LYMPH NODE AND BONE MARROW PATHOLOGY

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Incidence of malignant lymphomas in Montenegro

ABSTRACT

The diagnostis of malignant lymphomas (ML) on the teritory of Montenegro has been done solely in Clinical Hospital Center of Montenegro, so the incidence of diagnosed ML can be considered as the incidence in epidemiological sense. Comparing frequency and pathohystological profile of ML, which have been diagnosed from 1994 to1999 („immunohistochemical" period) by the same pathologist, there has been estimated ML frequency and has been checked influence of introduction of immunohistochemical diagnostic on ML pathohistological profiles. In the first period the diagnosis of ML has been done on HE, Giemsa, MGP, PAS and Gomori stained slides. In the second period it has been also used LSAB immuno- histochemical methodology for detection LCA, CD3,CD43, CD20, CD79, CD15 and CD30. The HNL:HL ratio in the first observed period has been 50.8%:49.2%. The same ratio in the "immunohistochemical" period has been 71.6% :28.4%. The most frequent are follicular center cell types NHL (54.8% in first, 69.1% in second period) but the immunoblastic type has been diagnosed in 16.1% and in 8.8% of patients. The NHL incidence in the second period (3,4/100000) significantly exceeds the same incidence from period of „morphological" diagnosis (1,5 /100000). The most frequent in both periods are follicular center cell types NHL (54.8% and 69.1%); small lymphocytic NHL has also been frequent (22.6% or 20.1%) The overdiagnose of HL in the period of pure „morphological" diagnose is one of reasons for established growth of NHL frequency in „immunohistochemical" period.

Key words: Lymphoma + epidemiology; Yugoslavia

INTRODUCTION

In SRJ, as well as in Montenegro, there is no centralized registration of malignant desease, so it is impossible to get the reliable information about the prevalence and incidence of malignant lymphomas (ML). Pathological diagnosis and the specific oncological therapy for the teritory of Montenegro has been done in practice only in CHC of Montenegro, so the most precise data can be get due to retrospection of pathohistological samples, and because of the relative isolation of Montenegro since 1991 up to now, collected facts can be used for the quite precise estimation of incidence of ML from the epidemiological aspect. Our previous study (1) of ML frequency and pathohisological patterns of Hodgkin (HL) and non-Hodgkin lymphomas (NHL), which has been done without the usage of immunohistochemical methods, showed the slow growth of the incidence of HL and NHL (maximal incidence of NHL in 1996 was 1,5\100000) with almost identical frequency of HL and NHL (rate 49 % : 51%) has suggested the overdiagnose of HL. From 1997 we have been using relatively narrow palete of specific antibodies in diagnose of ML. By comparation of frequency and PH patterns of ML diagnosed from 1994 to 1996 ("morphological" period) and from 1997 to 1999 ("immunohistochemical" period) by the same pathologist, we wanted (a) to continue the following of frequency diagnosed ML and (b) to check is it and how the introducing of immunohistochemical methodology has affected to pathohistological patterns of ML.

MATERIALS AND METHODS

In the first period (1994-1996) the ML diagnose has been done on HE, Giemsa, MGP, PAS and Gomori stained slides. In the second period (1996-1999) LSAB immunohistochemical methodology has also been used in detection of following antigenes : LCA, CD3, CD43, CD20, CD79, CD15 and CD30 (Dako). NHL have been diagnosed and classified according to criteria of Working Formulation, and HL according to Rye's criteria.

RESULTS

In the first period (1994-1996), in 30 patients were diagnosed HL, and in 31 patients NHL. In second one (1997-1999) HL was diagnosed in 27, and NHL in 68 patients. The NHL:HL ratio in the first period (31:30) was 50.8%:49,2 %, while the same ratio in second, #immunohistochemical# period was 71.6 %:28.4%. The ratio between male and female in first period was 1,1:1(16 :14), and in second one 1,7 :1(17:10). The male and female ratio in first period was 1,6:1(19:12) and in second 1,4:1(39:29). Table 1 shows that 7 (22.6%) and 14 (20.1%) NHL were small lymphocytic type (A), 17(54.8%) and 47(69.1%) belonged to follicular center cell types (B-G), but the large cell immunoblastic type (H) has been diagnosed in 5(16.1%) and in 6(8.8%) cases. In first period in 5(16.1%), and in second one in 8(11.8%) patients, NHL have been primary located extranodaly (stomach and small intestine mostly). 3(4.4%) NHL were T-cell type. The mean age of NHL deseased in the first period was 60 years, and 59 years in second. Mean age

Table 1. Frequency and pathohistological pattern of the HL in Montenegro

HL type	1994-1996		1997-1999	
	%	n	%	n
LH predominance	43,3	13	18,5	5
Mixed cellularity	23,3	7	44,5	12
Nodular sclerosis	20,0	6	33,3	9
Lymphocytic depletion	13,4	4	3,7	1
Σ	100,0	30	100,0	27

Table 2. Frequency and pathohistological pattern of the NHL in Montenegro

NHL	type	1994-1996		1997-1999	
		%	n	%	n
Low grade malignancy	A		7		14
	B	38,7	4	29,4	4
	C		1		2
	D		1		2
Intermediate grade malignancy	E	38,7	2	60,3	14
	F		5		11
	G		4		14
	H		5		6
High grade malignancy	I	22,6	1	10,3	1
	J		1		-
Σ		100,0	31	100,0	68

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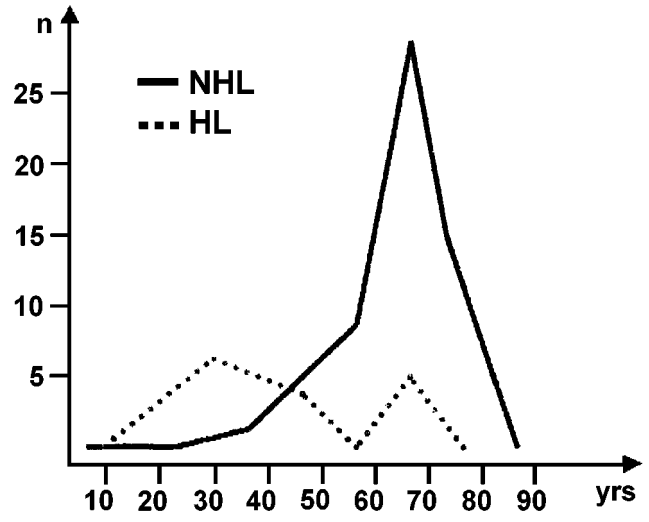
of HL deseased patients in first period was 39 and in second 47 years.

DISCUSSION

Comparing with the first three year's period (1994-1996), in second period (1997-1999) we have noticed the increase of diagnosed NHL (219%), and in the same time the increase of population in Montenegro was only 5 % : from averagely 638000 inhabitants in the first period up to averagely 655000 in second (2). The incidence of diagnosed ML in both periods can be considered as the incidence in epidemiological sense, with the possibility of discreatly higher value of the real desease incidence due to the fact that the part of population from northern parts of Montenegro is traditionaly oriented to medial institutions in Serbia. Migrations of population in both periods were significantly limited by nonmedical and medical reasons.

Figure 1. Age distribution of the HL and NHL diagnosed from 1997-1999 („Immunohistochemical" period)



The average incidence of NHL in the last three year's period has been 3,4\100000 inhabitants (range of 3,0-4,4) and significantly surpass the same value noticed in the period of „morphological" ML diagnose (maximal 1,5) We consider that overdiagnose of HL in the period of pure „morphological" diagnoses is one of reasons of so dramatic growth of NHL incidence, and the planned immunohistochemical material revision from the first period will show that number of NHL is diagnosed wrongly as HL. The last consideration has been indirectly confirmed with almost the same frequency of HL and NHL (49:51) noticed in period of „morphological" diagnose - this frequency of HL among all ML is far over values which have been mostly mentioned in literature (3,4,5), though it has been approuching to those which Lennert noticed in Germany between 1973 and 1976 : 43.6% HL : 56.4% NHL (6). ML incidence in Montenegro has been, with all mentioned limits, significantly lower (1-2 times) according to those in Hungary or Poland, while incidences in countries which are far away, geographically and\or socio-economically (Swicerland, Great Britain, France, Italy) have been surpassed many times.(5). Mostly diagnosed in both periods are NHL low and intermediate malignancy, follicular center cell types (54.8% and 69.1%), and small lymphocytic type (22.6% and 20,1%. Previous results compared with relevant data from Statistic year book of Montenegro (1) support the conclusion of epidemists (3) about existing characteristic NHL patterns , geo-socio-economicaly caused : Montenegro is undeveloped (69.1% follicular center cell types NHL) agricultural region on the border of Europe (20.1% small lymphocytic NHL), haven't been still integrated in modern economic, viral and chemical „flows" (8,85 immunoblastic type NHL), with low incidence of ML.

CONCLUSION

The most frequent in both periods are follicular center cell types NHL (54.8% and 69.1%); small lymphocytic NHL has also been frequent (22.6% or 20.1%) The overdiagnose of HL in the period of pure „morphological" diagnose is one of reasons for established growth of NHL frequency in „immunohistochemical" period.

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Immunohistochemical caracteristics of primary malignant bone tumors

ABSTRACT

A histological and immunohistochemical analisis os 86 primary malignant bone tumors was made in order to deterninate the immunophenotype of these neoplasms. The standard procedure of paraffin embeded tissue samples stained with Hemotixylin-Eosin was applied and immunohistochemical dyeing was made using avidin-biotin immunoperoxidase technique with the antibodies CKWS, vimentin, desmin, S-100 protein, NSE and LCA, for 20 osteosarcomas, 20 chondrosarcomas, 23 Ewing's sarcomas and 10 fibrosarcomas and antibodies IgA, IgG, IgM, kappa, lambda, LCA and vimentin for 13 myelomas. Chromogranin and synaptophysin were added to the group of Ewing's sarcomas. The presence of CKWS was demonstrated in 3 osteosarcomas, 1 chondrosarcoma and 2 Ewing's sarcomas. Vimentin was present in 17 osteosarcomas, 17 chondrosarcomas, 13 Ewing's sarcomas and 10 fibrosarcomas. Desmin was present in 1 osteosarcoma and 1 Ewing's sarcoma, S-100 protein in 7 osteosarcomas, 17 chondrosarcomas, 10 Ewing's sarcomas and 1 fibrosarcoma, NSE in 12 osteosarcomas, 16 chondrosarcomas, 14 Ewing s sarcomas and 1 fibrosarcoma, osteocalcin only in 10 cases of osteosarcoma. PNET-s showed positivity with vimentin, NSE, S-100 protein and synaptophisin. IgG positivity was demonstrated in 3 myelomas, kappa in 7, lambda in 6 cases of myeloma. None of the tumors stained with LCA, IgA, IgM and chromogranin. The antibody panel of immunohistochemical dyeing proved sucsessful in differentiation of particular types of PMBT within the histological pattern, but it did not provide an indication of strict specificity, except for osteocalcin antibody.

KEY WORDS: Bone Neoplasms; Immunohistochemistry

INTRODUCTION

Immunohistochemistry as a practical application of the immunological principles in cells and tissues is based on the relation between the tissue structural elements and chemical immunoglobulin activity, which actually represents the strong affinity between antigen and antibody1. In surgical pathology immunohistichemistry is a technique used for making objective diagnosis and solving some differential diagnostic problems. It is used in infective pathology, also for differenting neoplastic from reactive lesions, detecting hormone production and determining cells types and origin. Bone tumors are often a diagnostic problem to the pathologist, not only because of their rarity but also because they represent a small part of diagnostic experience of most general pathologist. The clinical manifestations of the most bone neoplasms are very unspecific, pain, swelling, general failure, but some of them like localization and age and radiographic appearance make the pathologist able for rational differential diagnosis. The knowledge of the immunohistochemical characteristics of primary malignant bone tumors (PMBT) would complete the diagnostic procedures in every day practice.

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MATERIALS AND METHODS

A histopathological analysis of 86 PMBT was made on the Institute of Pathology, Medical Faculty in Skopje, in period of 7 years. There were 20 osteosarcomas, 20 chondrosarcomas, 23 Ewing's sarcomas, 10 fibrosarcomas and 13 myelomas. The following techniques were used for making the diagnosis: paraffin embedding, 3-5 microns thick cuts, staining with Hematoxilin-Eozin, Van Gieson, PAS, reticulin and Gimsa. All the cases were additionally stained by avidin-biotin immunoperoxidase technique with the following antibodies: CKWS (DAKO, polyclonal, 1:400), desmin (DAKO, monoclonal, 1:100), vimentin (DAKO, monoclonal, 1:25), S-100 protein (DAKO, policlonal, 1:100), NSE (DAKO, monoclonal, 1:100), osteocalcin (SIGMA, 1:1), IgA (DAKO, monoclonal, 1:15), IgG(DAKO, monoclonal 1:50), IgM (DAKO, 1:50), kappa (DAKO, monoclonal 1:50), lambda (DAKO, monoclonal 1:50), chromogranin A (DAKO, 1:100), synaptophysin (DAKO, monoclonal, 1:100), LCA (DAKO, monoclonal, 1:100). The immunohistochemical results were gruped as: 1. negative- the tissue cellular elements were not coloured et all; 2. week positivity- approximatively 25% of the tissue cellular elements were coloured; 3. moderate positivity- approximatively 50% of the tissue cellular elements were coloured; 4. strong positivity- more than 50% of the tissue cellular elements were coloured. So a quatitative graduading of the results was made, using semiquantitative method in a free evaluation of the percentage by the pathologist. A statistical analysis was made ussing Crusscal-Wallis analysis, Mann-Whitney U test and Caplan-Maier life tables (statistical software SPSS). A panel of CKWS, vim, des, LCA, S-100, NSE and osteocalcin was used for histochemical staining of osteosarcomas, chondrosarcomas, Ewing's sarcomas and fibrosarcomas. Chromogranin and synaptophysin antibodies were applied to the group of Ewing's sarcomas. IgA, IgG, IgM, kappa, lambda, LCA and vim antiboddies were applied to the group of myelomas.

RESULTS

The subtypes of the primary malignant bone tumors were determined (Table 1).

The results of the immunohistochemical analisis are listed in Table 2.

None of the tumors stained with LCA, IgA, IgM and chromogranin and the 3 of PNET-s demonstrate vimentin positivity in 1/3, NSE in 3/3, S-100 in 1/3, synaptophysin in 3/3 and des in 1/3. Positive cases of PMBT showed a mixture of positive and negative cells with different intensity of colouring, except the cases where positivity was diffuse in all cellular elements (6 osteosarcomas, 8 chondrosarcomas, 12 Ewing's sarcomas, 6 fibrosarcomas and 9 myelomas). There was a highly significant statistical difference (p`0.01) for vim betveen the groups of Ewing's sarcoma and fibrosarcoma, for NSE between the groups of osteosarcoma-Ewing's sarcoma and osteosarcoma-fibrosarcoma, for S-100 between the groups of osteosarcoma - chondrosar-

Table 1. Subtypes of PMBT

Osteosarcoma		Chondrosarcoma		Ewing's sarcoma		Fibrosarcoma		Myeloma	
subtype / No cases									
osteoblastic	8	conventional	14	diffuse	12	low grade	4	mature	4
fibroblastic	3	mesenchimal	4	lobular	6	high grade	6	intermediate	2
chondroblastic	2	clear cell	1	organoid	2			imature	6
gigant cell rich	2	dedifferentiated	1	PNET	3			plasmablastic	1
small cell	3								
low grade central	1								
theleangiectatic	1								

Table 2. The immunophenotype of PMBT

Antibody	Osteosarcoma				Chondrosarcoma				Ewing's sarcoma				Fibrosarcoma				Myeloma			
	-	+	++	+++	-	+	++	+++	-	+	++	+++	-	+	++	+++	-	+	++	+++
CKWS	17	3			19	1			21	2			10							
VIM	3	3	8	6	3	4	6	7	10	5	5	3		1	3	6				
DES	19	1			20				22	1			10							
LCA	20				20				23				10							
NSE	8	9	3		4	5	6	5	9	2	4	8	7	2		1				
S-100	13	4	2	1	3	4	5	8	13	2	1	7	9	1						
OSTEOCAL	10	7	1	2		20			23				10							
IgA																	13			
IgG																	10	2	1	
IgM																	13			
KAPPA																	6			7
LAMBDA																	7		1	5

coma and significant difference (p`0.05) for the groups: osteosarcoma-Ewing's sarcoma and osteosarcoma-fibrosarcoma. Statistical analysis of other immunohistochemical parametars wasn't possible because of their small number. Caplan-Maier life tables showed the best percent of cumulative expected surviving for cases which obtained strong positivity of S-100 protein, NSE and vim in the whole group of PMBT.

DISCUSSION

Only a small group of PMBT were a real diagnostic problem. The most difficult was to differentiate small round cell tumors as were Ewing' sarcoma/PNET, small cell osteosarcoma and mesenchimal chondrosarcoma (2,6,9,14,16). The differentiation of small round cell tumors, by light microscopy, is allmost unpossible. Immunohistochemical dyeing didn't show significant differences between Ewing's sarcomas and PNET-s except synaptophysin dyeing which was positive et all 3 cases of PNET and negative in all Ewing's sarcomas. The small cell osteosarcomas demonstrated a presence of osteocalcin, which was absent in Ewing's sarcoma and mesenchimal chondrosarcoma. Tadashi Hasegawa at al (10) reported immunohistochemical study of 18 histologicly different subtypes of osteosarcomas in which a positivity of vim and osteocalcin was demonstrated. The presence of one or more neural markers indicates a Ewing's type of tumor, although NSE and S-100 can be found in osteosarcoma as well as in chondrosarcoma. A.M. Dierick (7) made an immunohistochemical studies of 45 Ewing's sarcoma and found out positive reaction with vim, NSE and synaptophisin and negative reaction with des, actin, LCA, keratin and chromogranin. According to H. Jurgen (11-13) positive NSE staining is not specific for Ewing's sarcoma, but for PNT and neuroblastoma. The immunohostochemical positivity of NSE and S-100 protein is not of much help in diagnosing mesenchimal chondrosarcoma. The reported results of K. Stefansson (17) about the presence of vim and S-100 in chondrosarcomas are similar with ours. The data about the presence of NSE in chondrosarcomas are usually discussed in mesenchimal type of this neoplasm. The differentiation of the malignant lymphomas is made easier by using lymphocitic markers, which by the rule are negative in the other malignant bone tumors (4,5,15). Resultats of our seria in the group of myelomas showed a presence of IgG in 23.07% and only light kappa and lambda chains in 76.92% of the cases. D. E. Berrgsagel (3) reported that myeloma cells secret IgG in 52% and light chains in 11%. Because of these reasons the group of myelomas need further more extensive examination. The antibody panel of immunohistochemical dyeing have been proved successful in differentiation of particular types of PMBT within the histological pattern, but it did not provide an indication of strict specificity, except for osteocalcin antibody.

CONCLUSION

The antibody panel of immunohistochemical dyeing proved sucsessful in differentiation of particular types of PMBT within the histological pattern, but it did not provide an indication of strict specificity, except for osteocalcin antibody.

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The incidence and prognostic evaluation of the histological changes in the bone marrow biopsies from patients with non Hodgkin's lymphoma (NHL)

ABSTRACT

The aim of this study was to evaluate the histological changes in the bone marrow in patients with diagnosed NHL, and to determine their prognostic significance. There were included 189 patients with diagnosed NHL on nodal and extra nodal tissue who had undergone the bone marrow biopsy for the purpose of staging the disease. The patients were followed for nine years with a minimal 4-month follow up. The clinical data were taken from the histories of the patients. Standard histochemical stainings as well as immunohistochemical staining were done on paraffin embedded bone marrow biopsies. The histological analysis of the bone marrow biopsies taken from the patients showed the presence of the lymphomatous infiltration in 79 cases or 41.8%, that didnít show a significantly different influence on survival between the groups. The presence of tumor myelopathy was found in 117 cases (61.9%) and the patients had worse survival than the others. The patients with osteolytic and osteoporotic changes (14.3% and 35.4% respectively) had worse survival than the patients with osteosclerotic (5.8%) and normal bone marrow trabecules (44.4%).The probabillity to survive was better in the group with normal cellularity than the aplastic bone marrow and hypercellular marrow spaces. The presence of dysplasia in the three cell lines was a bad prognostic factor: 32.8%, 29.8% and 29.1% of the patients with dysplasia survived after 36 months from the beginning of the disease, and for longer period these percents fall down to 10.9%, 9.9% and 9.7%. The increased number of eosinophils doesn't influence the survival. Changes in the stromal tissue didn't influence significantly the survival in the patients with NHL.

KEYWORDS: Lymphoma, Non-Hodgkin; Bone Marrow Examination; Prognosis

INTRODUCTION

The bone marrow biopsy is a useful diagnostic method with prognostic significance in the patients with NHL-s. Besides its role in determining the stage of the disease, this method enables a diagnosis of NHL when there is no lymphoma in the lymph nodes or the lymphoid tissue is bad preserved. The examination of the bone marrow biopsies in the patients with NHL-s enable identification of the architecture and behavior of the normal haemopoietic tissue and lymphomatous infiltration, that has a prognostic relevance (1,2). It was shown that involving of the bone marrow, histological type of the NHL and symptoms have the biggest prognostic significance. The survival is lower in the group of patients with bone marrow involved with NHL, absence of the reactive lymphoid nodules as well as with reduced cellularity (3,5). The aim of this study was to evaluate the histological changes in the bone marrow in patients with diagnosed NHL, and to determine their prognostic significance.

PATIENTS, MATERIALS AND METHODS

There were 189 patients with diagnosed NHL on nodal and extra nodal tissue who were submitted to the bone marrow biopsy for the purpose of staging the disease. Out of this number, in 19 patients the bone marrow was examinated by an analysis of the aspiration biopsies. We used the REAL classification of the NHL-s that included 12 cases with hairy cell leukemia. The patients were followed for nine year with a minimal 4-month follow up. The clinical data were taken from the histories of the patients. The trephine biopsies were taken at the initial diagnosis of the disease. The trephines were decalcified for 18 to 24 hours in 7.5% threechlor acetic acid in 10% buffered formaline and afterwards were paraffin embedded. One part of the biopsies was fixed in 2.5% glutaraldechyde and hard resin embedded. For histological analysis paraffin sections were stained with Hemalaun-Eosin, Giemsa, Reticulin ñ Gomori; immunohistochemical staining for lymphocyte markers was also performed by a Streptavidin biotin immunoperoxidase technique. The analysed histological changes in the bone marrow were determined on semi quantitative principles described by Bartl et al (4) as followes: presence of the remodeling of the marrow spaces, cellularity, qualitative and quantitative changes in the hemopoietic cell lines (erythroid, myeloid and megakariocytic), the presence of eosinophils, changes in the reticulin network and sinusoidal spaces, presence of edema, extravasated erythrocytes and deposition of haemosiderin. Statistical parametric and nonparametric analysis, as well as analysis for numerical and attributive features were done. Survival analysis was done according to Kaplan Meier life table.

RESULTS

Histological analysis of the bone marrow biopsies taken from the patients found lymphomatous infiltration in 79 cases or 41.8%. The bone marrow involvement was found more often in the group of lymphomas with indolent course (according to the REAL classification), and less often in the groups of the aggressive and very aggressive lymphomas, 22.8% and 12.7% respectively. Our survival analysis showed that there was no significant difference in the survival between the group with bone marrow involved with NHL and the group with uninvolved bone marrow.

The presence of disturbed histoarchitecture of the marrow spaces or tumor myelopathy was found in 117 (61.9%). In 38 of these patients there were no clear signs of a lymphomatous infiltration in the bone marrow ($Hi=10,663$, $DF=1$, $p<0,01$). However, the patients with tumor myelopathy showed worse survival than the others, and the difference was significant in the first 12 to 24 months of the disease. The probability for survival for this period was 77.3% and 70.1% for the patients, with regular histoarchitecture, and 54.8% and 52.4% for the patients with present tumor myelopathy. The changes in the bone marrow trabeculae remodeling were defined as osteolytic, osteoporotic, osteosclerotic and normal. The patients with osteolytic and osteoporotic changes (14.3% and 35.4% respectively) had worse survival than the patients with osteosclerotic (5.8%) and normal bone marrow trabecules (44.4%). The cellularity was determined as normocellular (32.3%), aplastic (10.6%), increased cellularity (13.2%), decreased cellularity (27.5%) and marrow with loosened fatty cells (16.4%). Kaplan Meier life table showed

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greater probabillity to survive in the group with normal cellularity than the aplastic bone marrow and hypercellular marrow spaces. The group with packed bone marrow had similar survival to the group with normal cellularity, owing to that they belong to the patients with hairy cell leukemia and small lymphocytic lymphomas, that usually have better survivals in all other analysis of clinical and histological parameters. The changes in the hemopoietic cell lines are shown in Table 1.

Survival analyses showed a bad influence of dysplasia in the three cell line on the survival of the patients: 32.8%, 29.8% and 29.1% of the patients survived for 36 months from the beginning of the disease, and for longer period these percents fall down to 10.9%, 9.9% and 9.7%. The presence of an increased number of eosinophils does not influence the survival. The reticulin network used to be normal (56.6%) , slightly increased (28%), dense network with collagen fibrosis (14.8%). For longer survival the mortality was increased in the group with slightly increased reticulin network. Changes in the sinusoidal spaces (collapsed, dilated - empty and dilated - full with haemopoietic cells) do not significantly influence on the survival in the patients with NHL-s. Other stromal changes in the bone marrow were: deposition of haemosiderin, extravasations of erythrocytes, presence of edema fluid, and changes in the reticuloendothelial cells. Comparative survival curves did not show any significant difference between the groups, except for the significantly shorter survival for the first 36 months in the groups that had hemosiderin deposition and edema fluid in the interstitial spaces.

Table 1. Distribution of the patients according to the maturation of haempoietic cell lines

Cell line	Regular maturation (%)	Arrest (%)	Dysplasia (%)	Hypolasia (%)	Other changes (%)
EL*	73 (38.6)	32 (16.9)	28 (14.8)	53 (28.0)	megalobl. (1.6)
GL**	78 (41.3)	21 (11.1)	38 (20.1)	51 (27.5)	eosinop. (21.2)
ML***	89 (47.1)	-	45 (23.8)	55 (29.1)	-

EL* - erythroid cell line, GL** - granulocytic cell line, ML*** - megakaryocytic cell line

DISCUSSION

We have analyzed 189 bone marrow biopsies taken from the patients with NHL for present histological changes in the histoarchitectural elements of the marrow tissue and evaluated their prognostic significance by Kaplan -Meier life table. In a study of 234 patients by univariate statistical analysis, it was found that the presence of lymphomatous infiltration, absence of lymphomatoid nodules, hypocellularity, myeloid hypoplasia, deposition of hemosiderin, increased number of mast cells and the age of the patients were bad prognostic factors (3). Other authors found that these parameters do not have any influence on survival in the patients with NHL-s (2). Our results showed that osteolytic and osteoporotic changes, as well as aplastic and hyperplastic bone marrow and dysplastic changes of the haemopoietic cells are bad prognostic factors. These results are in agreement with the results for the influence of the tumor myelopathy on survival in these patients. In fact, tumor myelopathy was consisted by the above-mentioned histological changes. The increased number of eosinophils is probably due to an increased production of eosinophylopoietin by tumor cells or stimulated normal T cells.

CONCLUSION

The increased number of eosinophils doesn't influence the survival. Changes in the stromal tissue didn't influence significantly the survival in the patients with NHL.

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Primary anaplastic large-cell lymphoma: morphologic, immunophenotypic and clinical characteristics

ABSTRACT

Anaplastic, CD30 positive, large-cell lymphoma (ALCL) is now a well recognized pathologic entity that accounts for 2% to 8% of all lymphomas. It is cytologically undifferentiated malignant lymphoma that need to be distinguished from a variety of other neoplasm using immunohistochemistry. A previously healthy 19-year-old female, 8 months after normal pregnancy and delivery, presented with mediastinal bulky disease, involvement of retroperitoneal lymph nodes and hepatosplenomegaly revealed by computerized tomographic (CT) scan. The diagnosis was obtained on review of the hematoxylin and eosin-stained slides and immunophenotype data. Immunohistochemical investigations of biopsy specimens revealed ALCL, null-cell lymphoma, positive to CD30 (Ki-1) antigen. Tumor cells showed also positive staining for vimentin and CD45 (leukocyte common antigen - LCA). Tumor cells were negative for B-cell marker (CD20), T-cell marker (CD3), epithelial membrane antigen (EMA), cytokeratin, S-100 and HMB-45. Immunophenotypic findings confirmed that ALCL, CD30 positive lymphoma, should be considered in the differential diagnosis of anaplastic tumor primary localized in mediastinum. The differential diagnosis may include anaplastic carcinoma, choriocarcinoma, malignant melanoma, sarcoma, Hodgkin's disease (HD) and histiocytic lymphoma.

KEYWORDS: Lymphoma, Large Cell, Ki-1; Antigens, CD30; Lymphoma, Non-Hodgkin; Immunohistochemistry

INTRODUCTION

One of the new members of the family of non-Hodgkin's lymphoma (NHL) which was not described in the older classification systems (Kiel and Workig Formulation), but defined in the revised European-American Lymphoma (REAL) (1,2) is anaplastic large-cell lymphoma (ALCL). It was first described in 1953. A consistent feature is the expression of the CD30 antigen, detected by the Ki-1 monoclonal antibody (mAb) (3). Before the availability of immunohistochemical staining by Ki-1 mAb, original diagnoses had been malignant histiocytosis and diffuse large cell immunoblastic NHL (4). Now, ALCL is a well recognized pathologic entity that accounts for 2% to 8% of all lymphomas.

CASE REPORT

The patient was a previosly healthy 19-year-old female, 8 months after normal pregnancy and delivery, who had a 2 weeks history of cough, left subscapular pain and easy fatiguability. She noted a great lump of left chest wall 10 days prior to her visit to the physician. She also reported a weight loss (10kg) in the previous 2 months. Physical examination revealed moderate hapatoslenomegaly with no palpable lymphadenopathy. The chest x-ray demonstrated a large anterior mediastinal mass and small pericardial effusion. CT scanning of the chest revealed moderate bilateral hilar adenopathy in addition to the anterior mediastinal mass. Abdominal CT revealed retroperitoneal lymph nodes enlargement and hepatosplenomegaly with involvement of peripancreatic, portal and splenic hilar lymph nodes. Laboratory studies revealed a hematocrit of 23%, Hgb 68g/L, platelets of 236x109/L and WBC of 14x109/L, with normal differential. The ESR was elevated at 75 mm/hr. Tests of liver and renal function were normal. The patient was HIV negative. During the hospitalisation, a right cervical lymph node was enlarged and its biopsy was performed.

RESULTS

The diagnosis was obtained on review of the hematoxylin and eosin-stained slides and immunophenotype data. Morphological features: The neoplastic infiltrate showed the typical appearance of common-type ALCL with diffuse involvement of a lymph node and cohesive growth pattern. The tumor was composed of large blastic cells with pleomorphic and eccentric nucleus with multiple (ussually) or single prominent nucleoli. Multinucleated forms (giant-cells) were rare. Mitoses were readily apparent (on the average two in high-power field). Non-neoplastic cells (lymphocytes and plasma cells) were scanty. Immunostaining of paraffin sections was performed using the method avidin-biotin immunoperoxidase. In order to avoid confusion of ALCL with anaplastic nonlymphoid tumors, we demonstrated negativity for cytokeratins, S-100 protein and HMB-45 antigens. Positive staining with vimentin and leukocyte common (CD45) antigen suggested the diagnosis of lymphoma. Anaplastic appearance of tumor cells and CD30 antigen expression revealed ALCL. Negative staining for CD3 (T-cell marker) and CD20 (B-cell associated antigen) determined #null# phenotype of tumor cells. Staining for EMA was also negative.

DISCUSSION

Immunophenotypic findings confirmed that ALCL should be considered in the differential diagnosis of anaplastic tumor primary localized in mediastinum. The differential diagnosis may include anaplastic carcinoma, choriocarcinoma, malignant melanoma, sarcoma, Hodgkin's disease (HD) and histiocytic lymphoma. The diagnosis of anaplastic carcinoma was excluded by negative cytokeratin staining. Choriocarcinoma was also considered because tumor appeared 8 months after termination of pregnancy. However, serum hCG levels didn't confirm gestational trophoblastic disease. Immunostaining for HMB-45 and S-100 protein was performed in the aim of excluding of malignant melanoma. Morphological features of tumor infiltration without inflammatory background and differential expression of CD30, CD45 and EMA was used in the separation of ALCL and HD (non-lymphocyte predominant). A great proportion (73%) of cases of ALCL expressed CD45+, CD30+, EMA±, EBV-, while in the same percentage of HD cases Reed-Sternberg cells (RS) were found to express the CD30+,CD45-, EMA-, EBV+ profile (9). Positive staining for CD30 readily permited the distinction of ALCL from hystiocytic malignancy. Single gold standard for the diagnosis of ALCL does not yet exist (7). The diagnosis requires both morphology and CD30 expression.

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The majority of tumors express T-cell associated antigens, many express neither T- nor B-cell- associated antigens („null" cells) and some cases express B-cell antigens (5). The recent availability of an antibody to the anaplastic lymphoma kinase (ALK) protein, which is highly associated with translocation t(2;5) (p23;q35) and a favourable prognosis can be useful as prognostic criterion for ALCL, but also can be applied to separate out the tumor subtype refered as ALK-lymphoma or „ALKoma" (8). The clinical presentation of this case was also adequate for the diagnosis of ALCL. ALCL occurs in all age groups, about 15-30% of the patients being under age 20 years (5,6). Clinically, two types of presentation are recognized: a systemic form (which may involve nodes or extranodal sites) and primary cutaneous form (5). Tumor that present with systemic extracutaneous disease are clinically more aggressive, but potentially curable with aggressive therapy (4).

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Adult Gaucher's disease - report of four cases

ABSTRACT

In this report, we describe four patients with "adult" Gaucher's disease. The clinical course of these patients was characterized by: progressive diffuse aseptic necrosis in the large bones and hepatosplenomegaly. Splenomegaly was followed by hypersplenism with anemia and thrombocytopenia because a splenectomy was performed. The dignosis of Gaucher's disease was made after Gaucher's cells were found on bone marrow biopsy. Tissue blocks were routinely processed. Slides were stained with HE (hematoxylin and eosin), staining for iron, and PAS (periodic acid - Schiff). Gaucher's cells were seen as large cells with granular or fibrillar distended cytoplasm, with the characteristic "wrinkled tissue paper", and excentric nuclei.

KEYWORDS: Splenomegaly; Hypersplenism; Gaucher's disease; Thrombocytopenia

INTRODUCTION

Gaucher's disease is an autosomal recessive lysosomal storage disease, resulting from a deficiency of the enzyme glucocerebcosidase, which is required for the lysosomal degradation of glycolipids. This leads to accumulation of the substrate glucocerebroside in phagocytic cells of the monocyte - macrophage lineage throughout the body. Gaucher's disease was first described by Philipe Gaucher in 1882. Mandelbaum in 1900 recognized the systemic nature of the disease. Rusca described a rapidly progressive fatal neurodegenerative type of the Type 2 disease in 1920. The "juvenile" form (type 3) of the disease was dercribed in Sweden in the 1950. In 1965, the deficient enzyme was discovered and the lysosomal nature of the disease was elucidated. Clinically, three subtypes are recognized: Type 1 (the adult form) is the prevalent form. It is characterized by sparing of the central nervous system. Type 2 (infantile form) is a rare acute form with progressive central nervous sistem manifestations leading to death before the age of 3 years. In type 3 (juvenile form) the neurological involment begins later, and the disease runs a more chonic course.

PATIENTS, MATERIALS AND METHODS

Case 1: A 19 year-old woman had progressive diffuse aseptic necrosis in the large bones, so called Erlenmeyer flask deformity, and progressive hepatosplenomegaly. The liver edge palpable at 8 cm. below the right costal margin. The spleen edge palpable at 16 cm. below the costal margin down to the pelvis. She had hypersplenism with anemia and thrombocytopenia. The

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diagnosis of Gaucher's disease was made after the bone marrow biopsy wich revealed typical Gaucher's cells. The splenectomy was performed because of severe anemia and thrombocytopenia. Pathological findings: The spleen weighed 4230 gr. It showed multiple nodules in the size of 0,5 - 2,5 cm. and areas of subcapsular infarcts. Histologically, a large proportion of the spleen tissue was infiltrated by mononuclear cells. These were histiocytic in nature. In HE preparations, the cytoplasm appeared pale and distended whereas nuclei were centrally located and displaced towards the periphery in others. These spaces contained typical Gaucher's cells with distended cytoplasm and eccentric nuclei. The fine "scroll - like" pattern in the cytoplasm is a characteristic feature. The periodic acid - Schiff staining showed strongly positive material in the cytoplasm of these histiocytic cells. This PAS - positive material appeared either granular of fibrillar in the typical Gaucher's cells.

Family history: her older brother was affected with the same disease.

Case 2: A 17 year-old man, with episodes of weakness, paleness, fever and epistaxis, chronic abdominal pain. During his hospitalization hepatosplenomegaly were observed. The spleen edge palpable at 6 cm. below the costal margin. He had hypersplenism with anemia and thrombocytopenia. The diagnosis of Gaucher's disease was made after the bone marrow biopsy where typical Gaucher's cells with the characteristic "wrinkeld tissue paper" apperance were found. PAS staining showed strongly postive granular or fibrillar material in the distended cytoplasm of the tipical Gaucher's cells. Family history: He was the only affected member in his family.

Case 3: A 65 year-old woman. The clinical symptoms developed sixteen years ago. She had diffuse hemorrhages and hemathomas, lymphnode enlargement, hepatosplenomegaly, chronic abdominal pain. The liver edge palpable at 4-5 cm. below the right costal margin. The spleen edge palpable at 15 cm. below the costal margin. She had hypersplenism with anemia, thrombocytopenia and pancytopenia. The diagnosis of Gaucher's disease was made after Gaucher's cells were found on the bone marrow biopsy. The bone marrow tissue contained typical Gaucher's cells stained for HE and PAS, and hemosiderin laden macrophages stained for iron. Family history: her brother died from enlarged "hepatosplenomegaly".

Case 4: A 66 year-old man with episodes of weakness, paleness, chronic abdominal pain and hepatosplenomegaly. The spleen edge palpable at 8 cm. below the costal margin. He had hypersplenism with anemia, thrombocytopenia and pancytopenia. The diagnosis of Gaucher's disease was made after Gaucher's cells were found on the bone marrow biopsy. Family history: he was the only affected member in his family.

DISCUSSION

The clinical course in these four patients was characterized by hepatosplenomegaly. Splenomegaly was followed by hypersplenism with severe anemia and thrombocytopenia. The diagnosis of Gaucher's disease was made after Gaucher's cells were found on the bone marrow biopsy. There was a correlation between the degree of spelnomegaly and the parameters of hypersplenism because a splenectomy was performed. Splenectomy was effective in correcting thrombocytopenia, anemia and eliminating the distress caused by the massively enlarged organ. The treatment in all ours patients was symptomatic.

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Meeting abstract
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Immunohistochemical analysis of the metastatic deposits with unknown primary location of the tumor

KEYWORDS: Immunohistochemistry; Neoplasms, Unknown

Immunohistochemical technique is an important additional method to the routine light microscopy in the diagnosis of the human neoplasms, especially important in the discovery of the metastatic deposits when their origin is not known. 16 metastatic foci with unknown location of the primary malignant process are explored in this paper. In order to get more precise data and verify the origin of the metastatic process, immunohistochemical analyses of the metastatic deposits was made. For the purpose of this paper, bioptic material form the Institute of Pathology, Medical Faculty in Skopje was used. The material was paraffin embedded, routinely stained with HE, and histochemically stained with Giemsa, PAS, Reticulin and Alcian Blue. For immunohistochemical analyses the Avidin-Biotin Peroxidase Method of Hsu was used on paraffin sections with the following panel of monoclonal antibodies: CKWS, CK13, CK18, CK19, ESA, Vimentin, CEA, Synaptophysin, NSE, HMB45, S-100, Desmin, Actin, LCA, CD3, CD20 HLA-DR. Out of 16 metastatic foci, 8 were located in lymph nodes, 3 in bone marrow, 3 in bone tissue and 2 in brain. The metastatic deposits in the lymph nodes in 6 cases were found to have glandular origin, with suggested primary site in the gastrointestinal tract, breast or lungs. The histological findings of foci of epithelial cells with signs of anaplasia, with growth pattern varying from solid sheets of cells to papillary structures, some of them PAS and Alcian Blue positive, implied further immunohistochemical analyses with marked positivity for CKWS, CK19, CK18 and CEA, while the lymph and mesenchimal markers remained negative. In one case metastasis from an amelanotic melanoma with finding of atypical cellular proliferation, marked pleomorphism of the cells, large nuclei and visible nucleoli, marked reticulin network, and absent melanotic pigment was found in a lymph node. In order to make differential diagnosis with a polymorphous liposarcoma, pleomorphous rhabdomyosarcoma or poorly differentiated squamous carcinoma, immunohistochemical analysis with marked positivity on S-100 protein and HMB45, and negativity on Desmin, Vimentin, CKWS (+/-) and MAC, was done. The metastasis in one lymph node originated from small cell lung carcinoma and it was made of oval and spindle oat-cells with scanty cytoplasm, forming strands or bundles, which immunohistochemically showed positivity for CKWS, CEA, S-100 protein, NSE, Synaptophysin and Chromogranin, and negativity for LCA and HMB45. The metastatic deposits in the bone marrow (total of 3 cases) in 2 cases showed epithelial neoplasm with positivity for CKWS, CK19, CK13 and CEA, with suggestion to exclude hypernephroma, gastrointestinal or lung carcinoma. In one case, a metastatic lymphoma was found in the bone marrow, with postitivity for LCA, CD20, HLA DR, while the residual myeloic cell line showed positivity for CD3 and MAC387. All three metastatic deposits in the bone biopsies had epithelial origin with positivity for CK18, ESA, CEA and marked positivity for p53. Both brain metastases had characteristics of epithelial origin of the tumor, with positivity for NSE and Chromogranin, which suggested that oat-cell carcinoma of the lung should be excluded. LCA negative finding excluded the differential diagnostic possibility of metastatic lymphoma. The immunohistochemical analyses of the metastatic deposits with unknown origin of the primary tumor node are of significant help in the differentiation of the process, associated to the basic histological methods and additional histochemical analyses.

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Correlation of cytologic and histopathologic findings of bone tumors

KEYWORDS: Bone Neoplasms; Cytodiagnosis

The cytologic analysis of imprints is a common procedure in many fields of cytology. The objective of this study is to determine the role of imprint cytology in the diagnosis of bone tumors. From 25 skeletal lesions in 22 patients imprints were made, air dried and stained with May-Grunwald Giemsa method. The findings were compared with histopathologic diagnoses. In eight patients with primary bone tumors cytologic and histopathologic findings were consistent in all cases, as well as in six patients with metastases. Of eight benign bone lesions cytologic and histopathologic diagnoses correlated in seven cases and in the patient with fibrous displasia of the bone, a cytologist suggested fibrosarcoma. Cytologic analysis of imprints of bone lesions is valuable for early orientation of the clinicians because the procedure of decalcination needs time.

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Histological and immunohistochemical characteristics of the plasma cell dyscrasias

KEYWORDS: Paraproteinemias; Immunohistochemistry

The aim of this paper is to show the role of the immunohistochemical technique in the differentiation of the reactive from malignant lymphoproliferative processes. Two cases of plasma cell dyscrasias from the autopsy material at the Institute of Pathology in Skopje, differentiated with the usage of the histological (HeEo, Giemsa) and immunohistochemical analyses, are shown. The method of Streptavidin Biotin Peroxidase Technique using a panel of monoclonal antibodies: kappa (k), lambda (l), IgA, IgG, and IgM was performed. The patients were male and a female, at the age of 65 and 57, respectively. Standard autopsy technique and vigorous histological and immunohistochemical analysis were used. In both cases, the basic pathological finding was proved to be plasma cell dyscrasia: Immunocytoma (case No. 1) and Plasmacytoma (case No. 2). The diagnosis was confirmed with immunohistochemical analysis, where atypical lympho-plasma cell infiltrates in the tissue specimens taken from the visceral organs were with monoclonal nature: k-, l+ (case No. 1) and k+, l- (case No. 2). Besides, the stainings for the other markers showed the following results: IgG+, CD10+, CD56+. The immunohistochemical technique is important for the differentiation of the monoclonal from polyclonal lympho-plasma cell dyscrasias, since they may appear in chronic inflammatory processes, too. The confirmation of the monoclonal characteristics of the same, implies the neoplastic proliferation in these two cases.

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Cytomorphological monitoring of patients with transplanted hematopoietic stem cell

KEYWORDS:Hematopoietic Stem Cell Transplantation; Hematopoiesis

The method of transplantation of hematopoietic stem cells was in early fifties very interesting for researchers when the first transplantation on experimental animals was performed. The first allogenic transplantation from unrelated donors in human medicine was done in 1958 in Paris (Mathe, et al.) on our physicists who had been accidentally radiated by lethal doses in the Institute in Vinca. All five of them who had been radiated had survived thanks to the application of this treatment method. In the early seventies, with the better knowledge of immunology and having discovered new hemiotherapeutic and immunosuppressive medicines, the intensive application of this type had begun. The source of stem cells was firstly from the bone marrow and later for the same purpose the peripheral blood was also used. In 1985. the team for transplantation was formed in the Military Medical Academy and since 1989. this type of treatment has been routinely applied, first in hematological diseases and later in the other diseases (solid tumors, immunodeficient conditions as well as in some congenital diseases). The task of this team was to determine the indications for the treatment, and then to select donors (HLA tipization, ABO-system) and to prepare a patient for the transplantation (hemiotherapeutic medicines, TBI). During transplantation it is predicted for a patient to receive through myeloinfusion an average 2 x 108 of mononuclear cells per body weight. The post transplantation phase is characterized with pancytopenia, the period of early hematopoiesis reconstitution (two weeks) and the period of late reconstitution (8 weeks for the hematopoiesis reconstitution and development of acute or chronic GvHD, but the immunologic reconstitution takes more - six months, while for the reconstitution of T lymphocytes it takes a year or two if GvDH exists). In the period from 1989. till March 2000. 88 TMCH were performed. In the diagnostic of hematological diseases FAB and MIK classifications were used (morphologic, immunophenotypic and karyotypic). The diagnostic was histopathologically confirmed but the other diagnostic procedures were used. According to indication there were: M-7, CML-23/T-4 and chronic phase 19, AML-9, SCID-1, AML-19, MDS-T-4, MH-9, NHL-8, Ewing-sa-2, M multiplex-3, Ca testis-1, Ca mammae-2. According to the type of transplantation there were 49 allogenic, 39 autologous and 7% were retransplanted. According to the source of hematopoietic stem cell there were 34 from the bone marrow and 54 from the peripheral blood. In all transplanted cases we controlled the reconstitution of hematopoiesis and it was measured by hematological parameters each day. Criteria for the allograft acceptance were the number of polymorphonuclear more than 500 and platelets over 20.000 during three days. The analysis of the bone marrow was done on 14th and 28th day. The cytogenic analysis (sex chromosome or cytogenic markers) was done after 40 days depending on the reconstitution of hematopoiesis. In all our patients (88) we registered the acceptance of allograft what meant the reconstitution of hematopoiesis. The further destiny depended on complications, i.e. GvHD development. Having compared the dynamics of hematopoiesis reconstitution, weather the source of stem cells was from the bone marrow or peripheral blood, faster recovery was noticed in the group who received stem cells from the peripheral blood what was in accordance with data from the large centers for TMCH. The faster recovery decreased the risk from complications due to cytopenia-infection and hemorrhagic syndrome.

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K I - 1 lymphoma - a case report

KEYWORDS: Lymphoma, Large-Cell, Ki-1; Immunohistochemistry

The authors have shown a patient, age 54, who was hospitalized in the Institute of Oncology after a diagnosed Hodgkin's lymphoma in another facility, for an adequate therapeutic treatment. After the analysis of the received histological material, the earlier diagnosis was brought into question, so additional pathohistological examinations were done. From the received paraffin block of the biopsy of a lymph node on the neck we prepared histological sections for histochemical analyses with PAS, Reticulin, Giemsa and MGP methods and imunohistochemical phenotyping with EMA, LCA, CD-3, CD-20, CD-30 (Ki-1) Ki-67 and Alfa 1 antichymotrypsin markers by „DAKO". The Negative control group consisted of original factory substrates, suggested by the manufacturer for each antibody. By a light-microscopic analysis of the results gained by using the mentioned histological techniques, the abnormal architecture of a lymph node was evidenced, in which a population of numerous, large, polymorphic, polygonal, multinuclear, anaplastic giant cells were noticed, with high nucleo-cytoplasmatic ratio which were PAS negative and discretely pyroninophilic. By imunohistochemical analyses an extremely positive reaction with LCA, moderate co-expresion with CD-3 and CD-20 (in ratio 60 % : 40 %) and positively in traces with EMA and Alfa 1 antichymotrypsin was evidenced. At the same time an extremely positive reaction of the described cells with CDK-30 (Ki-1), and negative Ki-67 was evidenced. Based on the evidenced morphology and the manifested histochemical and imunohistochemical quality of neoplastic cells in the lymph node the final diagnosis was given: Anaplastic large cell lymphoma type Ki-1.