appeared more prominent on TP in several cases, but this difference was not statistically significant. No significant differences in chromatin quality or nuclear size were noted.

The most important morphologic criterion in distinguishing small-cell carcinoma from other small round-cell lesions was nuclear molding. This criterion was absent in desmoplastic small round-cell tumor, Ewing's sarcoma, immature teratoma, lymphoma, neuroblastoma, rhabdomyosarcoma, and reactive lymph nodes. Molding was present in medulloblastoma and Merkel-cell carcinoma. However, the molding seen in these tumors was scarce and was only appreciated following examination of multiple microscopic fields. Single-cell necrosis was also characteristic of small-cell carcinoma on TP. Ten (71%) of 14 small-cell carcinomas showed single-cell necrosis, while only one case of rhabdomyosarcoma showed single-cell necrosis among the other small round-cell lesions. Although some degree of nuclear

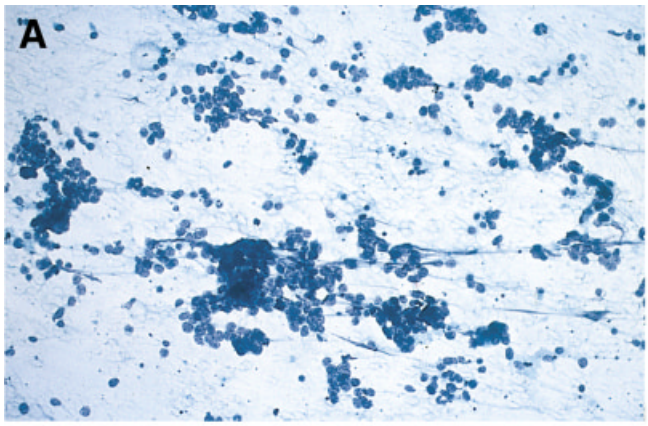

Fig. C-1A

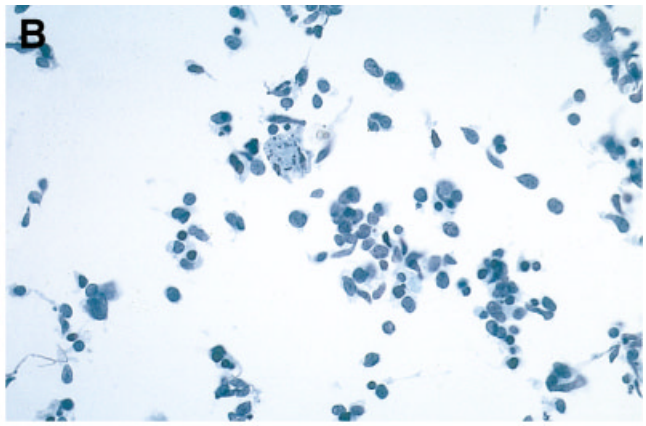

Fig. C-1B

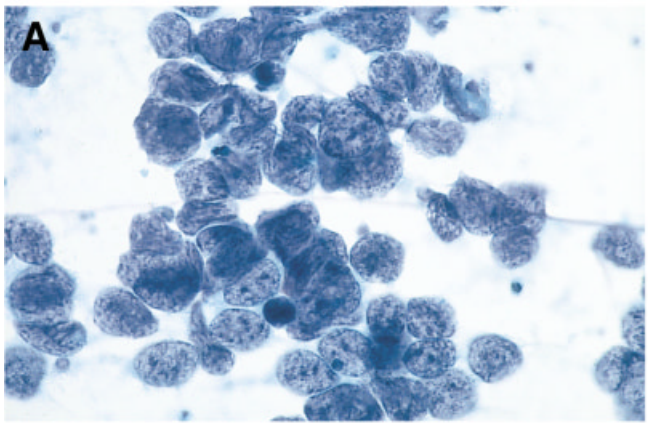

Fig. C-2A

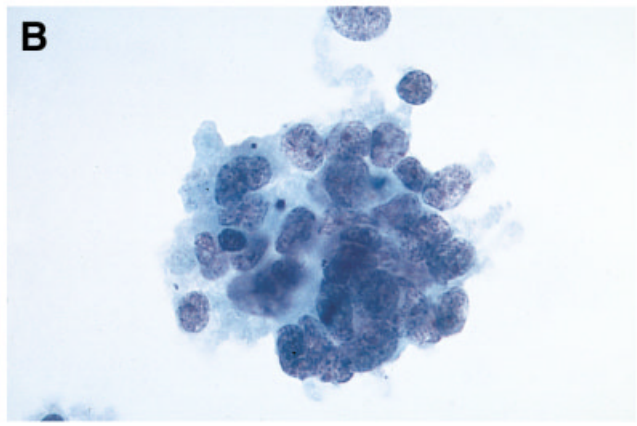

Fig. C-2B

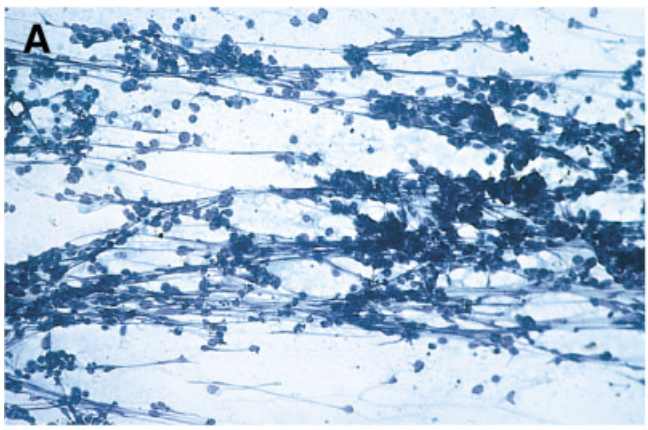

Fig. C-3A

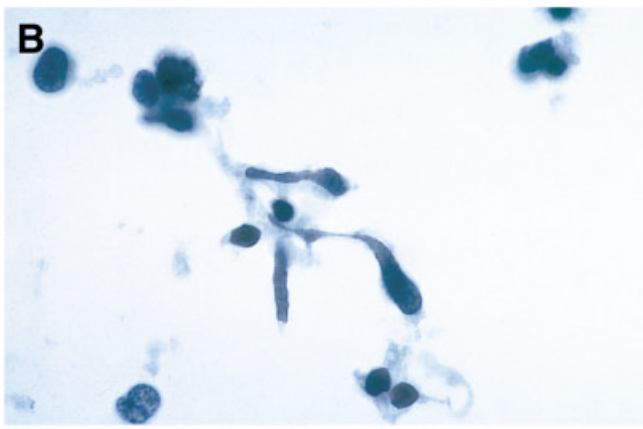

Fig. C-3B

Figs. C-1A–C-3B.

smearing, mainly in the form of nuclear elongation, was observed in all cases of small-cell carcinoma, this change was also seen in lymphoma, Merkel-cell carcinoma, reactive lymph nodes, and rhabdomyosarcoma. Generally, the nuclear smearing seen in the latter lesions was less prominent than that seen in small-cell carcinoma. Finely granular ("salt and pepper") chromatin was seen in all small-cell carcinomas but was also seen in immature teratoma, medulloblastoma, Merkel-cell carcinoma, and one case of rhabdomyosarcoma. The lack of cellular cohesion typical of lymphoma on CS was preserved on TP, and was useful in distinguishing lymphoid lesions from small-cell carcinoma.

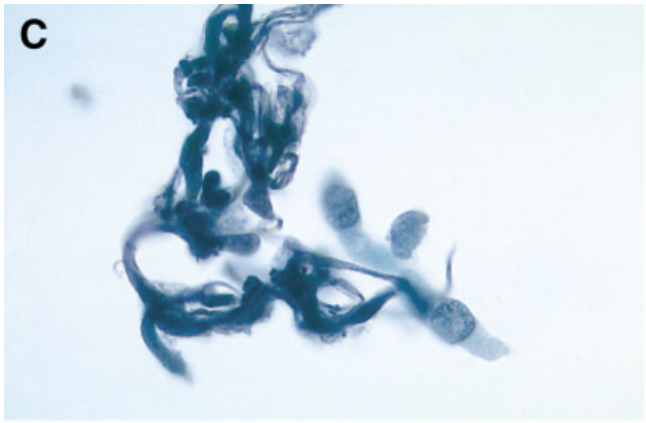

Fig. C-3C

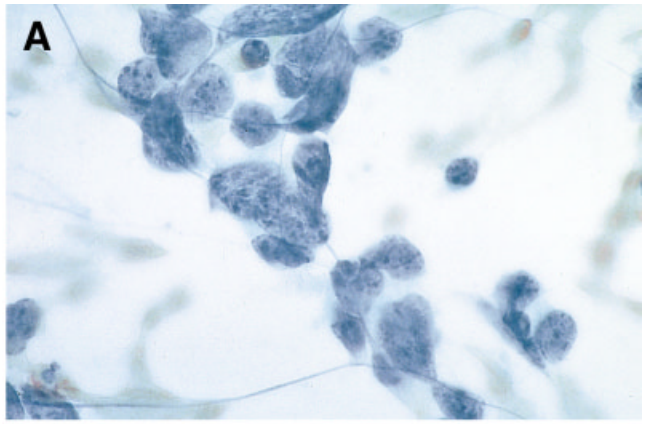

Fig. C-4A

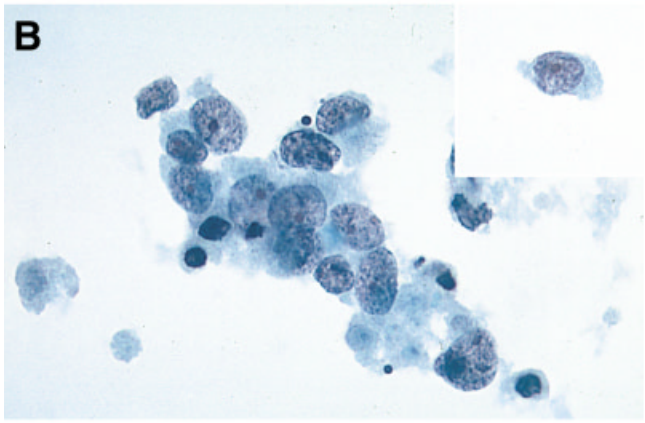

Fig. C-4B

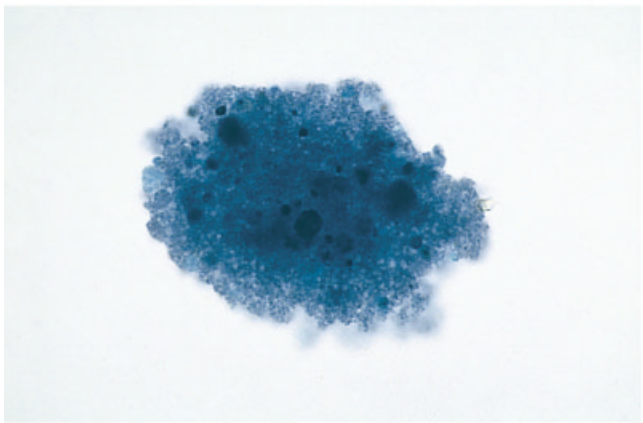

Fig. C-5

Figs. C-1–C5. Fig. C-1. A: Conventional smear of small-cell carcinoma, demonstrating sheets and clusters of small blue cells with single cells in background (Papanicolaou stain, ×100). **B:** Corresponding ThinPrep reveals smaller clusters and dispersed single cells (Papanicolaou stain, ×200). **Fig. C-2. A:** Conventional smear of small-cell carcinoma, demonstrating characteristic tight nuclear molding (Papanicolaou stain, ×1,000). **B:** Corresponding ThinPrep, demonstrating more subtle molding typically observed (Papanicolaou stain, ×1,000). **Fig. C-3. A:** Conventional smear of small-cell carcinoma, revealing long strands of nuclear material spread across large areas (Papanicolaou stain, ×200). **B:** Corresponding ThinPrep, showing nuclear elongation, without complete blurring of chromatin (Papanicolaou stain, ×1,000). **C:** Rarely, smearing was manifested on TP as a tangle of thread-like material (Papanicolaou stain, ×1,000). **Fig. C-4. A:** Conventional smear of small-cell carcinoma. Cytoplasm is not readily discernible (Papanicolaou stain, ×1,000). **B:** Corresponding ThinPrep, demonstrating thin rims or eccentric collections of pale cytoplasm (Papanicolaou stain, ×1,000). **Inset:** Single cell with eccentric collection of cytoplasm (Papanicolaou stain, ×1,000). **Fig. C-5.** Necrosis appeared on ThinPrep as scattered apoptotic bodies within droplets of amorphous material (Papanicolaou stain, ×1,000).

Nucleolar prominence, cytoplasmic qualities, nuclear size, and background could not be used reliably to separate small-cell carcinoma from other differential diagnostic considerations.

Discussion

We found subtle yet significant differences in the morphology of FNA of small-cell carcinoma on TP compared to CS. Although nuclear molding and smearing were present on both TP and CS, these features were less prominent on TP. The diffuse, tight nuclear molding characteristic of small-cell carcinoma on CS was represented on TP by focal, looser molding. The classic complete chromatin blurring induced during the preparation of CS was manifested on TP as nuclear elongation without complete loss of chromatin detail. Additionally, the amount of discernible cytoplasm

was greater on TP, appearing as a thin rim or eccentric collection. On TP, single-cell necrosis was observed less frequently, and nucleoli were more prominent in several cases. However, these differences did not reach statistical significance, possibly due to the relatively small number of cases included in the study. We also noted the occasional presence of conspicuous nucleoli in CS of small-cell carcinoma; in these cases, attention to nuclear size, molding, and chromatin texture was critical in arriving at a diagnosis of small-cell carcinoma. In spite of the subtle cytomorphologic differences observed between CS and TP, a confident diagnosis of small-cell carcinoma could still be rendered on each TP slide.

One important difference between CS and TP slides is the loss of background material. This is especially important in the diagnosis of thyroid and salivary gland lesions prepared by TP. Although we observed a cleaner background on TP-prepared aspirates of small-cell carcinoma compared to CS with respect to blood and inflammatory cells, the characteristic background, necrotic debris, and apoptotic bodies were present in the majority of TP. Necrotic debris on TP frequently appeared as droplets of amorphous material containing apoptotic bodies.

Several differences in processing method exist between CS and TP, which may account for the observed morphologic differences. In the preparation of a TP slide, aspirated material is rinsed directly into CytoLyt, a proprietary liquid fixative/transport medium, resulting in immediate wet fixation of the cells. Additionally, the mechanical forces associated with manual smearing are eliminated. These two effects may explain the increased amount of cytoplasm, decreased nuclear smearing artifact, and subtler molding seen on TP. TP processing also includes a mixing/homogenization step, which would explain the smaller clusters and increased number of single cells seen on TP.

The characteristic morphology of small-cell carcinoma was sufficiently preserved on TP to allow for a ready distinction from most other small round-cell lesions. The most helpful diagnostic criterion in this differential diagnosis was nuclear molding, which was absent in desmoplastic small round-cell tumors, Ewing's sarcoma, immature teratoma, lymphoma, neuroblastoma, rhabdomyosarcoma, and reactive lymph nodes. As might be expected, some degree of nuclear molding could be appreciated in medulloblastoma and Merkel-cell carcinoma; however, this feature was subtler than that seen in small-cell carcinoma. Single-cell necrosis was much more common in small-cell carcinoma than in other small round-cell lesions. However, this criterion was absent in 29% of small-cell carcinomas.

Differences in the cytomorphology of small-cell carcinoma on TP compared to CS were previously reported. However, to our knowledge, no published studies were devoted specifically to the morphology of FNA of small-cell carcinoma on TP. Hoerl et al. reported on a case of cervical small-cell neuroendocrine carcinoma in which the TP cervical smear lacked the characteristic nuclear molding and chromatin smearing ordinarily seen on CS.[10] The TP also closely mimicked squamous-cell carcinoma (small-cell type) and endometrioid adenocarcinoma. Hees and Lebeau compared TP and CS of mucoid bronchial washings and sputa and found that, in cases of small-cell carcinoma, tumor cells were "dissociated and often fewer in number than in conventional slides, where they are often attached in loose chains to mucus strands."[11] In an analogous fashion, differences in cytomorphology between TP and CS were observed in FNA of other tumors, most notably those of the breast and thyroid.[1,4,5,8,9]

As use of the ThinPrep technique gains popularity in the processing of FNA material, it becomes increasingly important for pathologists to be aware of cytologic alterations and potential diagnostic pitfalls. The classic cytologic features of small-cell carcinoma can be appreciated on TP; however, they are subtler and may require close attention to morphologic detail to be recognized. Due to the relatively small number of cases in this study, additional studies will be needed to confirm our findings.

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