Pol J Pathol 2004, 55, 2, 51-57 PL ISSN 1233-9687

Originals

Ewa Wolska-Szmidt[1], Anna Jakubowska[2], Karol Krzystolik[1], Maria Chosia[3]

Fine Needle Aspiration Biopsy and Molecular Analysis in Differential Diagnosis of Lymphoproliferative Diseases of the Orbit and Eye Adnexa*

[1]Department of Ophtalmology,
[2]Department of Genetics and Pathology,
[3]Department of Pathology, Pomeranian Medical University, Szczecin

The aim of the investigation was the assessment of the role of routine cytology and clonality evaluation using PCR in differential diagnosis of lymphoproliferative diseases of the orbit and eye adnexa. The investigations were carried out in cellular material collected via fine needle aspiration biopsy (FNAB) from 29 patients aged 31 - 82 years, including 17 women and 12 men. Apart from routine cytology, molecular-genetic studies were performed employing the PCR technique. In 21 cases histopathology was performed. In 2 patients, despite several attempts, FNAB failed to provide any diagnostic material for routine cytology. Based on cytology, non-Hodgkin's lymphoma was diagnosed in 11 patients and suspected in three. In 13 patients no firm diagnosis was possible based on cytological smears. The employment of PCR allowed for rendering the diagnosis more precise in 13 cases, confirming it in 13 patients, while in 3 cases the results of the above tests did not affect the final diagnosis. Clonality studies by PCR may be performed in material obtained through FNAB. Clonality assessment by PCR technique is very useful in differential diagnosis of lymphoproliferative disordes.

Introduction

Despite the fact, that fine needle aspiration biopsy (FNAB) is a commonly employed method in contemporary oncological diagnostic management it does not enjoy wide popularity in diagnosing lymphoproliferative diseases of the eye [5, 10, 13, 22, 31]. Benign and malignant lymphoproliferative lesions, as well as pseudotumors are among the most common tumors with primary location in the orbit or ocular adnexa [8, 14]. However, the morphological similarity of such diseases poses numerous problems in differential diagnosis, not only cytological, but also histopathological, particularly in differentiating benign lymphoproliferative lesions from small cell lymphomas. Thus, it is necessary to introduce additional methods to differential diagnosis.

To date, only a few reports describe flow cytometry performed in materials obtained through FNAB [3, 7, 20, 21, 29]. The literature does not provide any data on the use of PCR in FNAB-obtained materials in differential diagnosis of lymphoproliferative lesions within the orbit and eye adnexa. In the present investigations the authors attempted to assess the validity of routine cytology and clonality studies employing PCR in differential diagnosis of lymphoproliferative diseases involving the orbit and eye adnexa.

Material and Methods

The investigations were carried out on cellular material obtained through fine needle aspiration biopsy in 29 patients (17 women and 12 men) aged 31 - 82 years, who were treated or consulted at Department of Ophthalmology, Pomeranian Medical University in Szczecin, in the years 1989 - 2003. Nineteen patients presented with orbital or palpebral-orbital lesions, 7 - with palpebral, and three with conjunctival lesions (Fig. 1). In 15 cases the right orbit or ocular adnexa were involved, the lesions were situated on the left side in 11 patients, while 3 individuals presented with bilateral disease (Fig. 2). In 11 patients the disease had sudden onset. All the patients were subjected to fine needle aspiration biopsy, including 9 individuals in whom FNAB was performed under CT control (Fig. 3). In 20 patients with visible or palpable tumors, the biopsy did not require computed tomography control. The lesions were biopsied using a 22G needle (0.7mm in diameter), which was 3.75cm long in adults and 2.5cm in length in children in view of the risk of penetrating the superior orbital fissure or the intracranial cavity through the optic nerve canal [5]. The biopsies were

*The work was supported by the grant No 4 P05B 023 19 of the State Committee for Scientific Research (KBN)

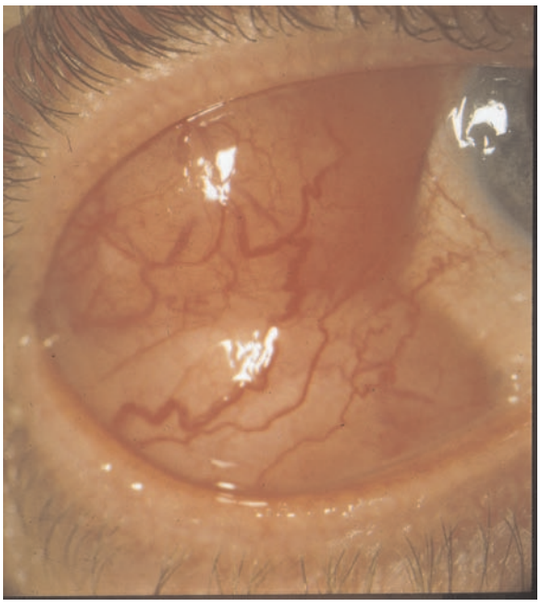

Fig. 1. Non-Hodgkin's lymphoma of conjunctiva (patient No 15).

performed inserting the needle through the conjunctiva, upper or lower crease or through the skin using the nearest approach. At least two biopsies were done and the material was aspirated to a 5 - 10ml syringe. Prior to the procedure, local anesthesia was applied to the conjunctival sac using 1% pantocaine or 0.5% alcaine. No complications were observed except subcutaneous hemorrhage in six patients and subconjunctival petechiae in two individuals. Histopathology was performed in 21 cases.

The FNAB-obtained materials served to prepare at least two smears, which were immediately fixed in 96% ethyl alcohol and stained with hematoxylin and eosin.

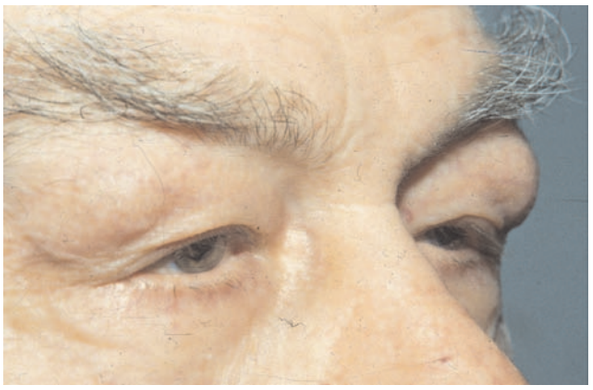

Fig. 2. Bilateral palpebral non-Hodgkin's lymphoma (patient No 7).

The material for investigating clonality using the PCR method was obtained from fine needle aspiration biopsy of the orbital tumor or ocular adnexa, with the bioptate placed in PBS, mixing 10ml of the latter with 1ml sodium versenate.

DNA isolation consisted of incubation with proteinase K (Sigma) at 37˚C, phenol and chloroform extraction and precipitation with 96% alcohol at -20˚C, followed by drying and dissolving in TE buffer.

To assess the clonality of B lymphocytes, the authors employed primers synthesized by TIB MolBiol, for permanent regions of immunoglobulin heavy chains:

- third skeletal region (3'V terminal): Fr3A-5'ACACGGC(G/T)(G/C)TGTATTACTGT3',
- VLJH region: GTGACCAGGGT(A/G/C/T)CCTTGGCCCCAG 3',
- J region: LHJ5'TGAGGAGACGGTGACC 3'.

The two-stage PCR reaction was performed using a Perkin-Elmer Model 480 thermostat. The end-product

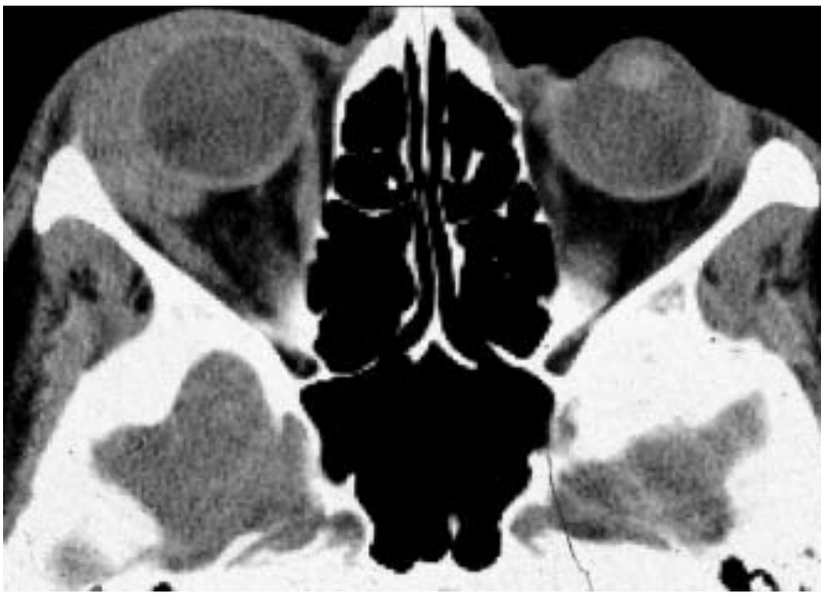

Fig. 3. CT scan: right side orbital and conjunctival tumor (patient No 15).

volume of the reactive mixture was 25µl. The PCR reaction was performed employing reagents manufactured by Peterfarm.

The mixture for PCR reaction contained 0.15µg DNA, 1.5mmol of each nucleotide (dNTP-mix), 2mmol of each primer (Fr3A and LJH 5'in stage I and Fr3A and VLJH in stage II), 2.5µl of buffer (concentrated 10x), 2U of thermostable polymerase Taq, and water to acquire the volume of 25µl. Subsequently, 50µl of mineral oil were added to each sample.

The PCR reaction parameters were as follows: denaturation at 94˚C, primer binding at 60˚C, elongation at 72˚C. The number of cycles was 30. In the first PCR the primers included Fr3A and LHJ and 30 cycles were applied. Five µl of the resultant product were diluted with H_2O to achieve the volume of 100µl. From this volume, 5µl were drawn and 20µl of the second PCR reaction (with the same composition) were added; in the second reaction the Fr3A and VLJH primers were employed and 20 cycles were performed.

To assess T lymphocyte clonality the authors used primers for TCRδ receptors complementary for 3' terminals of the following regions:

- Vδ1:
  PVD13' (5'CAAAGTACTTTTGTGCTCTTG3'),
- Jδ1:
  PJD 13 (5'GAGTTACTTACTTGGTTCCAC3').

The following primers for TCR(receptors were used:

- Vγ11: (5')TCTGGG/AGTCTATTACTGTGC(3'),
- Jγ11: (5')CCAGTGTTGTTCCACTGC(3').

The PCR reaction was performed as a two-stage process in a Perkin-Elmer Model 480 thermostat. The first PCR was subjected to 30 cycles. Five µl of the product obtained in the first PCR were diluted with H_2O to obtain the volume of 100µl. Five µl were drawn from this amount and 20µl of the second PCR reaction, containing the same primers, were added; subsequently 20 cycles were applied for TCRγ and 27 cycles for TCRδ.

The PCR reaction parameters were as follows: denaturation at 94˚C, primer binding at 55˚C for TCRγ and at 50˚C for TCRδ, elongation at 72˚C. Fifty µl of mineral oil were added to all the samples.

The PCR products were subjected to electrophoresis on agarose gel with ethidine bromide. The results were analyzed in ultraviolet light.

Results

Routine cytology was performed in all the patients. Table 1 lists the results of cytology and other tests. The final diagnosis was based on routine cytology and/or histopathology, as well as on PCR analysis. Cytology allowed for diagnosing non-Hodgkin's lymphoma in 11 patients and suspecting the disease in further 3 individuals. In 13 individuals the diagnosis was ambivalent, since cytology did not allow for a firm determination of the biology of the lesion (benign vs. malignant). In a female in whom the amount of material collected had been insufficient for cytology, histopathology allowed for the diagnosis of a pseudotumor. In one other case where routine cytology could not have been performed, the final diagnosis indicated lymphoma. Only in two lymphoma cases did cytology allow for identifying the type. These patients represented two cases of lymphoplasmocytic lymphoma, where the presence of plasma cells and plasmablasts facilitated the diagnosis. In four other patients the grade of lymphoma was suggested.

PCR studies were performed in all patients. In five of them, in whom primary cytological diagnosis had been inconclusive: NHL? BLPL? no clonality was observed and ultimately four of these individuals were diagnosed as benign lymphoproliferative lesions and one as a pseudotumor. Twenty two patients demonstrated clonality of lymphoid cell proliferation. In 18 cases there were B-cell non-Hodgkin's lymphomas (Fig. 4), including 10 patients with primary lymphomas of the orbit and eye adnexa. In the patient (No 8), who demonstrated clonal proliferation, histopathology allowed for establishing an ultimate diagnosis of a pseudotumor. In two lymphoma cases no clonality was noted in PCR. One of these patients (No 7) demonstrated clonal proliferation in flow cytometry and the final diagnosis was a low grade B-cell primary non-Hodgkin's lymphoma. In the other individual (No 6) histopathology allowed for diagnosing a primary MALT-type lymphoma. In three patients the rearrangement of Tδ lymphocyte receptor gene (Fig. 5) was demonstrated and the final diagnosis was a T-cell lymphoma.

Table 2 presents the comparison of preliminary cytological diagnosis and the clonality assessment employing PCR method. Table 3 illustrates the effect of clonality assessment on the final diagnosis. Based on PCR, the diagnosis was rendered more precise in 13 patients, confirmed in 13 cases, while in three individuals the results of the above tests did not affect the final diagnosis.

Discussion

The results presented in this report confirm the view that in the diagnostic management of lymphoproliferative lesions routine cytology is insufficient in establishing a reliable diagnosis, especially in primary lesions involving the orbit and eye adnexa. Despite the fact that in benign lymphoproliferative lesions the smears usually show cellular polymorphism associated with the presence of cells originating from germinal centers, while lymphomas generally demonstrate monomorphic character and the population is clearly dominated by a single cell type [4, 5], in many cases morphological similarity of benign lymphoproliferative lesions

TABLE 1

Tests results for the entire group of patients

Patient			Tests			Final diagnosis
No	Age	Sex	cytology	histopathology	PCR	
1	31	F	NHL? BLPL?	NHLs	no clonality	BLPL
2	81	M	NHL? BLPL?	Pseudotumor	no clonality	Pseudotumor
3	64	F	NHL? BLPL?	BLPL	no clonality	BLPL
4	69	F	NHL? BLPL?	BLPL?	no clonality	BLPL
5	64	M	NHL? BLPL?		no clonality	BLPL
6	64	F	NHL? BLPL?	NHL-lg MALT	no clonality	NHL-lg MALT
7	68	M	NHL-lg		no clonality	p-NHL-B-lg
8	54	F	too scant material	Pseudotumor	clonality B cells	Pseudotumor
9	52	F	NHL-LP		clonality B cells	p-NHL-LP
10	82	M	NHLs		clonality B cells	p-NHL-B-hg
11	71	M	NHL? BLPL?		clonality B cells	p-NHL-B-lg
12	62	F	NHLs		clonality B cells	p-NHL-B-lg
13	77	F	NHL? BLPL?	NHL-FL	clonality B cells	NHL-FL
14	66	M	NHL? BLPL?	NHL-lg	clonality B cells	p-NHL-B-lg
15	45	F	NHL? BLPL?		clonality B cells	p-NHL-B-lg
16	76	M	NHL-LP		clonality B cells	p-NHL-LP
17	43	F	NHL? BLPL?	NHL-lg MALT	clonality B cells	NHL-lg MALT
18	53	F	NHL		clonality B cells	p-NHL-B-lg
19	82	F	NHL-lg		clonality B cells	NHL-B-lg
20	63	M	NHL? BLPL?		clonality B cells	NHL-B-lg
21	63	M	NHL	NHL-B-hg	clonality B cells	NHL-B-hg
22	64	F	NHL-hg		clonality B cells	NHL-DLBCL
23	56	F	NHL		clonality B cells	NHL-B
24	69	M	NHL	NHL-DLBCL	clonality B cells	NHL-DLBCL
25	68	F	NHLs		clonality B cells	NHL-B-Ig
26	53	F	NHL		clonality B cells	NHL-B
27	48	F	NHL? BLPL?	NHL-lg?BLPL?	clonality B cells	p-NHL-T-lg
28	42	M	too scant material		clonality B cells	NHL-T
29	39	M	NHL-lg		clonality B cells	NHL-T-lg

Abbreviations: BLPL - benign lymphoproliferative lesion; Pseudotumor - inflammatory pseudotumor; NHL - non-Hodgkin's lymphoma; NHLs - non-Hodgkin's lymphoma suspected; p-NHL - primary non-Hodgkin's lymphoma; NHL-B - B-cell non-Hodgkin's lymphoma; NHL-T - T-cell non-Hodgkin's lymphoma; NHL-lg - low-grade non-Hodgkin's lymphoma; NHL-hg - high-grade non-Hodgkin's lymphoma; NHL-SLL-B - small lymphocytic B-cell lymphoma; NHL-DLBCL - diffuse large B-cell lymphoma; NHL-FL - follicular lymphoma; NHL-LP - lymphoplasmocytic lymphoma

and non-Hodgkin's lymphomas, especially when low-grade, is very high. The ability of establishing a final diagnosis in routine cytology is differently assessed by various authors. Steel et al. [26] estimated the accuracy of the method as 72%, Zeppa et al. [31] - as 83%, Zajdela et al. [30] - as 87%, while Dey et al. [6] reported the rate that was as high as 94%. In our material routine cytology allowed for a preliminary diagnosis of non-Hodgkin's lymphoma in 11/23 cases, while another 3 patients were suspected of NHL. The gravest problems were encountered while differentiating between benign lymphoproliferative lesions and malignant lymphomas. In as many as 13 cases of the total of 29 patients (45%) the result of cytology was inconclusive (NHL? BLPL?). Although routine cytology is a fast and simple test, it is, nevertheless, associated with a considerable percentage of inconclusive results. Moreover, only in two lymphoma patients (2/23) were the authors able to determine the type.

Molecular - genetic methods or flow cytometry, when combined with cytology, increases the diagnostic accuracy in non-Hodgkin's lymphomas and lymphoproliferative lesions [1, 2, 16, 29]. Sharara et al. [23], who collected tissue sections from 43 patients with lymphoproliferative lesions

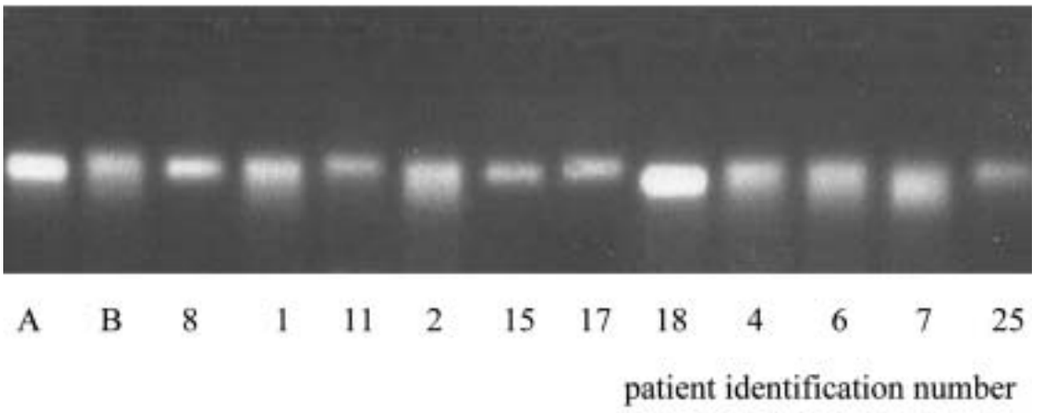

Fig. 4. Results of PCR tests for B cells; A - clonality (standard); B - no clonality (standard); 8, 11, 15 ,17, 18, 25 clonality B cells cases; 1, 2, 4, 6, 7 no clonality cases.

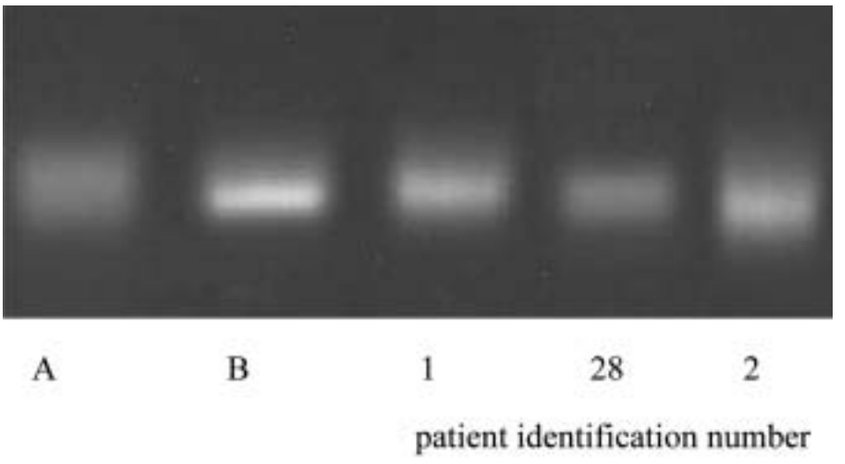

Fig. 5. Results of PCR tests for T cells; A - no clonality (standard); B - clonality (standard); 28 - clonality T cells case; 1, 2 - no clonality cases.

of the orbit and eye adnexa and subjected the materials to histopathological examinations combined with immunophenotype assessment in flow cytometry and PCR, decided that flow cytometry was more useful in evaluating clonality.

Nevertheless, PCR allows for detecting even small amounts of clonal cells, regardless of the presence of non-neoplastic lymphocytes or other types of cells [15, 17]. The method makes it possible to detect gene translocations, which are characteristic for lymphomas, even when such translocations are present only in one per 10^5 investigated cells [17]. While studying clonality employing the genetic-molecular method, one bases the test on translocations of immunoglobulin and T lymphocyte receptor (TCR) genes [12, 15, 17]. More than 90% of lymphomas involving the orbit and eye adnexa represent monoclonal B-cell lymphomas [11].

To assess clonality, various techniques may be employed, such as Southern blot and PCR.

In clonality studies of B-type lymphomas by PCR, the following primers are most frequently used: Fr3A, LJH and VLJH. The Fr3A primer is complementary to the 17 gene sequence of the human V segment. The LJH and VLJH primers are complementary to gene sequences of the 6^{th} J regions [28]. The binding site of the two latter primers is situated in the 5' part of the gene. When the above mentioned primers are employed, the resultant PCR products are 100 - 120 base pairs long. Wan et al. [28] determined the sensitivity of the method as 92% (24/26 cases) and believed it to be specific in detecting B lymphocyte clonality.

In the case of T-cell lymphomas, the assessment of clonality using the PCR method is based on detection of rearrangement of δ and γTCR (lymphocyte T receptor) chain genes. δ and γTCR chain genes undergo rearrangement early on when T lymphocytes change [9]. On the other hand, in the case of TCRδ, the 3' terminals of Vδ1 and Jδ1 regions, to evaluate which complementary primers have been employed, manifest approximately 60% of receptor gene translocations [27]. The products of the above mentioned reactions are 80 - 110 base pairs long [27]. Translocations of γTCR chain genes are detected by PCR in 74% of T-cell

TABLE 2

Cytological diagnosis vs. clonality assessment by PCR

Cytological diagnosis	Clonality assessment in PCR		Final diagnosis
	clonality	no clonality	
NHL	12	1_7	NHL
NHLs	1		NHL
NHL? BLPL?	7	1_6	NHL
NHL? BLPL?		4	BLPL
NHL? BLPL?		1	Pseudotumor
Too scant material	1		NHL
Too scant material	1_8		Pseudotumor
Total	22	7	

Subscript - patient identification number

proliferation cases [18]. PCR products obtained employing the Vγ11 and Jγ11 primers that are complementary to corresponding TCRγ regions are 75 - 95 base pairs long [19, 25].

In our material, PCR was performed in 29 patients. In 18/20 (90%) cases of B-cell lymphomas, B lymphocyte clonality was detected. In 2 cases no clonality was observed. These were one MALT type lymphoma (No 6) as well as primary low grade B-cell lymphoma (No 7). In 3/3 of T-cell lymphomas, lymphocyte clonality was detected. In 5/6 of benign lymphoproliferative lesions (4 BLPL and 1 pseudotumor), no clonality was noted. On the other hand, in one pseudotumor (No 8), diagnosed by histopathology, clonality was positive.

It is a well known fact that clonal proliferation is not a specific phenomenon characteristic of lymphomas and lymphocytic leukemias and, on the other hand, clonality may appear in some benign lymphoproliferative diseases [9, 12, 16]. Based on 12 cases of lymphoproliferative lesions of the eye with tissue samples studies using the PCR method, Sigurdardottir et al. [24] demonstrated the limited role of this test in assessing clonality, since in three cases where clonality had been demonstrated, only one was finally diagnosed as lymphoma, while two other lymphomas were polyclonal. Also Sharara et al. [23] observed clonal proliferation in 39% of chronic inflammatory lesions and in as much as 50% of benign lymphoproliferative and atypical lesions.

We believe that PCR may be employed in materials collected *via* fine needle aspiration biopsy as supplementary methods that together with clinical data complement cytology. The PCR technique requires an even smaller amount of material in comparison to flow cytometry, since changes resulting from gene translocation in the cells may be multiplied by PCR as many as 10^6 times.

TABLE 3

The effect of clonality in final diagnosis

Cytological diagnosis	Number of cases where the final diagnosis was affected by clonality			Final diagnosis
	confirmed	specified	exerted no effect	
NHL	12			NHL
NHLs	1			NHL
NHL? BLPL?		4		BLPL
NHL? BLPL?		1		Pseudotumor
NHL? BLPL?		7	$2^{**}_{6,7}$	NHL
Too scant material			1^{**}_8	Pseudotumor
Too scant material		1^*_{28}		NHL
Total	13	13	3	

*final diagnosis was establish based on T cells clonality;
**final diagnosis was establish based on histopathology;
subscript - patient identification number

References

1. *Braylan RC, Orfao A, Borowitz MJ, Davis BH:* Optimal number of reagents required to evaluate hematolymphoid neoplasias: Results of an international consensus meeting. Cytometry 2001, 46, 23-27.
2. *Cangiarella JF, Cajigas A, Savala E, Elgert P, Slamovits TL, Suhrland MJ:* Fine needle aspiration cytology of orbital masses. Acta Cytol 1996, 40, 1205-1211.
3. *Char D, Miller T:* Orbital Pseudotumor. Fine-needle aspiration biopsy and response to therapy. Ophtalmology 1993, 100, 1702-1710.
4. *Cossman J, Uppenkamp M, Sundeen J, Coupland R, Raffeld M:* Molecular genetics and the diagnosis of lymphoma. Arch Pathol Lab Med 1998, 112, 117-127.
5. *Czerniak B, Woyke S, Daniel B, Krzystolik Z, Koss LG:* Diagnosis of orbital tumors by aspiration biopsy guided by computerized tomography. Cancer 1984, 54, 2385-2389.
6. *Dey P, Radhika S, Rajwanshi A, Ray R, Nijhawan R, Das A:* Fine needle aspiration biopsy of orbital and eyelid lesions. Acta Cytol 1993, 37, 903-907.
7. *Dunphy CH, Ramos R:* Combining fine-needle aspiration and flow cytometric immunophenotyping in evaluation of nodal and extranodal sites for possible lymphoma: a retrospective review. Diagn Cytopathol 1996, 16, 200-206.
8. *Esmaeli B, Ahmadi MA, Manning J, McLaughlin PW, Ginsberg L:* Clinical presentation and treatment of secondary orbital lymphoma. Ophtal Plast Reconstr Surg 2002, 18, 247-253.
9. *Griesser H, Tkachuk D, Reis MD, Mak TW:* Gene rearrangements and translocations in lymphoproliferative diseases. Blood 1989, 76, 1402-1415.
10. *Gupta S, Sood B, Gulati M:* Orbital mass lesions: US-guided fine-needle aspiration biopsy - experience in 37 patients. Radiology 1999, 213, 568-572.
11. *Johnson TE, Tse DT, Byrne GE et al:* Ocular-adnexal lymphoid tumors: A clinicopathologic and molecular genetic study of 77 patients. Ophtal Plastic Reconstr Surg 1999, 15, 171-179.

12. *Korsmeyer SJ:* Antigen receptor genes as molecular markers of lymphoid neoplasms. J Clin Invest 1987, 79, 1291-1295.
13. *Krzystolik Z, Chosia M:* Biopsja aspiracyjna cienkoigłowa (b.a.c.) w guzach oczodołu. Okulistyka 2000, 1, 25-29.
14. *Laucirica R, Font RL:* Cytologic evaluation of lymphoproliferative lesions of the orbit/ocular adnexa: an analysis of 46 cases. Diagn Cytopathol 1996, 15, 241-245.
15. *Lubiński J:* Molekularno-genetyczne badanie przemieszczeń genów w diagnostyce chłoniaków i białaczek. Pat Pol 1989, 40, 137-148.
16. *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. Analyt Quant Cytol Histol 1988, 10, 391-397.
17. *Lubiński J, Ptaszyński K:* Molecular genetic analyses in lymphoma diagnosis. Postępy Biologii Komórki 1990, 17, 353-359.
18. *Lutz SC, Anderson SF, Wu CY, Townsend JC:* Non-Hodgkin's orbital lymphoma. Optom Vis Sci 2001, 78, 639-645.
19. *McCarthy KP, Sloane JP, Kabarowski JH, Matutes E, Wiedemann LM:* A simplified method of detection of clonal rearrangements of the T-cell receptor-γ chain gene. Diagn Mol Pathol 1992, 1, 173-179.
20. *Meda BA, Buss DH, Woodrruff RD, Cappellari JO, Rainer RO, Powell BL, Geisinger KR:* Diagnosis and subclassification of primary and recurrent lymphoma. The usefulness and limitations of combined fine-needle aspiration cytomorphology and flow cytometry. Am J Clin Pathol 2000, 113, 688-699.
21. *Nassar DL, Raab SS, Silverman JF, Kennerdell JS, Sturgis CD:* Fine-needle aspiration for the diagnosis of orbital hematolymphoid lesions. Diagn Cytopathol 2000, 23, 314-317.
22. *Orucevic A, Reddy VB, Selvaggi SM, Green L, Spitz DJ, Gattuso P:* Fine-needle aspiration of extranodal and extramedullary hematopoietic malignancies. Diagn Cytopathol 2000, 23, 318-321.
23. *Sharara N, Holden JT, Wojno TH, Feinberg AS, Grossniklaus HE:* Ocular adnexal lymphoid proliferations: clinical, histologic, flow cytometry, and molecular analysis of forty-three cases. Ophtalmology 2003, 110, 1245-1254.
24. *Sigurdardottir M, Sigurdsson H, Barkadottir RB, Kristjansdottir S, Agnarsson BA:* Lymphoid tumors of the ocular adnexa: a morphologic and genotypic study of 15 cases. Acta Ophtalmol Scand 2003, 81, 299-303.
25. *Slack DN, McCarthy KP, Wiedemann LM, Sloane JP:* Evaluation of sensitivity, specificity, and reproducibility of an optimized method for detecting clonal rearrangements of immunoglobulin and T-cell receptor genes in formalin-fixed, paraffin-embedded sections. Diagn Mol Pathol 1993, 2, 223-232.
26. *Steel BL, Schwartz MR, Ramzy I:* Fine needle aspiration biopsy in the diagnosis of lymphadenopathy in 1103 patients. Acta Cytol 1995, 39, 76-81.
27. *Vandenvelde C, Scheen R, Duys M, Corazza F, Van Beers D:* Fast PCR based therapeutic follow-up of T-cell malignancies. J Rev Fr Hematol 1991, 33, 293-297.
28. *Wan JH, Trainor KJ, Brisco MJ, Morley AA:* Monoclonality in B cell lymphoma detected in paraffin wax embedded sections using the polymerase chain reaction. J Clin Pathol 1990, 43, 888-890.
29. *Wolska-Szmidt E, Masiuk M, Krzystolik K, Chosia M:* Flow cytometry in the diagnosis of lymphoproliferative lesions of the orbit and eye adnexa in fine needle aspiration biopsy. Pol J Pathol 2003, 54, 253-259.
30. *Zajdela A, Vielh P, Schlienger P, Haye C:* Fine-needle cytology of 292 palpable orbital and eyelid tumors. Am J Clin Pathol 1990, 93, 100-104.
31. *Zeppa P, Tranfa F, Errico ME et al:* Fine needle aspiration (FNA) biopsy of orbital masses: a critical review of 51 cases. Cytopathology 1997, 8, 366-372.

Address for correspondence and reprint requests to :
Prof. M. Chosia, M.D., Ph.D.
Department of Pathology Pomeranian Medical University
Unii Lubelskiej 1, 71-252 Szczecin
phone: +48 91 4253484
e-mail: mchosia@sci.pam.szczecin.pl