Documenta Ophthalmologica **88:** 155–163, 1994.

The cytological, immunocytochemical and molecular genetic analysis in diagnosis of the neoplasms of the eye, eye adnexa and orbit

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Accepted 8 September 1994

Key words: Cytodiagnosis, Eye adnexa, Immunncytnchernistry, Molecular genetic examination, Neoplasm, Orbit

Abstract. The fine needle aspiration biopsy was performed in 91 patients including 57 cases of retrobulbar tumors done under CT control. All aspirates were cytologically examined. In 21 cases immunocytochemical examination was performed in order to distinguish poorly differentiated neoplasms. In 19 cases malignant lymphoma was distinguished from pseudolymphoma. Cytological diagnosis was confirmed by histopathological examination in 77 cases /84%/. One result was false positive, 3-false negative. The value of immunocytochemical methods in differential diagnosis of poorly differentiated neoplasms was stressed.

Introduction

A pathomorphological diagnosis whether histological or clinical is necessary for treatment decisions in patients with suspected neoplasm of the visual organ. Surgical biopsy with subsequent histopathological examination is the most valuable method in tumor typing. As an invasive diagnostic method it can cause spread of the neoplasm. The method which is practically equivalent to the above mentioned one in terms of the diagnostic value and yet much less traumatic is the fine needle aspiration biopsy (FNA) and cytologic diagnosis of the aspirate [8, 14, 17, 20].

The reliability of cytologic diagnosis depends on the degree of morphologic differentiation of the tumor. In poorly differentiated neoplasms it is possible to distinguish malignant cells from nonmalignant ones and merely make suggestion concerning their histologic type [3]. To specify the tumor type in cytologic smear different techniques are used, among them immunocytochemistry, which employs antibodies for linking cellular antigens and various methods of visualization of antigen - antibody complexes such as direct and indirect immunofluorescence or immunoenzymatic methods [1]. B cell lymphomas are characterized by monoclonal immunoglobulin light

chain expression (kappa or lambda). Conversely, in most benign lymphoid proliferations polyclonal immunoglobulin light chain expression is found. However, clonality in some lymphomas may not readily be revealed by immunocytochemistry. In such instances molecular genetic analysis which detects clonality may be helpful [18].

The purpose of this paper is to evaluate the usefulness and the reliability of cytological examination (supported in selected cases by immunocytochemical and molecular genetic analysis) in diagnosing the neoplasms of the eye, eye adnexa and orbit. The results of these examinations were compared with the final histopathological and / or clinical diagnosis.

Materials and methods

91 patients 6 months to 83 years old (mean age 52 years) with the clinical suspicion of the neoplastic tumor of the visual organ (44 males and 47 females) were examined. Full ophthalmological examination was performed in every patient. Additionally radiography of the orbit, paranasal sinuses and the skull was performed in 56 patients and pathological changes were found in 29 cases. Computed tomography (CT) of the orbit and skull was performed in 57 patients and pathological changes were found in all 57 cases.

FNA was done in 67 patients with the tumor located in the orbit or in the palpebro - orbital region, among them 44 under CT control and 23 without CT control, and in 15 patients with the tumor in the eye adnexa. In 9 cases of intrabulbar tumor the imprint smears after enucleation were made.

Pathomorphological diagnosis of the smears consisted of cytological examination in all 91 cases, immunocytochemical examination in 21 selected cases and molecular genetic analysis by Southern and polymerase chain reaction (PCR) method in 4 out of 19 patients with malignant lymphoma.

Results

The results were verified by histopathological examination and clinical follow up in 77 operated patients and by clinical observation in 14 unoperated patients.

The results are shown in Tables 1–7.

Discussion

In 95% of patients with tumor of the visual organ cytological diagnosis (in selected cases supported by immunocytochemical and molecular genetic

Table 1. Primary tumors of the eye adnexa

Final diagnosis	Site of primary tumor	No of cases	Cytology	Histo-pathology	Accuracy of the cytology with histop. or clinical diagnosis
Pilomatrixoma	Palpebra	2	2	2	1 +
Carcinoma basocellulare pigmentosum	Palpebra	3	3	3	3
Carcinoma planoepitheliale	Conjunctiva	2	2	2	2
Carcinoma sebaceum	Palpebra	1	1	1	0 o
Histiocytoma	Palpebra	1	1	1	1
Melanoma malignum	Palpebra	2	2	2	2
Melanoma malignum	Conjunctiva	4	4	4	4
Total		15	15	15	13

+ false positive - in cytology eyelid sarcoma
o false negative - in cytology benign tumor of the skin

Table 2. Primary orbital tumors

Final diagnosis	Site of primary tumor	No of cases	Cytology	Histo-pathology	Accuracy of the cytology with histop. or clinical diagnosis
	orbit	3			
Cystis dermoidalis			4	4	4
	orbito-intracranial	1			
	orbit	1			
Glioma			3	3	3
	orbito-intracranial	2			
Tumor mixtus	lacrimal gland	3	3	2	3
Adenocarcinoma	lacrimal gland	3	3	3	3
Carcinoma anaplasticum	orbit	1	1	1	1
Haemangiopericitoma	orbit	1	1	1	1
Fibrosarcoma	orbit	1	1	1	1
Rhabdomyosarcoma	orbit	3	3	3	3
Total		19	19	15	19

Table 3. Secondary tumors of the orbital

Final diagnosis	Site of primary tumor	No of cases	Cytology	Histo-pathology	Accuracy of the cytology with histop. or clinical diagnosis
Carcinoma planeopitheliale	Palpebra	1	1	1	1
Carcinoma planeopitheliale	Maxillary sinus	2	2	2	2
Carcinoma solidum	Supraorbital	1	1	1	1
Adenocarcinoma	Ethmoid sinus	1	1	1	1
Carcinoma anaplasticum	Ethmoid sinus	1	1	1	1
Progonoma pigmentosum	Subtemporal fossa	1	1	1	1
Meningioma	Cranical cavity	8	8	6	8
Primitive neuroectodermal tumor	Cranial cavity	1	1	1	0 o
Total		16	16	14	15

o false negative - in cytology meningioma

Table 4. Metastatic tumors of the orbit

Final diagnosis	Site of primary tumor	No of cases	Cytology	Histo-pathology	Accuracy of the cytology with histop. or clinical diagnosis
Rhabdomyosarcoma	Lower limb	1	1	1	1
Neuroblastoma	Retroperitoneal	1	1	1	1
Carcinoma anaplastium Adenocarcinoma Oat cell carcinoma	}Bronchus	3	3	3	3
Adenocarcinoma	Breast	3	3	3	3
Melanoma malignum	Arm	1	1	1	1
Total		9	9	9	9

In 4 cases orbital tumor was the first symptom of the disease

analysis) agreed with the final diagnosis. In 84% of patients the diagnosis was histopathologically verified and confirmed by clinical observation in the rest of the patients (11%). In 1 patient (1, 2%) false negative and in 2 patients false positive results were obtained. These data are similar to the data presented by Kenerdell (80% correct cytological diagnoses) [15].

Table 5. Intraocular tumors

Final diagnosis	Localization	No of cases	Cytology	Histo-pathology	Accuracy of the cytology with histop. or clinical diagnosis
Melanoma malignum	Intraocular	9	9	9	9
Melanoma malignum	Intraocular with orbital infiltration	4	4	4	4
Total		13	13	13	13

Table 6. Non Hodgkin's lymphomas

Final diagnosis	No of cases	Cytology	Immunocyto-chemistry	Molecular genetic	Histo-pathology	Accuracy of the cytology with histop. or clinical diagnosis
Lower grade malignancy lymphomas	16	16	3	4	8	16
Higher grade malignancy lymphomas	3	3	/	/	3	2 o
Total	19	19	3	4	11	18

o false negative - in cytology reactive lymphoid hyperplasia
In 15 cases palpebral tumor was the first symptom of the disease

The reliability of cytological diagnosis is very different and depends on the experience of the person performing FNA and cytopathologist who makes the diagnosis.

In neoplasms of epidermal origin the material is the easiest to be obtained. The definite cytological diagnosis was made in high malignancy lymphomas whereas cytologic differentiation of low malignancy lymphomas, pseudo-tumors and reactive hyperplasias of lymphatic system was difficult. These cases sometimes required repeated biopsy and additional immunocytochemical examinations.

Table 7. Results immunocytochemical diagnosis

	Cytological diagnosis	No of Cases	VIM	KER	LCA	HMB 45	DES	Final diagnosis
Final diagnosis	Melanoma malignum	9	9/9	0/6	/	6/9	/	Melanoma malignum
	Lymphoma malignum	2	2/2	0/1	2/2	/	/	Lymphoma malignum
	Carcinoma	3	2/2	3/3	/	/	/	Carcinoma
	Meningioma	1	1/1	0.1	/	/	/	Menigioma
Initial diagnosis Cellulae neoplasmaticae malignae								
	Lymphoma malignum	1	1/1	/	1/1	/	/	Lymphoma malignum
	Rhabdomyosarcoma	3	2/2	/	0/1	/	3/3	sarcoma
	Carcinoma	2	2/2	2/2	0.1	/	/	Carcinoma
	Total	21	19/19	5/13	3/5	6/9	3/3	

Palpebro - orbital lymphomas consisted mostly of small lymphatic cells and were most difficult to distinguish from benign hyperplasias of the lymph nodes. In such cases the initial cytologic diagnosis of a lymphoma allows to direct the clinical haematological examination and the cytological diagnosis can subsequently be supported by immunocytochemical and molecular genetic analysis [9, 21, 2]. Quick diagnosis and prompt treatment is especially important in cases of highly malignant lymphoma.

In 4 patients orbital metastases were recognized with the help of cytologic examination without previous symptoms of primary lesion. The cytological diagnosis allowed to limit the number of examinations in search for a primary lesion. In cases of tumors located in the apex of orbit the application of FNA under CT control eliminates the need of craniotomy to obtain a biopsy and this is stressed by many authors [8, 15, 16, 2]. In our material all the changes of posterior part of the orbit were accurately recognized by cytology.

In lacrimal gland tumors cytological examination can distinguish the benign from malignant tumors, and therefore it helps to establish the extention of surgical treatment.

The cytological examination should be performed not only in diagnostically difficult cases of eye adnexa tumors. Adenocarcinoma, sebaceous carcinoma of Meibomian or Zeiss gland can be clinically misdiagnosed as

blepharitis, conjunctivitis or chalasion. According to Zimmermann and other authors almost half of these malignant tumors are incorrectly recognized as chalasion [2, 10, 11, 12, 19, 22]. In our material one case of sebaceous carcinoma was cytologically misdiagnosed as a benign tumor of the skin adnexa. Only the local recurrence and rapidly expanding metastases led to verification of the slides and to proper diagnosis. For palpebral lesions a conventional biopsy may seem to be a more reliable diagnostic method than FNA biopsy because more material is obtained and most lesions will be resected anyhow. Nevertheless, FNA biopsy may be useful since small anatomical dimensions of the eye adnexa not always make possible the surgical biopsy without damaging their function and some lesions may require only nonsurgical treatment. In some cases the cytologic diagnosis prior to surgery may help to plan in detail the extension of surgical treatment. It helps also to decide about the nonsurgical treatment (e.g. irradiation).

In 23% of cases cytological examination was supported by immunocytochemical one. Successful use of immunocytochemistry in diagnostic cytology depends on the diagnostic question to be answered (which should be based on the morphology of tumor cells and on clinical data) and the antibodies available. Some markers are very specific (e.g. PSA - Prostate specific antigen) others that are present in a wide variety of tumors can still have a diagnostic role if used to answer a specific question and used together with other markers. For instance vimentin which is present in a wide variety of sarcomas, lymphomas, melanomas and also in some carcinomas (in coexpression with keratin) can support the diagnosis of melanoma in carcinoma vs melanoma dilemma if tumor cells are keratin negative. However, in most such cases antibodies to other markers e.g. HMB-45 or S-100 protein are needed. The presence of vimentin was observed in smears of all nine melanoma cases and the presence of HMB 45 antigen in 6 of 9. Over 90% melanoma cells in aspirates showed positive vimentin reaction. The HMB 45 antibody is described as highly specific for melanoma cells, but of low sensitivity which was also confirmed in our material [7].

If the diagnostic question is small cell anaplastic carcinoma versus malignant lymphoma antibodies to vimentin, LCA and keratin are sufficient to make the decisive diagnosis [5]. However, there are some exceptions i.g. Ki-1 positive anaplastic large cell lymphoma [16].

Leucocyte common antigen can not distinguish between for instance pseudolymphoma, lymphoid hyperplasia or malignant lymphoma. In such cases molecular genetic analysis may help. In 4 immunocytochemical examinations which were performed in patients with suspected malignant lymphomas, the presence of vimentin and LCA (leucocyte common antigen) in all 3 cases was observed.

In 2 cases of anaplastic carcinomas in initial cytological diagnosis malignant cells were recognized and carcinoma suspected. The definite diagnosis of carcinoma was achieved by the use of immunocytochemical examination, where coexpression of keratin and vimentin was found. In 3 cases of highly differentiated carcinomas before extensive surgical treatment the initial cytologic diagnosis was confirmed by the immunocytochemical examination which showed coexpression of keratin and vimentin. The occurrence of keratin is characteristic for all types of carcinomas [1, 6].

In 3 cases of rhabdomyosarcoma the presence of desmin was observed and in 2 of these vimentin was also coexpressed. Many authors [1, 4, 13, 23], consider desmin as the most specific marker in diagnosis of rhabdomyosarcoma. Other markers such as vimentin, actin, myoglobin are considered as less specific. For the adequate diagnosis the results obtained by immunocytochemical methods must be analysed together with the clinical data of the patient and the morphology of the cells in the smears [3].

In 4 of 19 patients with suspected malignant lymphoma molecular genetic analysis was carried out. Clonality was found in 3 patients. Negative result was obtained in 1 patient using both Southern and PCR method which is a technique of extraordinary sensitivity [11] . In this case malignant lymphoma was diagnosed on the basis of clinical symptoms, cytologic examination and changes in myelogram.

Conclusions

1. Cytological examination supported in selected cases by immunocytochemical and molecular genetic analysis is a method of low invasiveness which makes possible prompt diagnosis of the visual organ neoplasms.
2. High degree of correlation of cytological diagnosis and small number of false positive and false negative results make this method highly reliable and useful.

References

1. Bibbo M. Comprehensive cytology in: B. Saunders Company. Philadelphia 1990.
2. Cavanagh HD, Green WR, Goldberg HK. Multicentric sebaceous adenocarcinoma of the Meibomian gland. Amer J Ophthalmol 1974, 326–332.
3. Chosia M. Use of immunocytochemical techniques in differential diagnosis of neoplasms in fine needle aspirates. Pomorska Akademia Medyczna. Szczecin Poland 1991.
4. Cintorino M et al. Expression of actin isoforms and intermediate filament proteins in childhood orbital rhabdomyosarcoma. J Submicrosc Cytol Pathol 1989, 21: 409–419.
5. Domagała W et al. Differential diagnosis of lymph node aspirates by intermediate filament typing of tumor cells. Acta Cytol 1986, 30: 225–234.

6. Domagała W et al. Decisive role of intermediate typing oftumor cells in the differential diagnosis of difficult fine needle aspirates. Acta Cytol 1987, 31: 253–266.
7. Domagała W et al. Immunocytochemical criteria in the differential diagnosis of malignant melanoma versus carcinoma, lymphoma and sarcoma in fine needle aspirates. Pat. Pol. 1991, 42: 73–78.
8. Dresner Sc, Kennerdell J S, Dekker A. Fine needle aspiration biopsy of metastatic orbital tumors. Surv Ophthalmol 1983, 27: 397–398.
9. Ellis J H et al. Lymphoid tumors of the ocular adnexa. Ophthalmol 1985, 92: 1311–1324.
10. Gamel J W, Eiferman R A, Guibor P. Mucoepidermoid carcinoma of the conjunctiva. Arch Ophthalmol Chicago 1984, 102: 73–731.
11. Gibbs R A. DNA amplification by the polymerase chain reaction. Anal Chem 1990, 62: 1202–1214.
12. Graham J, Joung S E, Luna M. An unusual case of metastatic carcinoma of the eyelid. Amer J Ophthalmol 1978, 86: 400–402.
13. Holbach L et al. Zur immunozytochemischen Diagnose embryonaler Rhabdomyosarcome der Orbita. Klin Mbl. Augenheil 1989, 195: 190–195.
14. Jakobiec F A, Chattock A. Aspiration cytodiagnosis of eyelid tumors. Arch Ophthalmol 1979, 97: 1907–34.
15. Kennerdell J S et al. Orbital fine needle aspiration biopsy. Amer J Ophthalmol 1985, 99: 547–551.
16. Koss L G, Woyke S, Olszewski O. Aspiration Biopsy. Cytologic Interpretation and Histologic Bases. Sec ed Iqaku - Shoin, New York, 1992.
17. Krzystolik Z. Clinical value of fine needle aspiration biopsy and computed tomography in diagnosing pathological changes of the orbit and ocular adnexa. Pomorska Akademia Medyczna. Szczecin - Poland 1989.
18. Lubiński J, Chosia M, Huebner K. Molecular genetic analysis in the diagnosis of lymphoma in fine needle aspiration biopsies. I. Lymphomas versus benign lymphoproliferative disorders. Anal Quant Cytol Histol 1988, 10: 391–398.
19. Mansour A M, Hidayat A A. Metastatic eyelid disease. Ophthalmology 1987, 94: 667–670.
20. Midena E et al. Fine needle aspiration biopsy in ophthalmology. Surv Ophthalmol 1985, 29: 410–422.
21. Morgan G, Harry J. Lymphatic tumors of interdeterminate nature: a 5 year follow up of 98 conjunctival an orbital lesion. Brit J Ophthalmol 1978, 62: 381–383.
22. Perlman E, Mc Mahon R L. Sebaceous gland carcinoma of the eyelid. Amer J Ophthal 1978, 86: 699–703.
23. Shields J A, Shields C L. Orbital Rhabdomyosarcoma. Arch Ophthalmol 1987, 105: 700–701.
24. Vogiatzis K V. Lymphoid tumors of the orbit and ocular adnexa: A long term follow - up. Ann Ophthalmol 1984, 1046–1055.

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