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Original Paper

The Clinical Use of Fine Needle Aspiration Cytology for Diagnosis and Management of Children with Neuroblastic Tumours

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This study presents the results of fine needle aspiration cytology in a series of 26 consecutive children with neuroblastic tumours. The cytological spectrum varied from undifferentiated small tumour cells to mature ganglion cells in a fibrillar background. In 24 children with neuroblastic tumours at onset the cytological diagnosis was correct in 21 cases, whereas two aspirates yielded nondiagnostic necrotic material and a fibrillar material without tumour cells, respectively. One necrotic lymph node aspirate was initially incorrectly diagnosed as lymphoma, but the diagnosis was later revised to neuroblastoma. Suspected signs of disease progression or relapses were confirmed ($n = 9$) or ruled out ($n = 1$) using aspiration cytolology. The diagnostic accuracy in the complete series was 97% (31/32) in cases with adequate smears. Immunocytochemistry confirmed the cytological diagnosis in 14 of 15 cases and was decisive in one. Elevated catecholamine metabolites in urine was detected in all children with a cytological diagnosis of neuroblastoma. General anaesthesia was only performed when coincidental invasive investigations ($n = 13$) were to be carried out or if the aspiration was intrathoracic ($n = 6$). It is concluded that aspiration cytology in conjunction with immunocytochemistry offers a safe, rapid and accurate diagnostic method which may be useful, together with analyses of catecholamine metabolites in urine, in the clinical management of children with neuroblastic tumours. © **1998 Elsevier Science Ltd. All rights reserved.**

Key words: neuroblastoma, ganglioneuroblastoma, ganglioneuroma, cytology, childhood cancer, immunocytochemistry
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INTRODUCTION

NEUROBLASTOMA, INCLUDING ganglioneuroblastoma, is the third most common malignant disease and the most common extracranial solid tumour in childhood [1]. Neuroblastic tumours are derived from primitive neuroectodermal cells (neuroblasts from the neural crest) normally forming the sympathetic ganglia, the adrenal medulla and the paraganglia of Zuckerkandl. The tumours may arise at any of these locations. The morphological subtypes neuroblastoma, ganglioneuroblastoma and benign ganglioneuroma correspond orderly to the spectrum of normal maturation of the sympathetic nervous system [2]. Ganglioneuroma is composed of mature ganglion and Schwannian cells organised with fascicles in stroma. Any degree of immaturity in this benign variant negates the diagnosis of ganglioneuroma. Neuroblastoma and ganglioneuroblastoma are the malignant variants of neuroblastic tumours and the most common subtypes, which belong to the small round cell tumours of childhood also including lymphoma, embryonal rhabdomyosarcoma and Ewing's sarcoma. The differential diagnosis of these tumours can be difficult using morphology alone. However, if the tumour cells are characterised with immunostaining, most cases can be accurately subclassified [3–5].

At the Karolinska Hospital, Stockholm, Sweden fine needle aspiration cytology (FNAC) has been used for many

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years to diagnose childhood tumours and we recently reported a high accuracy in diagnosing lymphomas and rhabdomyosarcomas [6, 7]. To our knowledge, there is only one previous report concerning the clinical utility of FNAC in the treatment of children with neuroblastoma [8] and none of children with ganglioneuroma. The aim of the present report is to describe the cytomorphology and immunocytochemistry of all three subtypes of neuroblastic tumours in FNA material. In addition, the role of FNAC for diagnosis and clinical management of neuroblastic tumours is described.

PATIENTS AND METHODS

Patients

During 1985–1994 a total of 52 children with neuroblastoma (including ganglioneuroblastoma) and 6 with ganglioneuroma were diagnosed at the Department of Paediatrics, Karolinska Hospital. In 26 children (13 girls), FNAC was used, according to clinical decision, in the diagnostic examination of primary disease ($n = 24$, pts 1–21 and 24–26, Table 1) and at suspected progressive disease ($n = 3$, pts 12, 23–24, Table 1) and at suspected relapses ($n = 6$, pts 1, 3, 9, 14, 19, 22, Table 1). Patients 22 and 23 had a primary histological diagnosis and were aspirated at relapse and progressive disease, respectively. 1 additional child, in whom FNAC correctly confirmed a relapse, was not included because of insufficient clinical information. The median age of these 26 children was 3.4 years (range newborn to 14.7 years). The median time with symptoms before attending the Karolinska Hospital was 1 month (range 1 day to 11.7 years). The signs or symptoms were abdominal mass ($n = 7$); tumour of the scalp ($n = 3$); orbital swelling ($n = 2$); multiple skin lumps ($n = 1$); local or diffuse pain ($n = 4$); coughing ($n = 6$); loose stools ($n = 3$); urinary incontinence ($n = 1$); and fever with ($n = 5$) or without ($n = 2$) other symptoms.

Out of 26 children in this series, 7 children (pts 2, 11–13, 23–25, Table 1) did not achieve complete remission and died after 1.2 years median follow up. 19 children achieved first remission and 14 of them are alive without disease (including all 5 with ganglioneuroma) after 1.6–9.5 years follow up (median time; 5.2 years). 11 (of these 14) children were in first and 3 in second remission. 8 children relapsed (pt 1 four times, pt 9 twice, pts 3, 7, 17, 19, 22 and 26 once). 5 of these 8 children are dead of disease.

Clinical investigations and staging

The children were investigated with selected imaging studies including X-ray, computerised tomography (CT), magnetic resonance tomography (MRT), ultrasonography and 99Tc bone scintigram. 10 patients underwent meta-iodobenzylguanidine (MIBG) scintigram [9]. All children but 1 infant with neuroblastoma (pt 2, Evan's stage = IVS) were evaluated for urinary catecholamine metabolites in 12-h urine collections (Table 1). Bone marrow biopsies and aspirates were obtained from 19 children (including most neuroblastoma but not all ganglioneuroma). Plasma concentrations of neuropeptide Y(NPY) were determined in all 22 children diagnosed after December 1986 [10]. The children were staged according to the Evan's system [11] (Table 1) and from 1989 also using the INSS criteria [12].

Fine needle aspiration cytology

Aspiration was performed by cytopathologists using the Franzen technique [13]. A 23 or 25 gauge needle (0.6 or 0.4 mm outer diameter respectively) was used. The smears were directly assessed and if the yield or quality was deemed suboptimal, additional aspirations were performed at the same occasion. The number of passes varied from 1–4. The cellular smears were air dried and stained with May–Grünwald Giemsa, or methanol fixed and stained using the Papanicolaou method [13]. FNAC was performed in 24 patients for primary disease within a median time of 1 day (range 0–35 days) after admission. Aspirations were made from primary tumours in the abdomen ($n = 9$), the thorax ($n = 7$) and pelvis ($n = 3$, transabdominally). In 5 children (pts 2, 3, 11, 13 and 14) with primary abdominal tumours, the aspirations were initially performed from metastases only (skin, skull, skull, inguinal lymph node and skull, respectively). In addition, four aspirates were made from suspected metastases in 3 children with progressive primary tumour disease (pts 12, 23 and 24). FNAC was also performed in 6 suspected relapses (pts 1, 3, 9, 14, 19 and 22) as described in Table 1. Thus, 34 aspiration procedures were accomplished (24 at initial work up and 10 at recurrent or progressive disease) from a total of 26 patients. Six smears from 6 children were used for image cytometric analysis of DNA content after Feulgen staining [14].

Immunocytochemistry

Aspirates from 15 patients (12 at initial work up) were used for immunological characterisation as previously described [15]. Briefly one part of the aspirated material was suspended in phosphate-buffered saline and cytospins prepared without washing the cells. Cytospin preparations were used to avoid heavy background staining. The air dried cytospins were fixed in acetone and stained by a three step alkaline phosphatase technique. The following available monoclonal antibodies (MAbs) (Dakopatts Ltd, Copenhagen, Denmark) were used: Vimentin Dako M 7020 1/200, cytokeratin, EMA Dako M 613 1/50, leucocyte common antigen Dako M701 1/100, desmin Dako M 760 1/50 and MIC-2 Dako 12 E7. Polyclonal antibodies against NSE Dako M 873 1/50 and S 100 Dako Z 311 1/500 were also used.

Histology

Surgery was performed in 25 children on 32 occasions. Surgical material was fixed in formalin and stained with haematoxylin eosin for routine histological classification. Material was also used for immunohistochemistry.

Cytomorphological classification

The neuroblastic tumours in this series were retrospectively classified cytologically into neuroblastoma, mixed neuroblastoma and ganglioneuroma according to Joshi and coworkers [2]. In neuroblastoma, neuroblasts constitute the exclusive cellular component of the tumour and there is minimal stroma. In ganglioneuroma, the exclusive cells are mature ganglion cells with neurites and mature Schwann cells. There is also a rich stroma component. Any degree of immaturity negates ganglioneuroma. In mixed neuroblastoma the tumour is composed of a mixture of mature and immature cells [2].

Treatment

The patients were treated with surgery, chemotherapy and radiotherapy based on clinical stage and age according to national and international protocols. 1 patient in primary disease (pt 13, Table 1) was palliatively treated only.

Table 1. Diagnosis, treatment and follow-up

Patient	Age (yrs)	Primary site	Stage*	Catecholamines†	FNAC site diagnosis‡	First treatment§	Histology	Clinical follow-up	Morphological diagnosis of Rel/PD	Outcome/ follow-up time
1	6.3	THO	I	- -+	PT/MNB	Surg	MNB	Rem/4 Rel, all at prim site	Nr 1:FNAC = MNB Nr 2:Hist = MNB Nr 3:Hist = MNB Nr 4:Not done	DOD/7.9y
2	Newb	ABD	IVS	000	Skin/NB	Surg	NB	No Rem	Not done	DOD/7 w
3	1.9	ABD	IV	+++	Bone/NB	Cyto	MNB	Rem/1 Rel, Skel	FNAC = NB	DOD/2.6 y
4	8.5	THO	I	+- -	PT/GN	Surg	GN	Rem		NED/9.5 y
5	5.6	THO	I	- - -	PT/GN	Surg	GN	Rem		NED/9.3 y
6	1.5	THO	I	++-	PT/GN	Surg	GN	Rem		NED/9.2 y
7	0.4	ABD	IVS	++-	PT/NB	Cyto	NB	Rem/1 Rel, Prim Site	Not done	DOD/0.9 y
8	5.9	THO	I	- - -	PT/GN	Surg	GN	Rem		NED/8.6 y
9	5.0	ABD	IV	+++	PT/NHL	Cyto	MNB	Rem/2 Rel, all at primary site	Nr 1:Hist = NB Nr 2:FNAC = NB	DOD/2.2 y
10	4.8	PEL	III	+++	PT/MNB	Cyto	GN	Rem		NED/7.7 y
11	5.7	ABD	IV	+++	Scalp/NB	Cyto	NB	No Rem	Not done	DOD/2.0 y
12	6.1	ABD	III	+++	PT/NB	Cyto	MNB	No Rem	Prim site:Hist = NB Neck:FNAC = NB	DOD/1.9 y
13	5.1	ABD	IV	++0	LN/NB	Pall	Not done	No Rem	Not done	DOD/0.1 y
14	3.4	ABD	IV	+++	Scalp/NB	Cyto	NB	Rem Rel?	FNAC = adenitis	NED/5.4 y
15	Newb	PEL	II	++0	PT/MNB	Surg	MNB	Rem		NED/5.3 y
16	14.7	THO	II	+-0	PT/IS	Surg	GN	Rem		NED/5.1 y
17	8.5	ABD	II	++0	PT/MNB	Surg	MNB	Rem/1 Rel, LN	Hist = MNB	NED/4.8 y
18	0.6	PEL	III	++0	PT/NB	Surg	NB	Rem		NED/4.8 y
19	11.4	ABD	IV	++0	PT/MNB	Cyto	MNB	Rem/1 Rel, liver	FNAC = NB	DOD/0.7 y
20	2.5	ABD	III	++0	PT/MNB	Surg	MNB	Rem		NED/4.2 y
21	1.0	ABD	III	++0	PT/NB	Cyto	NB	Rem		NED/3.6 y
22	Newb	ABD	IVS	+++	Not done	Surg	NB	Rem/1 Rel, skin	FNAC = NB	NED/2.9 y
23	0.7	ABD	IV	+00	Not done	Surg	NB	No Rem	Skin:FNAC = NB	DOD/0.8 y
24	1.2	ABD	IV	-++	PT/NB	Cyto	NB	No Rem, sinus LN	FNAC = NB FNAC = NB	DOD/1.2 y
25	1.3	ABD	IV	++-	PT/NB	Cyto	NB	No Rem	Not done	DOD/0.7 y
26	2.4	THO	II	- -0	PT/Necrotic material	Surg	NB	Rem/1 Rel, prim site	Hist = NB	NED/1.6 y

*Stage according to Evans' system [11]; †Cat = Catecholamines in the following order: Dopamine, Homovanilic Acid and Vanillylmandelic Acid, + = above the upper 3 Sd level according to age, - = normal and 0 = not done; ‡Metastatic sites are given; §First treatment after initial aspiration procedure.

Newb, newborn; Prim, primary; THO, thorax; ABD, abdomen; PEL, pelvis; FNAC, fine needle aspiration cytology; PT, primary tumour; LN, lymph node; IS, insufficient material; NB, neuroblastoma; GN, ganglioneuroma; MNB, mixed neuroblastoma according to Joshi and colleagues [2]; NHL, non-Hodgkin's lymphoma; Surg, surgery; Cyto, cytostatics; Pall, palliative treatment; Hist, histology; Rem, remission; Rel, relapse(s); No Rem, no remission; Skel, skeletal; PD, progressive disease; NED, no evidence of disease; DOD, dead of disease; y, years; w, weeks.

Sedation

Local skin application of anaesthetic ointment or short general anaesthesia mostly without tracheal intubation was used.

RESULTS

Cytomorphology and immunocytochemistry

Smears from all 13 cases (11 at primary diagnosis) of neuroblastoma often showed the tumour cells arranged in loose sheets, although single cells were frequent (Figure 1). In Giemsa stains the individual cells were oval to round, had a scanty, fragile cytoplasm which stained light blue to grey and a slightly irregular nucleus with granular chromatin and lacked nucleoli in most cases. Mitotic karyorrhectic cells were seen (Figure 1a). In clusters, the cells were occasionally separated by a fibrillary material which stained light grey-blue to pink (Figure 1c). Fibrillary material and pseudorosettes (Homer–Wright rosettes) were rarely seen in direct smears (Figure 1d). However, in Giemsa stained cytospin preparations fibrillary material was easily observed in all (Figure 2a) but one case and pseudorosettes in 6 of the 14 examined (Table 2; Figure 2b). We therefore found that Giemsa stained cytospin material could be of great value in the diagnostic examinations but not sufficient as the only diagnostic modality.

Mixed neuroblastoma (ganglioneuroblastoma) often yielded cellular smears which presented three different cell types: neuroblasts, mature ganglion cells and intermediate variants (Figure 3). The neuroblasts were identical to those found in neuroblastomas. The ganglion cells had abundant well-defined granular cytoplasm and excentric round nuclei with prominent nucleoli. Some ganglion cells were binucleate or multinucleated (Figure 3). The intermediate cells varied in size from that of the neuroblast to the mature ganglion cell. They often presented a large granular cytoplasm. Smears from ganglioneuroma were sparse and showed a few mature ganglion cells in a background of fibrillar fragments which stained pink in Giemsa and a protein precipitate (Figure 4). Few intermediate cells were found while neuroblasts were lacking.

Immunocytochemistry of tumour cells in neuroblastoma ($n = 8$) and mixed neuroblastoma ($n = 7$) (Table 2; Figure 5) showed positivity for NSE in 12/15 (80%), S-100 in 5/8 (63%) and vimentin in 8/13 (62%), but were mostly negative for epithelial, lymphoid and myogenic markers. Aspirates from ganglioneuroma gave such a typical cytological picture that no immunocytochemistry was necessary to corroborate the diagnosis.

Diagnosis of primary disease

In 21 children presented in Table 1 with primary disease (pts 1–8, 10–15, 17–21, 24 and 25) a diagnosis of neuroblastoma ($n = 11$), mixed neuroblastoma ($n = 6$) and ganglioneuroma ($n = 4$) was made using cytomorphology alone or in combination with immunocytochemistry ($n = 13$). However, immunostaining was decisive for diagnosis in 1 child (pt 13). Catecholamine metabolites in urine were elevated in all 16 children cytologically diagnosed as neuroblastoma (neuroblastoma and mixed neuroblatoma, Table 1), but only in 2 of

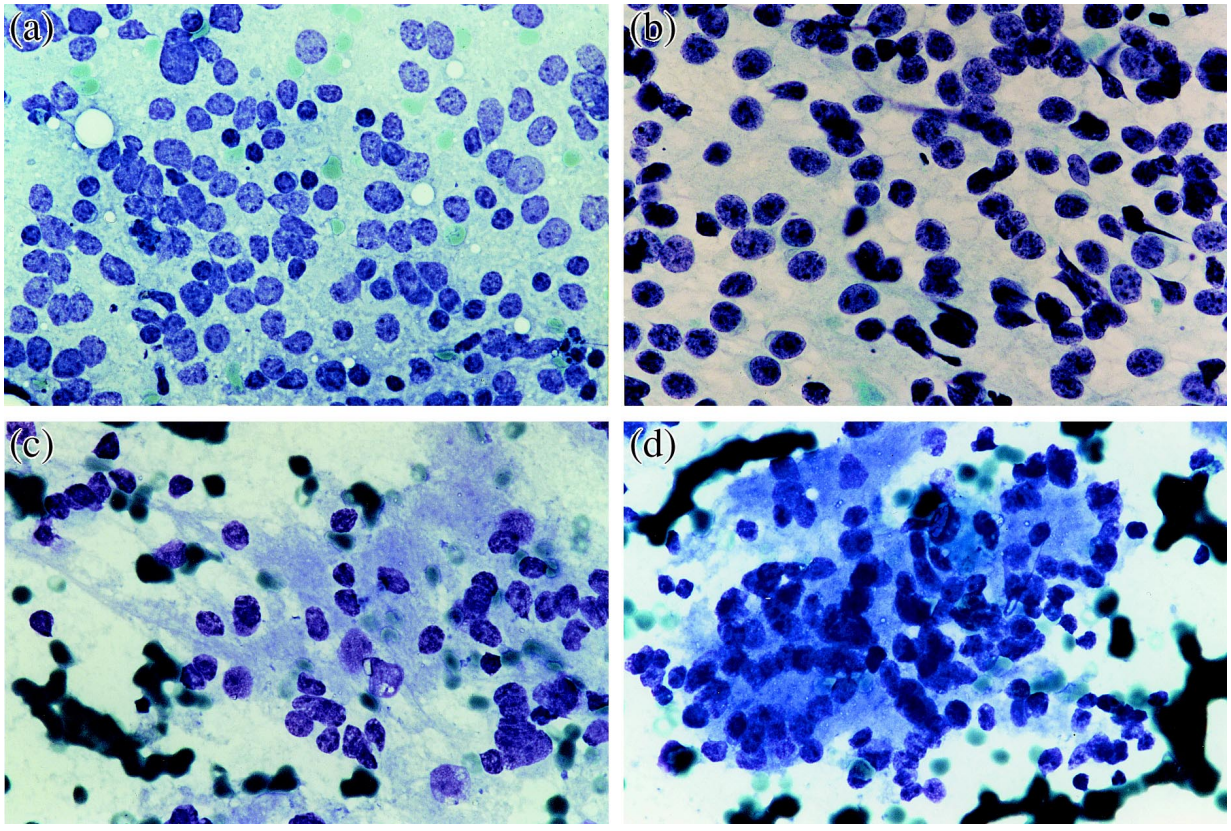

Figure 1. FNA smear from neuroblastoma showing mostly dispersed small cells with round or oval nuclei with scanty cytoplasm stained with May-Grünwald Giemsa (a, ×160) and Papanicolaou technique (b, ×160). A mitotic karyorrhectic cell is seen in the right hand lower corner of (a); (b) neuroblastoma cells with cytoplasmic processes and fibrillar material (May-Grünwald Giemsa; ×160); (c) neuroblastoma cells with round or oval nuclei forming pseudorosettes around fibrillar material (May-Grünwald Giemsa; ×160).

Table 2. Immunocytochemistry and cytospin findings in children with neuroblastoma and mixed neuroblastoma

Immunomarkers								Cytospin findings	
Subtype positivity	LC	CK	Vim	Desmin	NSE	S-100	Mic-2	Neuropil	Rosettes
Neuroblastoma ($n=8$)	0/8	0/6	5/8	0/8	7/8	1/3	0/2	7/8	2/7
Mixed neuroblastoma ($n=7$)	1/3	0/1	3/5	0/4	5/7	4/5		7/7	4/7
Total ($n=15$)	1/11	0/7	8/13	0/12	12/15	5/8	0/2	14/15	6/14

LC, leucocyte common antigen (CD45); CK, creatine kinase; Vim, Vimentin; NSE, neurone specific enolase.

4 children with ganglioneuroma (P 0.03, Fisher's exact test). These results, together with the clinical stage of disease and age, directly influenced the selection of therapy. 10 children with resectable tumours diagnosed as ganglioneuroma (pts 4–6, 8), neuroblastoma (pts 2, 18) and mixed neuroblastoma (pts 1, 15, 17, 20) underwent surgical excision of the primary tumour. Histology confirmed the pre-operative cytological diagnosis.

In 10 children with primarily nonresectable tumours with the cytological diagnosis of neuroblastoma (pts 3, 7, 11, 12, 14, 21, 24 and 25) and mixed neuroblastoma (pts 10 and 19), chemotherapy was started within 2 days after the aspiration procedure (range 0–6 days). The tumours responded to therapy in all but 1 child (pt 10). Surgery was performed after a median time of 3 month following cytostatic therapy, and the primary tumours could be excised radically in 7 and partially in 2 (pts 11 and 14). In 1 child (pt 25) the tumour had decreased in size but was inoperable and an incisional biopsy was made. In all but 1 (pt 10) histological diagnosis confirmed the cytological diagnosis of neuroblastic tumour subtype. In patient 10 (Table 1) 2.5 months of pre-operative chemotherapy had no measurable effect on tumour size. After removal of the tumour, the histological examination suggested it to be a ganglioneuroma. The neuroblastic cells described in the pretreatment aspirated material could not be found. Patient 13 (Table 1) had an inoperable stage IV tumour which was diagnosed as neuroblastoma on aspirated cells. He received palliative treatment only, and died 1 month after diagnosis. No autopsy was performed.

In patient 9 (Table 1) masses in the left fossa supraclavicularis and abdomen were initially aspirated. Cytomorphology of this mostly necrotic material was interpreted as high grade non Hodgkin's lymphoma, likely B cell derived since occasional cells were positive for B-cell markers. Cytostatic

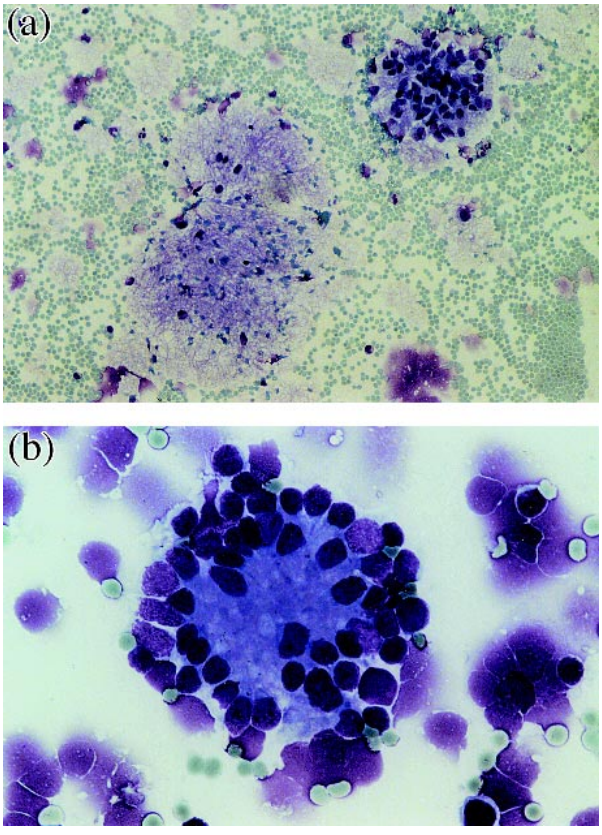

Figure 2. Cytospin preparation of cell suspension of aspirate from neuroblastoma showing tufts of fibrillary material (a) MGGx40 and pseudorosette around neuropil (b) MGG×160.

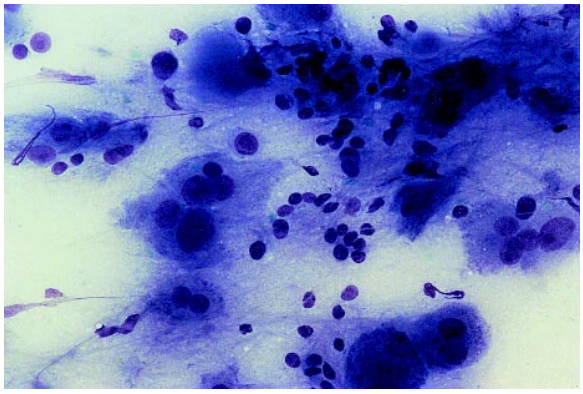

Figure 3. FNA smear from mixed neuroblastoma showing neuroblasts and mature ganglion cells with abundant cytoplasm and excentric nuclei with prominent nucleoli (MGG×80).

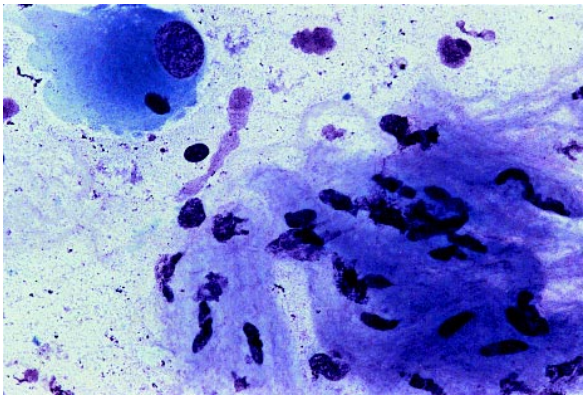

Figure 4. FNA smear from ganglioneuroma presenting a ganglion cell with abundant cytoplasm and excentric nuclei and fragment of fibrillary material with spindle shaped cells from neuroma component (MGG×160).

therapy according to a lymphoma protocol was started but the tumour showed progression. Elevated plasma NPY and urinary catecholamine metabolites were noted. The cytological material was reviewed and a diagnosis of neuroblastoma was suggested. A surgical biopsy of the supraclavicular metastasis revealed a NSE-positive mixed neuroblastoma. This child died after a second relapse confirmed by FNAC (Table 1).

Patient 16 was admitted at 14 years of age with an asymptomatic thoracic tumour detected at 3 years of age following an X-ray due to a heart murmur. Multiple aspirations of the tumour only yielded fibrillar material without tumour cells. Histology after surgical excision revealed a ganglioneuroma. The patient is alive with no evidence of disease 4.8 years after surgery.

A paravertebral tumour in the left and apical part of the chest cavity in a 2-year old girl (pt 26) was aspirated twice but only necrotic cells were found. Surgical excision of the tumour was performed and histology showed over 90% necrotic tissue with few small clusters of neuroblastic cells. Urinary excretion of dopamine and HVA was normal as was NPY in plasma. She was in stage I (according to Evans) and received no further therapy. 7 months later she experienced a local relapse. A second surgical excision was followed by chemotherapy. At follow-up 1.6 years later she was in continous second remission.

During the study period, 1 child was 'falsely' diagnosed with neuroblastoma. A differential diagnosis of Ewing's sarcoma by cytology was shown to be correct.

Diagnosis of progressive disease at primary and metastatic sites

Subsequently progressive disease was confirmed at primary site by surgery (1 occasion, pt 12) and at metastatic sites by FNAC (4 occasions, pts 12, 23 and 24), (Table 1).

Diagnosis of recurrent disease

In 9 children (Table 1), there were 13 suspected relapses. FNAC was performed in 6 patients (pts 1, 3, 9, 14, 19 and 22) and a diagnosis of recurrent tumour was made in all except patient 14. This resulted in chemotherapy in 2 children (pts 1 and 22), and palliative treatment in 3 (pts 3, 9 and 19). 1 boy (pt 1) had three more local thoracic relapses for which surgical excision was made in two and confirmed relapses. Surgery also confirmed local relapse in 2 other patients (pts 9 and 26). For two relapses (pt 1 in fourth and pt 7 in first remission) no morphological confirmation was revealed.

Patient 14 (Table 1) was on retinoic acid therapy at first remission when she presented an enlarged lymph node, which clinically indicated a relapse. The lymph node was aspirated and cytology showed a reactive lymphadenitis. This diagnosis was corroborated by immunocytochemistry.

The use of general anaesthesia at the aspiration procedure

For three of the 34 aspiration procedures insufficient information an anaesthesia was available. For 12 of the 31 procedures (6 of 13 transabdominal and 6 of 10 superficial aspirations) no general anaesthesia was used. In 11 of these children, no further coincidental invasive investigation was carried out. In the other 19 FNAs, short general anaesthesia was used in all thoracic cases ($n = 6$) and in 13 patients where painful other investigations were also to be carried out. Tracheal intubation was generally not used and the child was awake within 15 minutes after finishing invasive procedures.

Side-effects of fine needle aspiration

There were no immediate or late local or systemic complications noted in the 25 children undergoing FNAs. The aspiration did not compromise any other procedure. There were no signs of seeding in the needle track in any of the children.

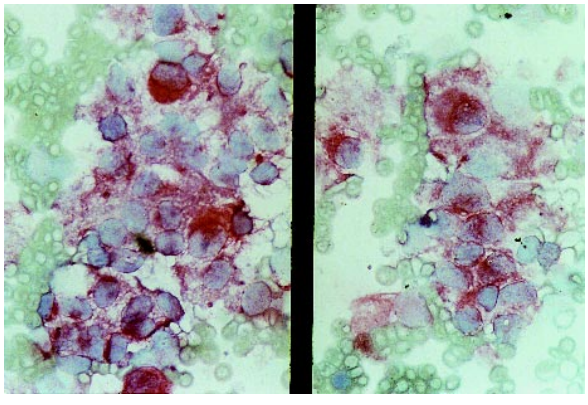

Figure 5. Immunocytochemistry on cytospin preparation of aspirate from neuroblastoma showing vimentin (right) and NSE (left) positivity (alkaline phosphatase×160).

DISCUSSION

The most common malignant childhood tumours are the small round cell type which may be difficult to subtype on cytology and histology. There are, however, several reports on the usefulness of FNAC in this field [3, 4, 6, 7, 16, 17]. Electron microscopy and immunocytochemistry have been shown to aid cytomorphology in the correct subclassification of these tumours [3–6]. Some reports have described cytomorphology of neuroblastoma [2, 18, 19]. In the present study, we had an overall diagnostic accuracy of 91% (31/34) and 97% (31/32) in cases with sufficient cellular material. For an optimal cytological evaluation of the smears, we found it was important to avoid a mixture of peripheral blood and, when this was possible, one smear was sufficient for diagnosis. In 1 patient (pt 9 Table 1), a necrotic aspirate from a lymph node was diagnosed as lymphoma, mainly based on the identification of few intact lymphoid cells using immunocytochemistry. The lack of response to chemotherapy and increased catecholamines led to review of the aspirated material and the diagnosis was changed to neuroblastoma. This diagnosis was confirmed on surgical biopsy material. Two cases had insufficient material due to almost acellular aspirates from 1 patient (pt 16) with a ganglioneuroma and necrotic material from a case (pt 26) of neuroblastoma. Ganglioneuromas may show only few ganglion cells in a fibrillar stroma. Obviously the few diagnostic cells may be missed using either FNA biopsy or an incisional biopsy.

It is noteworthy that Giemsa stained cytospin preparations from aspirated cells made it possible to reveal fibrillary material (neuropil) in all but one case of the most undifferentiated neuroblastic tumours (neuroblastoma, $n = 11$ and mixed neuroblastoma, $n = 4$). Cytology, immunocytochemistry and catecholamines identified this case as a true neuroblastoma. These fibrillary features could rarely be seen in direct smears in these most immature neuroblastoma which is in agreement with other reports [18, 19].

The use of immunocytochemistry on cytospin preparations confirmed the cytological diagnosis in all but the case (pt 9) which was erroneously diagnosed as lymphoma. This mistake, however, occurred at the beginning of our experience with immunocytochemistry. Currently, we use immunocytochemistry to confirm our cytological diagnosis which, in the case of most neuroblastomas, can be accurate using cytomorphology alone. Basically, differentiating neuroblastoma and ganglioneuroblastoma (in this study both designated as mixed neuroblastoma) are generally easy to diagnose with typical ganglion cells intermixed with small round cells of varying maturation in direct smears and nearly always with a fibrillary background in cytospin preparations. In undifferentiated neuroblastoma with homogenously small round cells, immunostaining can be essential for separation from other small round cell tumours of childhood. This can be accomplished with a rather limited panel of antibodies such as those directed to LCA (CD45), desmin, myoglobin, NSE and MIC-2. In this study, we found that immunocytochemistry was decisive in only one case (pt 13). One interesting finding in this study is that immunostaining showed vimentin positivity in 62% (8/13). The positive cells were few in most cases. Formalin fixed paraffin embedded tumour tissue from neuroblastoma are considered to be vimentin negative which distinguishes them from Ewing's sarcoma [1, 3, 20, 21]. This discrepancy is likely to be explained by a higher sensitivity of immunostaining in cytospin preparations used in our study. However, we have now completed a study using paraffin embedded material from 8 cases and found that vimentin can be detected in neuroblastoma cells if the sections are treated in a microwave oven (B. Fröstad, Falu Hospital, Sweden). According to a recent paper [22] vimentin was initially expressed by nearly all neural precursor cells *in vivo* and it has been suggested to play a role in initiation of neurite outgrowth in neuroblastoma cells.

In the 21 cases where primary tumours were correctly diagnosed surgery was the first treatment of choice after cytological diagnosis in 10 patients in whom the clinical asessment indicated it possible to resect all the tumour without sacrificing vital structures. In 10/21 children preoperative chemotherapy was started within 2 days based on the cytological diagnosis. In 1 child (pt 10) histology showed ganglioneuroma (after 2.5 months chemotherapy) in contrast to the cytological diagnosis of mixed neuroblastoma, in which the smears demonstrated clusters of small cellular neuroblastic cells together with mature ganglion cells and stroma with fascicles. Thus, this small cell component had disappeared after treatment. The urinary catecholamine excretion in this patient was high at diagnosis (dopamine, HVA and VMA).

Much progress has been made during the last 10 years in understanding the clinical behaviour of the neuroblastic cell at the molecular genetic level [23–25]. It has been found that the pattern of genetic markers is convincingly correlated to disease activity and outcome. This fact has resulted in a proposal for a molecular phenotyping staging system determining, together with clinical stage and age, the most appropriate choice of treatment [25]. We have recently performed molecular genetic analyses on stored frozen FNAC material from 16 children with NT. Fifty separate analyses (image cytometry or FISH for ploidy, FISH or PCR for 1p deletion, FISH for N-myc) resulted in clear information in 48 (B Fröstad, Falu Hospital, Sweden). These preliminary results indicate that aspirated material can also be used for molecular tests, which today are considered crucial for prognostic evaluation [26].

Aspiration smears do not allow evaluation of tumour architecture, which is of importance for diagnostic and prognostic evaluation according to histopathological systems [1, 27–29]. According to the International criteria for diagnosis of neuroblastma (INSS), FNAC is considered suboptimal because of the lack of architectural information [12]. However, unequivocal tumour cells from bone marrow aspiration in combination with increased excretion of urinary cathecolamines are accepted as minimum criteria for diagnosis, but by definition restricted to stage 4S and 4 patients. Our experience is that FNAC of primary or metastatic tumours yields a tumour cell population, which will allow not only a conclusive cytological diagnosis, but will also be sufficient for most ancillary studies that today are considered of prognostic value. This gives a more clear-cut interpretation of cytomorphology and immunocytochemistry. The use of this technique should be possible in neuroblastoma patients regardless of clinical stage.

Our present series describes the high accuracy in the diagnostic procedure and if aspiration material could be used for up-front analysis of selected molecular markers this might in the future be sufficient for assessment of prognosis and selection of treatment in most children with neuroblastic tumours. Chemotherapy is probably the first choice of treatment after clinical assessment in many children. A study has indicated that complications may be fewer for delayed surgery after tumour reducing chemotherapy especially in infants [30].

In conclusion, our study shows that FNAC has a high level of diagnostic accuracy and may be useful in combination with catecholamine analyses for conclusively diagnosing neuroblastomas in children. Fine needles with outer diameters of 0.4 to 0.6 mm are sufficient to aspirate cells both for cytomorphology and ancillary studies, such as immunocytochemistry and molecular genetics, from most neuroblastic tumours. It is also possible to store material for ancillary studies or research purposes. The procedure is relatively atraumatic. It can immediately be repeated in case of insufficient smear material and does not necessarily require general anaesthesia. In our series, it was accomplished within 1 day of admission to the department and treatment could be started immediately after clinical assessment. There were no problems with wound healing and no long-term sequelae.

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