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Diagnostic Accuracy of Fine Needle Biopsy for Metastatic Melanoma and Its Implications for Patient Management

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Background: The use of fine needle biopsy (FNB) for the diagnosis of metastatic melanoma can lead to the early removal and treatment of metastases, reduce the frequency of unnecessary surgery, and facilitate the staging of patients enrolled in clinical trials of adjuvant therapies. In this study, the accuracy of FNB for the diagnosis of metastatic melanoma was investigated.

Methods: A retrospective cohort study was performed with 2204 consecutive FNBs performed on 1416 patients known or suspected to have metastatic melanoma. Almost three-quarters (1582) of these FNBs were verified by either histopathologic diagnosis following surgical resection or clinical follow-up.

Results: FNB for metastatic melanoma was found to have an overall sensitivity of 92.1% and a specificity of 99.2%, with 69 false-negative and 5 false-positive findings identified. The sensitivity of the procedure was found to be influenced by six factors. The use of immunostains, reporting of the specimen by a cytopathologist who had reported > 500 cases, lesions located in the skin and subcutis, and patients with ulcerated primary melanomas were factors associated with a significant improvement in the sensitivity of the test. However, FNBs performed in masses located in lymph nodes of the axilla and FNBs that required more than one needle pass to obtain a sample were far more likely to result in false-negative results.

Conclusions: FNB is a rapid, accurate, and clinically useful technique for the assessment of disease status in patients with suspected metastatic melanoma.

Key Words: Cytology—Diagnosis—Diagnostic accuracy—Fine needle biopsy—Melanoma—Pathology.

Fine needle biopsy (FNB) is frequently used in the diagnostic workup of clinically or radiologically detected mass lesions that are suspicious for metastatic melanoma. By determining whether they represent metastatic melanoma, the use of FNB in melanoma patients can expedite detection of metastases, leading to earlier removal and treatment; facilitate the staging of patients enrolled in clinical trials of adjuvant therapies (particularly in deep-seated lesions); reduce the frequency of unnecessary surgery; and assist in the planning of the most appropriate surgery.

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FNB is a swift, minimally invasive and cost-effective technique employed in the diagnostic workup of mass lesions occurring in a wide variety of organs.[1,2] The technique has been extensively evaluated in assessing the nature of lesions located in the tissues of the breast, thyroid, lung, liver, pancreas, lymph nodes, salivary glands, and kidneys, among other locations.[3–6]

There have been several previous case studies assessing the use of FNB in patients with melanoma. The majority of these studies lacked sufficient case numbers to precisely determine the diagnostic accuracy of FNB.[7–10] However, in 1986, Perry and colleagues[11] analyzed almost 300 FNBs from melanoma patients and found the procedure to be accurate, with a sensitivity of 86.5% and a specificity of 96.1%. Since this study, there have been important changes in the procedure, including improvements in immunochemical characterization and radiological guidance. In 2000, Voit and colleagues[12] published a study of 739 FNBs from melanoma patients with palpable suspicious lymph nodes or small lesions that were only detectable by ultrasound B-scan examination. Although the authors reported a sensitivity of 97.8% and a specificity of 100.0%, the sensitivity depended on lesion size.

The objective of this retrospective cohort study was to evaluate the diagnostic accuracy of the FNB procedure in the detection of metastatic melanoma. To accomplish this, a very large consecutive sample was collected of FNBs performed on melanoma patients who attended the Sydney Melanoma Unit (SMU), Sydney, Australia. This large sample also allowed evaluation of the effect of several clinicopathologic features and factors related to the procedure on the diagnostic accuracy of FNB.

MATERIALS AND METHODS

Patients

For all patients with melanoma who attended the SMU and gave informed consent, clinical and histologic details of their disease were recorded on the SMU database, and follow-up information was entered prospectively. Patients with suspicious clinically palpable or radiologically identified mass lesions, detected by a variety of imaging modalities, were further investigated by FNB. All SMU patients who underwent an FNB that had been reported by cytopathologists from the Department of Anatomical Pathology at the Royal Prince Alfred Hospital in Sydney, Australia, between January 1992 and December 2002 were identified from the SMU database, and their clinical records and FNB reports were reviewed. Details of the patients are provided in Table 1.

TABLE 1. *Patient characteristics*

Characteristic	n	%
Sex		
Male	888	62.7
Female	528	37.3
Age at melanoma diagnosis (y)		
10–30	110	7.8
31–40	135	9.5
41–50	256	18.1
51–60	303	21.4
61–70	302	21.3
70–80	219	15.5
81+	62	4.4
Unknown	29	2.0
Cancer diagnosis		
Other cancer diagnoses	159	11.2
More than one other cancer diagnoses	15	1.1
No. of primary melanoma lesions		
1	1236	87.3
2	148	10.4
≥3	32	2.3
No. of FNBs		
1	953	67.3
2	287	20.3
3	98	6.9
>3	78	5.5
Total no. of patients	1416	100.0

FNB, fine needle aspiration biopsy.

FNB Procedure

For palpable lesions, the FNBs were performed by the reporting cytopathologist or a trainee pathologist under their supervision. Following localization and stabilization of the lesion with one hand, a hollow bore needle (22G, 23G, or 25G) was inserted directly into the mass and the needle was moved swiftly in and out for approximately 10 seconds. Aspiration with a syringe was not used in the vast majority of cases, hence our preference for the term 'fine needle biopsy' and 'fine needle aspiration biopsy'. In our experience, the use of a needle without an attached syringe allows better control of the movement of the needle. Furthermore, aspiration often yields blood, promotes clotting and hampers optimal interpretation of cytologic detail. The procured material was ejected from the needle onto glass slides by pushing air from a syringe through the needle. The material was spread evenly across the slide using another glass slide. One slide was air-dried and stained immediately with Diff-Quik (Lab Aids, Narrabeen, NSW, Aus-

TABLE 2. *Categories of cytodiagnoses*

Characteristic	Total n	%	Confirmed	Not confirmed
Positive for Metastatic Melanoma				
Malignant cells—melanoma	1089	49.4	805	284
Suspicious for Metastatic Melanoma				
Malignant cells—suspicious for melanoma	44	2.0	39	5
Malignant cells—unknown cancer	33	1.5	22	11
Suspicious for malignancy	40	1.8	35	5
Negative for Metastatic Melanoma				
Malignant cells—other cancer	92	4.2	47	45
No malignant cells—other cells present	416	18.9	308	108
No malignant cells—scant other cells	296	13.4	204	92
No malignant cells—no other cells	175	7.9	122	53
No malignant cells—procedure not performed	19	0.9	0	19
Total	2204	100	1582	622

tralia) and another fixed in alcohol and later stained by the Papanicolaou method.[2] Residual material was washed into Hanks balanced salt solution for later preparation of cell blocks using the serum-prothrombin method[2] or by cytocentrifuge preparations, to be used for immunochemistry. The air-dried slides were examined by the cytopathologist at the time of the procedure. Further passes were performed if necessary, depending on the amount and type of cellular material obtained.

Review of Clinical Material and Follow-up

The accuracy of the FNB procedure in diagnosing metastatic melanoma was evaluated by two reference standards: a) histopathologic evaluation of the excised lesion (1120 cases) or b) follow-up in those cases for which histologic material was not available (462 cases). The duration of follow-up was 6 months or greater (mean 50.2 months, median 45.7 months, range 6.1–144.4 months) in 456 cases. In six cases, the length of follow-up was less than 6 months (mean 4.2 months, median 4.0 months, range 3.0–5.7 months). The mass was considered benign if it was stable in size or resolved after clinical follow-up.

Cytodiagnosis

Cytodiagnoses were categorized as positive, suspicious, or negative for metastatic melanoma (Table 2). Cases were considered positive for metastatic melanoma if the specimen included sufficient numbers of well-preserved malignant cells with typical cytological features, pigment, and/or confirmatory immunochemistry for a confident diagnosis of melanoma to be made. On verification, these samples were classified as truly positive (n = 800, 50.5%) or falsely positive (n = 5, 0.3%).

Samples that contained cells from unclassified/unspecified malignancies or cases categorized as suspicious for melanoma (those with small numbers of atypical cells, poorly preserved cells, and cells that lacked specific features of melanoma, such as cytoplasmic pigment, and where insufficient material was present for immunochemistry) were classified as suspicious for metastatic melanoma. These were determined to be true suspicious (n = 78, 4.9%) or falsely suspicious (n = 18, 1.1%) results after verification.

FNBs classified as negative for metastatic melanoma contained no material that could be diagnosed as metastatic melanoma. They included cases containing malignant cells diagnosed as another neoplasm or b) various amounts of cellular material from the tissue of the site that was sampled. After verification, these procedures were found to be either true negative (n = 612, 38.7%) or false negative (n = 69, 4.4%).

Statistical Analysis

All analyses were performed using the S-PLUS software package (Insightful Corporation, Seattle, WA) and Microsoft Excel, version 2000 (Microsoft, Redmond, WA). Diagnostic accuracy of FNB for metastatic melanoma was measured by the sensitivity and specificity of the test.

The effect of the type of tissue involved by melanoma, anatomic location, and 15 clinicopathologic and procedural factors were analyzed. These factors included features of the primary melanoma such as tumor thickness, dermal mitotic rate, presence of ulceration, predominant cell type and histopathologic subtype of melanoma; patient attributes such as sex, American Joint Committee on Cancer (AJCC)/International Union Against Cancer (UICC) stage,[13] age at FNB and location of the FNB (local or distant to the primary lesion); aspects of the FNB procedure

TABLE 3. *Fine needle biopsy procedures that could not be performed*

Location	Explanation for failure	Confirmation	Histology
Left neck	Unable to locate lesion	Negative—follow-up	–
Left face (subcutis)	Pain	Not confirmed	–
Right face	Unable to locate lesion	Not confirmed	–
Left neck (LN)	Unable to locate lesion	Negative—surgery	No evidence of malignancy
Right breast	Adjacent to prothesis	Negative—follow-up	–
Right neck	Pain	Not confirmed	–
Right axilla (LN)	Unable to locate lesion	Negative—follow-up	–
Right neck	Pain	Negative—surgery	No evidence of malignancy
Left neck (LN)	Pain	Negative—follow-up	–
Left neck (LN)	Unable to locate lesion	Negative—follow-up	–
Right groin	Pain	Negative—surgery	No evidence of malignancy
Left axilla (LN)	Unable to locate lesion	Positive—surgery	Melanoma
Right axilla (LN)	Unable to locate lesion	Negative—follow-up	–
Right neck (LN)	Pain	Not confirmed	–
Left axilla (LN)	Unable to locate lesion	Not confirmed	–
Left neck	Unable to locate lesion	Negative—follow-up	–
Thyroid	Unable to locate lesion	Negative—follow-up	–
Right axilla (LN)	Unable to locate lesion	Negative—follow-up	–
Right sternum (LN)	Unable to locate lesion	Not confirmed	–

LN, lymph node.

such as number of needle passes, needle size, the experience (based on caseload) of the reporting cytopathologist; year of procedure, use of immunostains; and presence of necrosis. Statistical significance was determined by the 95% confidence intervals (95% CIs) of these parameters. When comparing different samples, a two-sample test for binomial proportions was used. All equations used have been described elsewhere.[14,15]

RESULTS

In the 11-year study period, 2204 consecutive FNBs were performed in 1416 patients. Nineteen FNBs (0.9% of all FNB cases) were excluded from the analysis because the procedure could not be performed, i.e., no aspirate was obtained (Table 3). In half of these cases, the lesion could not be located (inappropriate referrals), but patient pain tolerance and difficulties with the procedure also contributed.

Verification of the remaining 2185 FNBs was partial; in 1582 (71.8%), the true disease status was confirmable by either histopathologic evaluation of the excised lesion or by clinical follow-up (Fig. 1). A total of 1120 FNBs were verified by histopathologic evaluation of the excised lesion; all confirmed positive FNBs and FNBs with false-positive or negative results were verified histologically. In those cases (mostly FNBs with negative results) where histopathologic material was not available (462 cases), verification was made by clinical follow-up. The mass was considered benign if it was stable in size or if it resolved after clinical follow-up. The duration of follow-up was >6 months in 456 cases and 3 to 6 months in 6 cases. Of the verified FNBs, 1435 (90.7%) were palpation-guided FNBs performed by trainee cytopathologists or cytopathologists, while 147 (9.3%) were image-guided FNBs performed by radiologists or other physicians.

A total of 603 FNBs (27.4%) could not be verified; this was due to loss to follow-up, particularly by death in patients with advanced metastatic melanoma, uncertainty regarding sites (where FNBs from more than one site were obtained at the same visit to the clinic), and where there were multiple metastases, which led to uncertainty in lesion location and the correlation of FNB and histopathologic results. Unconfirmed FNBs differed from confirmed procedures in several ways. They were more likely to be from lesions located in visceral organs or to be in patients with AJCC/UICC stage IV disease. FNBs with inconclusive cytodiagnoses were more likely to be followed up with clinical observation or further biopsy.

Confirmation of FNBs was unaffected by the year of procedure, the number of needle passes during sampling, the use of immunostains, the reporting cytopathologist, or the age or sex of the patient.

Approximately 12% of the patients were diagnosed with additional cancers (most commonly breast cancer, colorectal cancer, and chronic lymphocytic leukaemia), with some suffering from multiple types of other cancers. Multiple primary melanomas occurred

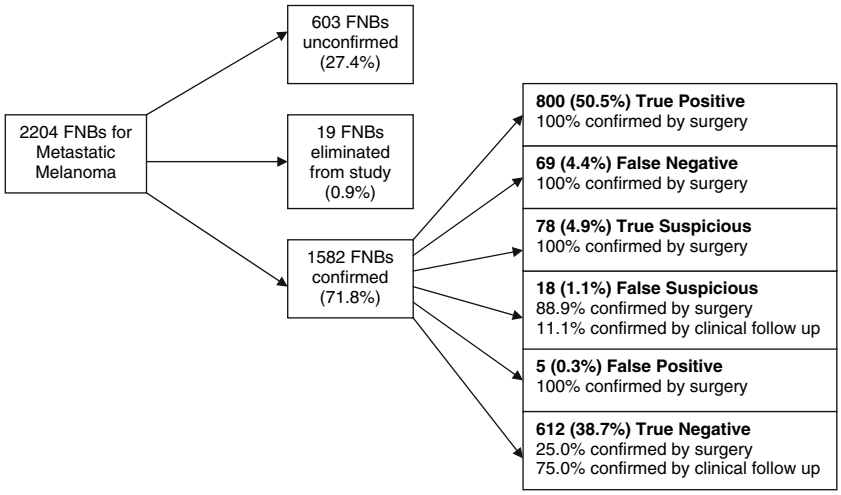

FIG. 1. Flow diagram showing fine needle biopsy (FNB) result distribution.

TABLE 4. *False-positive fine needle biopsy findings for metastatic melanoma*

Cytodiagnosis	Histology	Location	Comment
Melanoma	Metastatic adenocarcinoma	Right axillary (LN)	–
Melanoma	Metastatic papillary carcinoma	Left supraclavicular fossa (LN)	–
Melanoma	Hematoma	Left axilla	–
Melanoma	Chronic osteomyelitis	Left skull (bone)	S100 positive[a]
Melanoma	Metastatic adenocarcinoma	Right axilla	–

LN, lymph node.
[a]S100-positive histiocytes were identified in the excision specimen, which probably caused the misdiagnosis.

in an eighth of this patient population. Almost a third of the patients underwent multiple FNBs. These procedures were performed both concurrently and sequentially.

There were 1582 FNB procedures for metastatic melanoma with histologic verification or clinical follow-up. The overall sensitivity was 92.1% (95% CI, 93.7–90.0) and the specificity was 99.2% (95% CI, 99.7–98.1). Five cases were determined to be false positive, resulting in a false-positive rate of 0.6% (Table 4). The false-negative rate was 10.2%, with no metastatic melanoma identified in 69 FNB cases in which metastatic melanoma was identified by later histologic evaluation (Fig. 2).

The large numbers of confirmed cases permitted detailed analysis of this procedure for metastatic melanoma. The effect of FNB site was studied (Table 5). Lymph node tissue was the most common site for FNB evaluation, with 926 procedures, 753 of them confirmed. The sensitivity and specificity of FNB for metastatic melanoma in lymph nodes were not significantly different compared with that for all sites. However, the sensitivity for FNBs performed on lymph nodes located in the axilla was approximately 9% less compared with FNBs performed on

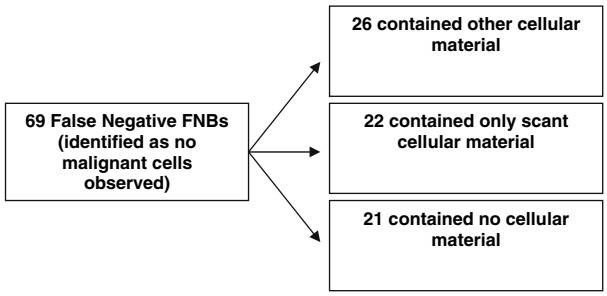

FIG. 2. Distribution of false-negative fine needle biopsy (FNB) cytodiagnoses.

lymph nodes in other locations ($z = -3.9$, $P = 0.0001$). Increased FNB sensitivity (by 4%) was found when the procedure was performed on lesions located in the skin and subcutis compared with other sites. However, this was only of borderline significance ($z = 1.9$, $P = 0.05$). Too few confirmed FNBs were conducted on visceral organs ($n = 79$) to allow conclusions to be drawn regarding the success of the procedure in these locations.

Fifteen clinicopathologic and procedural factors were analyzed to assess their effect on the diagnostic accuracy of FNB for metastatic melanoma (Table 6).

TABLE 5. *Diagnostic accuracy of FNB: Effect of tissue type and anatomic location*

Location	n	Confirmed	%	TP	FN	TS	FS	FP	TN	Sn	(95% CI)	Sp	(95% CI)
All FNB	2204	1582	71.8	800	69	78	18	5	612	0.92	(0.90–0.94)	0.99	(0.98–1.00)
Lymph nodes	926	753	81.3	413	43	41	7	3	246	0.91	(0.88–0.93)	0.99	(0.97–1.00)
Neck	235	185	78.7	104	3	15	4	1	58	0.97	(0.92–0.99)	0.98	(0.91–1.00)
Axilla	383	313	81.7	155	29	12	2	2	113	0.84[a]	(0.78–0.89)	0.98	(0.94–1.00)
Groin	274	233	85.0	144	11	11	0	0	67	0.93	(0.88–0.96)	1.00	–
Other	34	22	64.7	10	0	3	1	0	8	1.00	–	1.00	–
Skin and subcutis	711	504	70.9	270	17	26	4	0	187	0.94[b]	(0.91–0.96)	1.00	–
Head and neck	131	98	74.8	54	5	7	0	0	32	0.92	(0.82–0.96)	1.00	–
Trunk	288	196	68.1	101	4	6	2	0	83	0.96	(0.91–0.99)	1.00	–
Limbs	292	210	71.9	115	8	13	2	0	72	0.93	(0.88–0.97)	1.00	–
Visceral organs	176	79	44.9	30	3	5	2	0	39	0.91	(0.76–0.97)	1.00	–
Liver	56	22	39.3	7	1	1	0	0	13	0.88	(0.53–0.98)	1.00	–
Lung	94	43	45.7	18	2	4	1	0	18	0.90	(0.70–0.97)	1.00	–
Other	391	246	62.9	87	6	6	5	2	140	0.94	(0.87–0.97)	0.99	(0.95–1.00)

TP, true positive; FN, false negative; TS, true suspicious; FN, false suspicious; FP, false positive; TN, true negative; SN, sensitivity; SP, specificity; 95% CI, 95% confidence interval.

[a]Axilla lymph nodes had significantly reduced sensitivity compared with other sites ($z = -3.9$, $P = .0001$).

[b]Skin and subcutis FNBs had significantly increased sensitivity compared with other sites ($z = 1.9$, $P = .05$).

The FNB confirmation rate was similar for all clinicopathologic factors, with the exception of AJCC/UICC stage and location (distant or regional to the primary lesion), as described above.

Patients with an ulcerated primary lesion experienced slightly better FNB test sensitivity compared to those with no ulceration ($z = 1.9$, $P = 0.05$). AJCC/UICC stage, anatomic location, year of procedure, needle size, sex, age at FNB, primary lesion thickness, dermal mitotic rate, predominant cell type, histologic subtype of melanoma, and presence of necrosis did not affect the diagnostic accuracy of the test (Table 6).

Three factors related to the conduct of the procedure affected FNB test accuracy. These were the number of needle passes needed to collect the sample, the number of FNBs for metastatic melanoma the cytopathologist had reported during the study period (caseload), and the use of immunostains. Samples that required only one attempt at FNB had a sensitivity increase by >10% compared to samples with more than one attempt ($z = 4.8$, $P < 0.001$). Sensitivity fell in a linear manner with each subsequent attempt (Fig. 3). Cytopathologists who had caseloads of >500 FNBs (2 cytopathologists) had greater FNB test sensitivity by about 5% compared to those who had reported <500 FNBs (16 cytopathologists) ($z = 2.4$, $P = 0.02$). Finally, FNB tests in which immunostains were used had better sensitivity ($z = 4.8$, $P < 0.001$) (Table 6).

DISCUSSION

Metastatic melanoma is an aggressive tumor with a high mortality rate. Patients with primary melanomas that are at high risk of metastasizing who attend the SMU are followed closely for evidence of metastatic disease with radiological investigations and regular clinical follow-up. Early surgery for metastatic disease may lead to longer disease-free periods and may ultimately improve survival in some of these patients. This underscores the importance of early detection of metastatic disease.

In some melanoma treatment centers, FNB has been used for many years to verify clinically suspicious lesions and radiological abnormalities in patients with melanoma, prior to traumatic or costly surgical or adjuvant treatment. FNB is a cost-effective, rapid procedure that is well tolerated by patients when performed by proficient operators.[2] However, the ability of the test to accurately diagnose suspicious lesions as metastatic melanoma has been infrequently studied.

Sensitivity and specificity are measures that are used extensively in the FNB literature. They were chosen as the best measures of diagnostic accuracy of FNBs in patients with melanoma because of the binary quality of the data.[16] In this study, a large number of consecutive FNB procedures (2204) for metastatic melanoma were analyzed, with 1582 procedures confirmed by either histopathology after surgical resection or by clinical follow-up. This cohort represents more FNB procedures in melanoma patients than all previous reported series combined.[7–12] The overall sensitivity of FNB for metastatic melanoma was 92.1%, and the specificity was 99.2%. These results are superior to those obtained by Perry and colleagues[11] in their study of 298 (261 confirmed) cases of metastatic melanoma FNB more than 20 years ago (sensitivity of 86.5% and specificity

TABLE 6. *Diagnostic accuracy of fine needle biopsy effect of clinicopathologic factors*

Factor	N	Confirmed	%	TP	FN	TS	FS	FP	TN	SN	(95% CI)	SP	(95% CI)
All FNB	2204	1582	71.8	800	69	78	18	5	612	0.92	(0.90–0.94)	0.99	(0.98–1.00)
AJCC/UICC stage at FNB													
Stage I	400	323	80.8	128	9	22	6	3	155	0.93	(0.88–0.97)	0.98	(0.95–0.99)
Stage II	705	569	80.7	283	31	24	6	1	224	0.90	(0.86–0.93)	1.00	(0.98–1.00)
Stage III	836	561	67.1	310	24	27	5	1	194	0.93	(0.90–0.95)	0.99	(0.97–1.00)
Stage IV	263	129	49.0	79	5	5	1	0	39	0.94	(0.87–0.97)	1.00	(0.91–1.00)
Location													
Regional	1340	1077	80.4	617	58	58	6	4	334	0.91	(0.89–0.93)	0.99	(0.97–1.00)
Distant	864	505	58.4	183	11	20	12	1	278	0.94	(0.90–0.97)	1.00	(0.98–1.00)
Use of immunochemistry													
Yes	583	406	69.6	316	6	26	8	1	49	0.98[a]	(0.96–0.99)	0.98	(0.90–1.00)
No	1621	1176	72.5	484	63	52	10	4	563	0.88	(0.86–0.91)	0.99	(0.98–1.00)
Year													
1992–1994	392	285	72.7	144	15	15	5	1	105	0.91	(0.85–0.94)	0.99	(0.95–1.00)
1995–1997	555	414	74.6	216	15	13	6	1	163	0.94	(0.90–0.96)	0.99	(0.97–1.00)
1998–2000	693	500	72.2	241	21	26	3	2	207	0.92	(0.88–0.95)	0.99	(0.97–1.00)
2001–2002	564	383	67.9	199	18	24	4	1	137	0.92	(0.87–0.95)	0.99	(0.96–1.00)
Sex													
Male	1370	994	72.6	500	48	45	10	1	390	0.91	(0.89–0.93)	1.00	(0.99–1.00)
Female	834	588	70.5	300	21	33	8	4	222	0.93	(0.90–0.96)	0.98	(0.96–0.99)
Age at FNB													
≤50 y	572	414	72.4	198	12	22	1	2	179	0.94	(0.90–0.97)	0.99	(0.96–1.00)
> 50 y	1632	1168	71.6	602	57	56	17	3	433	0.91	(0.89–0.93)	0.99	(0.98–1.00)
No. of FNB attempts													
1	426	309	72.5	232	4	3	2	0	68	0.98[b]	(0.96–0.99)	1.00	(0.95–1.00)
2	518	371	71.6	185	13	21	3	0	149	0.93	(0.89–0.96)	1.00	(0.97–1.00)
3	315	219	69.5	79	13	12	6	0	109	0.86	(0.77–0.92)	1.00	(0.97–1.00)
≥4	192	136	70.8	33	17	21	1	1	63	0.66	(0.52–0.78)	0.98	(0.92–1.00)
Unknown	753	547	72.6	271	22	21	6	4	223	0.92	(0.89–0.95)	0.98	(0.96–0.99)
Needle size[c]													
22G	133	60	45.1	22	2	4	1	0	31	0.92	(0.74–0.98)	1.00	(0.89–1.00)
23G	173	138	79.8	66	7	8	2	0	55	0.90	(0.81–0.95)	1.00	(0.93–1.00)
25G	910	664	73.0	351	24	39	7	1	242	0.94	(0.91–0.96)	1.00	(0.98–1.00)
Necrosis present													
Yes	67	41	61.2	24	4	9	0	0	4	0.86	(0.69–0.94)	1.00	(0.51–1.00)
No	2137	1541	72.1	776	65	69	18	5	608	0.92	(0.90–0.94)	0.99	(0.98–1.00)
Pathologist caseload													
< 100 cases	164	104	63.4	51	7	5	4	0	37	0.88	(0.77–0.94)	1.00	(0.91–1.00)
100–500 cases	651	474	72.8	227	29	27	3	2	186	0.89	(0.84–0.92)	0.99	(0.96–1.00)
> 500 cases	1389	1004	72.3	522	33	46	11	3	389	0.94[d]	(0.92–0.96)	0.99	(0.08–1.00)
First primary Breslow thickness													
≤2 mm	950	705	74.2	332	23	34	8	4	304	0.94	(0.90–0.96)	0.99	(0.97–0.99)
> 2 mm	919	662	72.0	367	38	33	6	0	218	0.91	(0.87–0.93)	1.00	(0.98–1.00)
Unknown	335	215	64.2	101	8	11	4	1	90	0.93	(0.86–0.96)	0.99	(0.94–1.00)
First primary ulceration													
Yes	571	407	71.3	247	16	18	1	0	125	0.94[e]	(0.90–0.96)	1.00	(0.97–1.00)
No	1009	757	75.0	360	40	35	10	4	308	0.90	(0.87–0.93)	0.99	(0.97–1.00)
Unknown	624	418	67.0	193	13	25	7	1	179	0.94	(0.90–0.96)	0.99	(0.97–1.00)
First primary lesion mitotic rate (/mm^2)													
≤1	414	300	72.5	136	10	12	4	2	136	0.93	(0.88–0.96)	0.99	(0.95–1.00)
1 to < 4	481	352	73.2	170	19	20	2	1	140	0.90	(0.85–0.93)	0.99	(0.96–1.00)
4 to < 8	332	237	71.4	141	7	8	1	0	80	0.95	(0.91–0.98)	1.00	(0.95–1.00)
≥8	392	296	75.5	175	22	14	4	0	81	0.89	(0.84–0.93)	1.00	(0.95–1.00)
Unknown	585	397	67.9	178	11	24	7	2	175	0.94	(0.90–0.97)	0.99	(0.96–1.00)
First primary lesion histologic subtype													
Desmoplastic	130	97	74.6	42	6	6	3	0	40	0.88	(0.75–0.94)	1.00	(0.91–1.00)
SSM	512	370	72.3	168	10	22	4	1	165	0.94	(0.90–0.97)	0.99	(0.97–1.00)
NM	461	343	74.4	198	20	14	4	1	106	0.91	(0.86–0.94)	0.99	(0.95–1.00)
Other	754	549	72.8	277	26	22	4	1	219	0.91	(0.88–0.94)	1.00	(0.97–1.00)
Unknown	347	223	64.3	115	7	14	3	2	82	0.94	(0.89–0.97)	0.98	(0.92–0.99)

TABLE 6. *Continued*

Factor	N	Confirmed	%	TP	FN	TS	FS	FP	TN	SN	(95% CI)	SP	(95% CI)
First primary lesion predominant cell type													
Epithelioid	443	329	74.3	185	17	13	4	0	110	0.92	(0.87–0.95)	1.00	(0.97–1.00)
Spindle	137	89	65.0	47	6	9	0	0	27	0.89	(0.77–0.95)	1.00	(0.88–1.00)
Mixed	173	111	64.2	58	6	8	3	0	36	0.91	(0.81–0.96)	1.00	(0.90–1.00)
Unknown	1451	1053	72.6	510	40	48	11	5	439	0.93	(0.90–0.95)	0.99	(0.97–1.00)

TP, true positive; FN, false negative; TS, true suspicious; FN, false negative; FP, false positive; TN, true negative; SN, sensitivity; SP, specificity; 95% CI, 95% confidence interval; SSM, superficial spreading melanoma; NM, nodular melanoma.

[a]FNBs that used immunostains had significantly increased sensitivity compared with those that did not ($z = 4.8$, $P < .001$).

[b]FNBs obtained in one pass had significantly increased sensitivity compared with FNBs which required more than one pass ($z = 4.8$, $P < .001$).

[c]The size of the needle used for the FNB procedure was not known in 988 cases.

[d]FNBs reviewed by a cytopathologist who reported >500 cases had significantly increased sensitivity compared with FNBs reported by cytopathologists who had reported <500 cases ($z = 2.4$, $P = .02$).

[e]FNBs obtained from patients with an ulcerated first primary lesion had increased sensitivity compared with FNBs from patients without ulcerated first primary lesions ($z = 1.9$, $P = .05$).

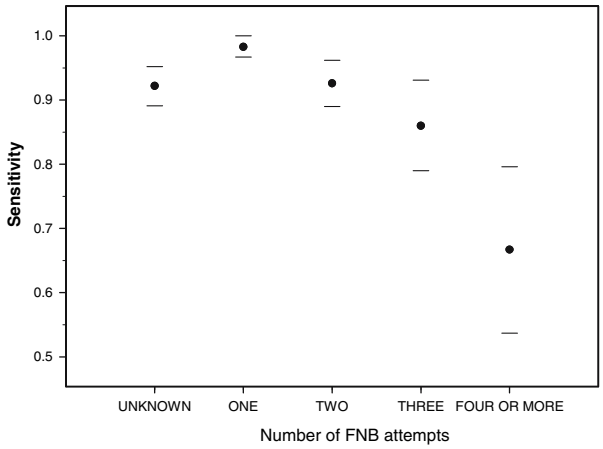

FIG. 3. Sensitivity of fine needle biopsy (FNB) in the diagnosis of metastatic melanoma. Sensitivity is reduced in a linear manner as the number of FNB attempts needed to obtain the sample increases. Bars, 95% confidence intervals.

of 96.1%). Other similar case series did not have the power to determine the true diagnostic accuracy of FNB because of their low numbers of FNBs (between 56 and 108 confirmed cases).[7–10] Voit and colleagues[12] examined 739 FNBs for metastatic melanoma and found the technique to be highly sensitive (97.9%) at their institution. However, regional node basins in their patient population were routinely evaluated by ultrasound B-scan, and a third of FNB cases were performed under ultrasound guidance.

Studies of FNB are frequently retrospective investigations and thus verification bias may be a hazard in these studies. Not only are there different reference tests, which are determined by the results of the FNB procedure, namely histopathology after surgical resection for all positive FNB tests and clinical follow-up for most negative procedures, but verification is also partial. Not every consecutive FNB can be verified. Differential verification can lead to overestimation of the measures of diagnostic accuracy.[17,18] This bias is related to the quality of the reference tests. Confirmation of melanoma metastases by histopathologic examination of tissue removed after surgery is better at identifying true disease status than clinical follow-up of patients with negative FNB results.[19] Melanoma metastases may regress spontaneously, resulting in failure to identify all false-negative results by clinical follow-up, or they may cause truly positive findings to be incorrectly classified as false positives if the lesion resolves before it is excised or during follow-up.[20,21]

In this study, there were differences in those FNBs that were verified by the reference tests compared with those that were not. Unconfirmed FNBs were more often performed on nonpalpable lesions in visceral sites, and in patients with advanced disease. Verification rates of negative, positive, and suspicious FNBs were 68.2%, 73.9%, and 82.1%, respectively.

Despite the high specificity rate, as a result of the large number of confirmed biopsy samples, five false-positive findings were detected (Table 4). The cytologic features of these cases have been reviewed in more detail elsewhere.[22] In all cases, misinterpretation of the cellular material had occurred, three of which were found to be adenocarcinoma. Two of these represented metastatic breast adenocarcinoma in axillary nodes, and one was a metastatic papillary adenocarcinoma of renal origin. Two other false-positive cases were caused by the misinterpretation of large histiocytes or reactive fibroblasts as metastatic melanoma cells.

In 69 FNBs, no metastatic melanoma cells were identified cytologically; however, the presence of

metastatic melanoma was subsequently identified by histopathologic examination (false negatives) (Fig. 2). Astute clinical and/or radiologic surveillance results in the detection of small suspicious lymph nodes. Difficulties in performing FNBs on such small suspicious lymph nodes contribute to some of the false-negative diagnoses (particulary small, mobile axillary nodes). In fact, approximately a third (n = 21) of these biopsy samples (which were reported as "no malignant cells identified") did not contain any cellular material, suggesting that an absence of sampling of the malignant cells was the reason for the false-negative result. Further investigation is usually necessary to identify the cause of the mass lesion. Surgical examination is usually the next step when the index of clinical suspicion is high.

There were several sources of difficulty in performing the FNB procedure for metastatic melanoma. Often it occurred when there was a failure to locate the suspicious lesion because of its small size or its location was not communicated with adequate precision. Failure to identify metastatic melanoma occurred when cellular material showed too few typical morphologic characteristics of metastatic melanoma, or these characteristics were destroyed or masked by necrosis.

The large number of confirmed FNB in this study permitted the analysis of subgroups. Most FNB for metastatic melanoma were performed in lymph nodes as well as skin and subcutaneous tissues. No difference in sensitivity was noted among these tissue groups. However, FNBs of lymph nodes of the axilla were significantly less sensitive compared with those performed at other sites and with FNBs performed in lymph nodes of the groin and neck. A number of factors are likely to contribute to the low sensitivity of FNBs performed in the axilla, including the greater difficulty in gaining access to and locating lymph nodes, particularly those high in the axilla, and the presence of large amounts of fatty tissue in axillary lymph nodes ("horseshoe" nodes).

Fifteen clinicopathologic factors were examined for their effect on the accuracy of FNB for metastatic melanoma, most of which had no effect on the diagnostic accuracy of the test. However, the following four variables did influence the sensitivity of the FNB procedure. (1) Ulceration of the primary lesion led to a small increase in the sensitivity of the test ($P = 0.05$). (2) Additional needle passes were performed if the initial sampling failed to obtain sufficient cellular material; thus, the number of needle passes is an indicator of the difficulty in obtaining a sample from a specific lesion. FNBs that were performed in only one needle pass were found to have superior sensitivity than those which required additional passes to obtain an adequate sample. Furthermore, the sensitivity of the test seemed to decrease in a linear manner with each subsequent pass. (3) Training and experience have been shown to significantly influence the interpretation of FNB of the breast,[23] and in this study, we found that the caseload (i.e., level of experience) of the cytopathologist who reviewed the slides influenced the sensitivity of the test. Those cytopathologists who reviewed >500 metastatic melanoma FNB samples in the study period performed better. Those cytopathologists who performed <100 reviews seemed to do as well as those who had a caseload of 100 to 500 cases. (4) The use of immunostains was associated with improved sensitivity of the test. However, this may reflect the fact that immunochemistry was only performed in those cases in which a sufficiently high cellular yield was obtained, in which case it may have been the high cellular yield that lead to an improvement in sensitivity, rather than the use of immunostains per se. The diverse cytological presentation of metastatic melanoma may also be a factor in the association of the use of immunochemistry with increased sensitivity.[24,25] Nasiell et al.[26] found that immunochemical characterization was necessary to conclusively diagnose $>50\%$ of metastatic melanomas that presented with an equivocal cytological picture. However, immunological characterization cannot be considered definitive when the FNB lacks typical cytologic features expected of metastatic melanoma. For example, one of the false-positive cases exhibited S100 positivity, whereas two of the false-negative FNBs contained melanoma cells that seemed to be S100 negative. This suggests that cytopathologists should be cautious when reporting the results of immunostains on limited samples.

Our study shows that FNB for metastatic melanoma is a procedure with very high specificity and good sensitivity. Several clinicopathologic factors were found to influence the diagnostic accuracy of the test for metastatic melanoma. These included factors relating to the original primary melanoma lesion, location of the sampled lesion, and factors relating to the performance of the test. The SMU employs an on-demand FNB service with assessment of the cytologic material and delivery of a provisional result to the clinician at the time of the patient's visit. This helps guide subsequent diagnostic and therapeutic measures, and it reduces costs (e.g., by decreasing the need for additional patient visits to the clinic). A multidisciplinary approach involving clinicians,

pathologists and radiologists enables an efficient and cost-effective management strategy in melanoma patients.

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