Diagnosis of Melanoma Aspirates on ThinPrep®:
The University of Michigan Experience

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The purpose of this study was to compare the cytologic features of melanoma fine-needle aspirates (FNAs) prepared by ThinPrep® (TP) with those in conventional smears (CS) and to identify any diagnostic pitfalls. Fifty-one aspirates diagnosed as melanoma were obtained, 36 of which were prepared by both TP and CS. The preparations were evaluated for cellularity, cell aggregates, cellular appearance, melanin pigment, cytoplasmic, and nuclear features. Categorical data were analyzed by the chi-square test and continuous data by the Wilcoxin-signed rank test. Correlation was determined by Spearman's test for bivariate correlations (rho). Good correlation between the two methods was identified for the following features: cellularity, cell type, bi/multinucleated cells, cytoplasmic features, NC ratio, and presence of macronucleoli. TP exhibits coarser chromatin compared to CS ($P = 0.005$*). Six of 36 CS contained large cellular groups; none of the TP contained them (*$P = 0.018$*). Twenty-five of 36 CS contained intranuclear inclusions as opposed to 12/36 TP (*$P < 0.001$*). The number of inclusions was significantly reduced on TP. The amount of intracellular melanin was the same with both techniques. Background melanin was markedly reduced on TP except when either trapped by fibrin or attached to cellular clusters (*$P = 0.006$*). Background blood was also markedly reduced on TP (*$P < 0.005$*). In summary, the cytological features of TP and CS for FNA evaluation of melanoma correlate well; however, one needs to be aware of the cytologic alterations introduced by TP. TP is a sufficient preparation method in the diagnosis of melanoma FNA aspirates when performed by clinicians. It is also a useful adjunct in bloody or low-cellular aspirates, where it tends to reduce the background blood and concentrate the cells.* Diagn. Cytopathol. 2002;26: 334–339. © 2002 Wiley-Liss, Inc.

Key Words: melanoma; fine-needle aspiration; ThinPrep®

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Received 10 July 2001; Accepted 16 January 2002
Published online in Wiley InterScience (www.interscience.wiley.com). DOI 10.1002/dc.10099

Over the last 5 yr, ThinPrep® (TP), an automated slide processing technique, has been gaining popularity as a collection and preparation method. The technique requires collection of the specimen in a methanol-based solution (Cytyc, Marlborough, MA, Cytolyt™), preservation in a methanol-based preservative (Cytyc, PreservCyt™), and preparation of the slides by using a filter and a vacuum chamber resulting in a 20-mm circular deposit with evenly dispersed cells.[1]

In gynecologic cytology, TP has proven to give equal or superior performance when compared to conventional smears (CS).[2–7] However, the role of TP in nongynecologic cytology is not well defined and studies have shown that while they may correlate well with CS, experience and familiarity with the cytologic alterations introduced by the technique are essential.[8–17] In a previous study, we suggested that TP induces cytologic changes that may hamper the diagnostic accuracy in fine-needle aspirates (FNAs) of melanoma. The decreased detection of intranuclear inclusions and background melanin are among the most significant alterations.[9]

While melanoma is seldom aspirated for a primary diagnosis, it is a common source of FNAs in establishing a recurrent lesion. In many institutes where aspirates are primarily performed by the treating clinicians, the aspirate may be entirely rinsed in CytoLyt™ in an attempt to avoid the technical problems associated with inappropriate smear preparation.

The objectives of this study are: to compare the cytologic features of melanoma FNAs prepared by TP with those prepared by CS, and to identify any diagnostic limitations or pitfalls that may result from the above-mentioned cytologic alterations and define the usage of TP in diagnosing melanoma aspirates.

Materials and Methods

Fifty-one cases diagnosed as melanoma between July 1996 and September 2000 were identified from the files of the

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Table I. Site of Fine Needle Aspiration

Location	*Number*
Lymph Nodes	26
Lung	1
Soft tissues/skin	22
Parotid gland	2
Total	51

Pathology Laboratory at the University of Michigan. All of the cases were obtained by FNA. Table I summarizes the site of the aspirates.

Thirty-six of the FNAs were performed by cytopathologists and 15 were performed by treating clinicians. FNAs performed by cytopathologists were obtained with a minimum of four passes into the lesion. For each pass a drop is deposited on one slide and a CS is prepared by the two-slide pull technique. One slide is air-dried and stained with Diff-Quik for immediate interpretation; the other slide is alcohol fixed and stained with the Papanicolaou method. The needles were rinsed in CytoLyt™ for preparation of TP.[1] Only cases with at least one TP smear having adequate cellularity were included in this study; however, only 36 cases had corresponding CS. TP obtained by direct patient-to-vial technique without CS were used as a control group for the potential bias introduced by sample splitting. Although we reviewed all smears available, we performed our comparison and scoring on the fixed smears to avoid the additional bias of air-dried smears and to ensure homogeneity of preparations.

The TP and CS preparations were evaluated for multiple features: cellularity, cell aggregates (size, cohesiveness), cellular appearance (plamacytoid, spindled, multinucleation), melanin pigment (intracellular and extracellular), cytoplasm (dusty, vacuolation), and nuclear features (chromatin texture, nuclear cytoplasmic (NC) ratio, mitotic activity, intranuclear inclusions, nucleoli). All features were scored quantitatively or qualitatively. Categorical data such as cellularity (high vs. low) were analyzed by the chi-square test and continuous data such as the number of intranuclear inclusions by the Wilcoxin-signed rank test. Correlation was determined using the Spearman's test for bivariate correlations (rho).

Results

Diagnoses

Fifty of the 51 patients had a history of histologically confirmed melanoma and one was primarily diagnosed by FNA. A follow-up biopsy was available on 35 cases. The diagnoses of melanoma were confirmed in 34/35 cases (97%). On subsequent biopsy, one case diagnosed as melanoma on FNA revealed malignant epithelial neoplasm of neural crest origin.

Cellularity, Cellular Grouping, Cell Types

There was good correlation of cellularity between the two methods (rho = 0.59). Six of the 36 CS contained larger groups of melanoma cells (defined as occupying at least half of a ×40 field). TP, including the control group, had single and small clusters of cells ($P = 0.018$). (Fig. C-1A,B).

The cell types identified in the 36 CS are: plasmacytoid/epithelioid 20/36 (55.5%), spindled 6/36 (16.7%), and mixed cell type 10/36 (27.7%). The corresponding TP were in agreement except for one case, which was classified as spindled on CS and mixed cell type on TP. The number of bi/multinucleated cells per case had a good correlation between the two methods (rho = 0.64). The number of the giant cells ranged between 0–9 per case. Twenty-six of 36 CS, 23/36 corresponding TP, and 13/15 of the control group did not have any giant cells. Due to the paucity of giant cells, a significant correlation could not be determined.

Background

The loose melanin pigment in the background was markedly reduced in TP. The pigment was retained, however, when either trapped by fibrin or attached to clusters of melanoma cells ($P = 0.006$). The amount of intracellular pigment was retained in TP preparations and correlated well with CS. (Fig. C-2A,B). Similar to the reduction in background melanin, there was a reduction in the background red blood cells, lymphocytes, and necrotic debris ($P < 0.001$). Comparable findings were seen in the control group.

Cytoplasmic/Nuclear Features

The cells were well preserved and air-drying artifact was virtually absent on TP. Dusty cytoplasm was identified in 33/36 CS, 34/36 corresponding TP (rho = 0.78) and 14/15 control group. Vacuolated cytoplasm was identified in 2/36 CS, 1/36 correlating TP and 1/15 control group. In only 1/36 CS was clear cytoplasm identified. No clear cytoplasm was identified using TP. Both the NC ratio (rho = 0.64) and the presence of macronucleoli (rho = 0.78) correlated well between TP and CS. TP exhibited coarser chromatin compared to CS ($P = 0.005$). Twenty-seven of the 36 CS contained intranuclear inclusions, compared to 12 of the 36 TP ($P < 0.005$). The mean number of intranuclear inclusions were 4, 0.9, and 1 in the CS, TP, and control group, respectively (Fig. C-3A,B). Tables II, III, and IV show a detailed comparison between TP and CS.

Discussion

Melanoma is the seventh most common malignancy in the United States.[18,19] Regional metastasis, including satellitosis and regional lymph node involvement, are seen in two-thirds of patients.[20] Early detection of both primary and metastatic melanoma is important for initiating appropriate therapy, and for this purpose FNA provides a rapid and accurate tool for diagnosis. Frequently, such aspirates are

Table II. Comparison Between TP and CS — General Features

	Split samples (n = 36)			*Control Group (n = 15)*
Feature	*CS (n = 36)*	*TP (n = 36)*	*P* value	*TP alone*
Cellularity				
High	26 (72%)	15 (42%)	NS	9 (60%)
Low	10 (28%)	21 (58%)		6 (40%)
Cellular Aggregation				
Large clusters	6 (17%)	0 (0%)	0.018	2 (13%)
Small clusters and single cells	30 (83%)	36 (100%)		13 (87%)
Background				
Melanin present	15 (42%)	9 (25%)	0.005	1 (7%)
Blood/lymph present	31 (86%)	2 (11%)	<0.001	0 (0%)
Cell Type				
Plasmacytoid	20 (56%)	20 (56%)	NS	13 (87%)
Other	16 (44%)	16 (44%)		2 (13%)
Giant Cells				
Present	10 (28%)	13 (36%)	NS	2 (13%)
Absent	26 (72%)	23 (64%)		0 (0%)

performed by the treating physician when a new mass is discovered in a follow-up visit, especially in institutes where pathologists do not offer such services.

In such settings, where the clinicians predominantly perform the FNAs, collecting the material in CytoLyt™ is gaining popularity. There are two reasons for this. First, it is faster, easier, and it bypasses the need to train the clinical personnel to prepare their smears on site. Such smears were frequently limited by technical problems such as thick smears, poor cellular preservation, air-drying artifact, extensive smearing, and obscuring blood. Second, it affords the laboratory better control on the sample procurement and number of slides prepared. While many of the technical problems could improve through close communication between the laboratories and physicians, it has been the experience of many laboratories that such problems periodically recur due to turnover of staff and trainees.

While cytologic features of melanoma are well-defined on CS,[18,21–26] the effect of the cytologic alterations introduced by the TP method on such features and their influence on the diagnostic accuracy has not been evaluated to date. This is particularly significant when a diagnosis of recurrence is established based on TP.

We have reviewed TP and corresponding CS to identify any cytologic differences between the two techniques and to define the potential diagnostic problems/pitfalls of TP in the diagnosis of melanoma FNAs. Overall, TP and CS have a good correlation; however, a number of cytologic artifacts are introduced by TP. As previously noted,[8,17] large cellular clusters observed in CS were fragmented into smaller clusters in TP. Many studies have commented on the reduction of background blood, inflammatory infiltrate, and small loose particles such as melanin, hemosiderin, necrotic debris, etc., in TP.[2,8–11,17] We have also observed similar effects in our study. Loose melanin was lost unless entrapped by fibrin or attached to cell clusters. However, intracellular melanin was well preserved in the TP technique.

The frequency of intranuclear inclusions was markedly reduced by the TP. We postulate that this is most likely due to the balling-up effect as a result of wet fixation. We recently reported[9] a similar reduction of intranuclear inclusions in TP of aspirates of papillary carcinomas of the thyroid.

The cytoplasmic and the nuclear features remain essentially unchanged by the TP technique. Similar to our findings, Leung et al.[10] found that the NC ratio was preserved and cytoplasmic features were unchanged. They also noted that the chromatin patterns were enhanced and nucleoli were more prominent in TP. Many studies showed better nuclear detail with the TP technique.[8–13] We have not noticed any loss of cytological detail as described by Perez-Reyes et al.[17]; however, we did observe some nuclear shrinkage.

Several studies have reported reduced screening time with the TP technique.[14,15] While we have not specifically evaluated this aspect in our study, it is our experience that the lack of obscuring factors enhances screening and evaluation of the smear.

In summary, while it is best to have the aspirate performed by the pathologist and optimally prepared by the laboratory staff, this is not always the case and many institutes receive specimens as outside consultations or as part of outreach services, where onsite assistance is not possible. In the former situation, we recommend the performance of traditional smears. We routinely rinse our needles in CytoLyt and decide onsite whether we need a cell block, TP, or both. We elect a cell block if we have adequate material and/or we need further immunostains for confirmation. We elect TP only if the needle rinse is low in

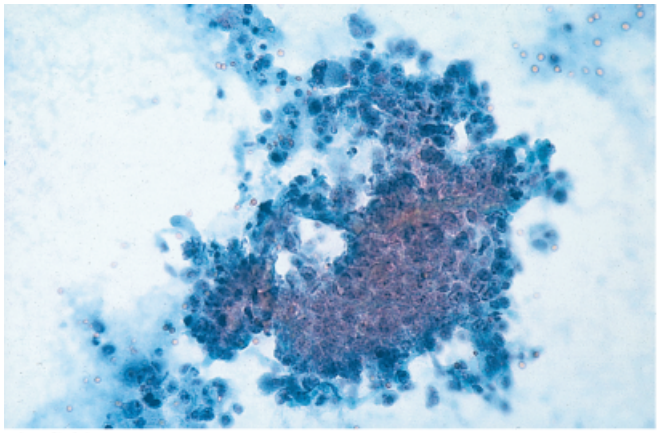

Fig. C-1A

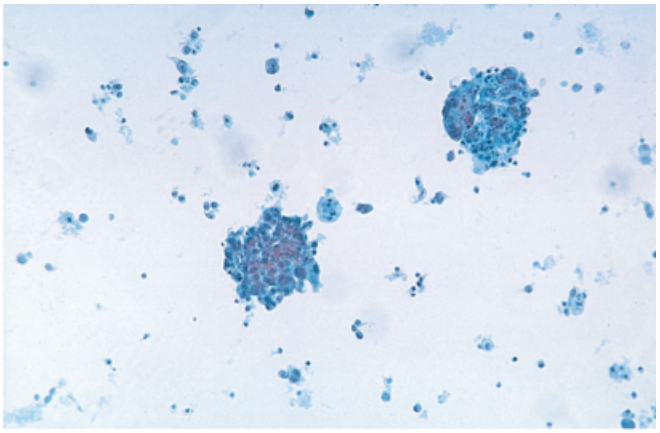

Fig. C-1B

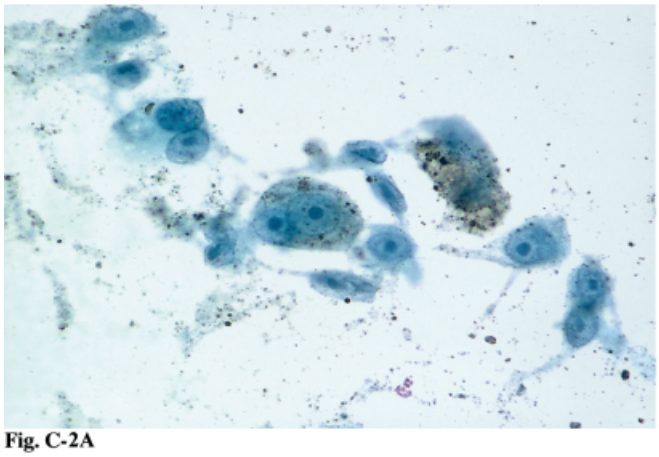

Fig. C-2A

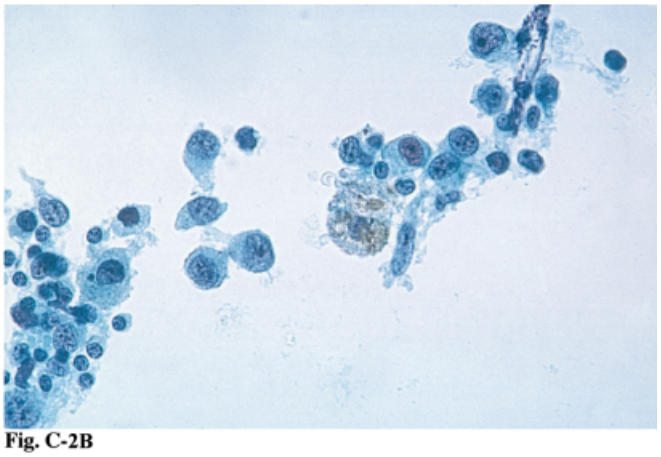

Fig. C-2B

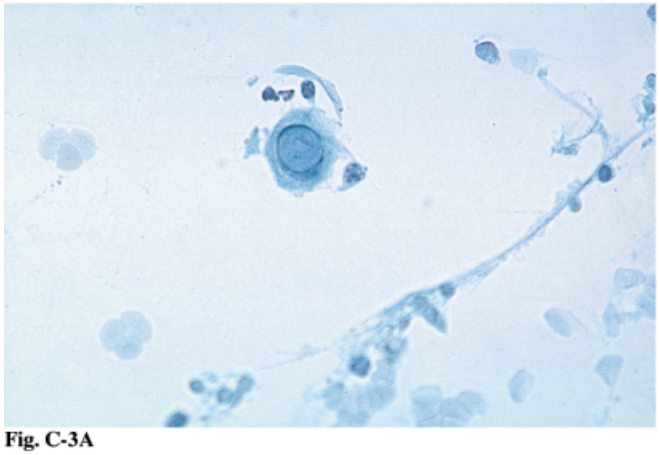

Fig. C-3A

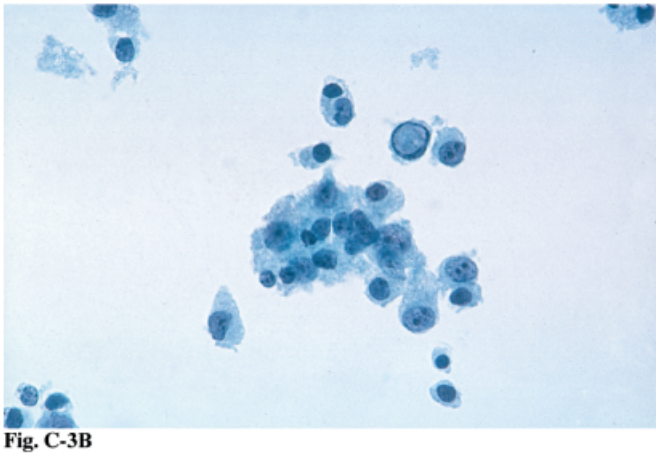

Fig. C-3B

Fig. C-1. **A**: Fine-needle aspirate of melanoma showing large cellular clusters in CS (Papanicolaou stain, ×320 magnification). **B**: Fine-needle aspirate of melanoma, showing small clusters and single cells on TP (Papanicolaou stain, ×160 magnification).
Fig. C-2. **A**: Fine-needle aspirate of melanoma showing loose background as well as intracellular melanin within melanocytes and histiocyte in CS (Papanicolaou stain, ×640 magnification). **B**: Fine-needle aspirate of melanoma showing the loss of background melanin, but retention of the intracellular pigment (Papanicolaou stain, ×500 magnification).
Fig. C-3. **A**: Fine-needle aspirate of melanoma showing intranuclear inclusions within a melanocyte. Many of these inclusions were seen in other areas of the smear (Papanicolaou stain, ×640 magnification). **B**: Fine-needle aspirate of melanoma showing a rare intranuclear inclusion within a melanocyte on TP (Papanicolaou stain, ×500 magnification).

cellularity and may not be adequately presented in a cell block. We occasionally perform both cell block and TP if the FNA is excessively bloody and would like to ensure a good Papanicolaou-stained slide.

Few studies utilized immunostains performed on TP slides; however, experience with such a methodology is limited and it is not yet a standard of practice. In addition, it is important to bear in mind that TP is not necessarily

Table III. Comparison Between TP and CS — Cytoplasmic and Nuclear Features

	Split sample (n = 36)			*Control group (n = 15)*
Feature	*CS (n = 36)*	*TP (n = 36)*	*P* value	*TP alone*
Multi/binucleated cells/smear				
< 5 cells	7 (20%)	7 (20%)	NS	5 (33%)
> 5 cells	29 (80%)	29 (80%)		10 (67%)
Cytoplasm				
Dusty	33 (92%)	33 (92%)	NS	14 (93%)
Vacuolated	2 (5%)	3 (8%)		1 (7%)
Clear	1 (3%)	0 (0%)		0 (0%)
Prominent nucleoli				
Present	28 (78%)	29 (80%)	NS	15 (100%)
Absent	8 (22%)	7 (20%)		0 (0%)
Nucleocytoplasmic ratio				
Increased	26 (72%)	29 (80%)	NS	13 (87%)
Chromatin				
Coarse	25 (69%)	34 (94%)	0.005	14 (93%)
Vesicular	8 (22%)	1 (3%)		0 (0%)
Fine	3 (8%)	1 (3%)		1 (7%)
Intranuclear inclusions				
Present	27 (75%)	12 (33%)	<0.005	3 (20%)
Absent	9 (25%)	24 (67%)		12 (80%)

Table IV. Summary: Differences Between TP and CS

Feature	*TP*	*CS*	*P* value
Cellular clustering	Smaller clusters and single cells	Larger clusters	0.018
Background melanin	Markedly reduced	Present	0.005
Background blood, lymphocytes, and necrotic debris	Markedly reduced	Present	<0.001
Nuclear chromatin	Coarser	Finer	0.005
Intranuclear inclusions	Reduced	Present	0.005

optimal for immunostaining of low cellular samples, as a limited number of slides would be obtained. This limitation is because the sample is consumed as the TP processor continues the application of vacuum and suction until either the filter is saturated or the sample is depleted.

We believe that TP alone is a sufficient preparation method in the diagnosis of melanoma FNA aspirates when performed without pathology assistance. However, the pathologist needs to be aware of the cytologic alterations introduced by the TP technique (Table IV) and take it into consideration, especially when TP is used alone, to avoid a misclassification. In cases of bloody and of low cellular aspirates, TP is recommended as a useful adjunct where it tends to reduce the obscuring background and concentrates the few cells in a relatively small area.

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