OUR EXPERIENCE IN THE FLOWCYTOMETRIC DIAGNOSIS OF MALIGNANT HEMOPATHIES

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Abstract: *The discovery of monoclonal antibodies made possible the immunological investigation of malignant hemopathies, facilitating the positive diagnosis. We are presenting several cases of acute leukaemia, chronic lymphoblastic leukaemia and one of multiple myelomas in which flowcytometry was useful for establishing the diagnosis. Today, flowcytometry represents an indispensable instrument for the malignant hemopathies study.*

Keywords: *immunological diagnosis, flowcytometry, acute leukaemia, chronic lymphocytic leukaemia, multiple myelomas.*

Rezumat: *Descoperirea anticorpilor monoclonali a făcut posibilă investigarea imunologică a hemopatiilor maligne, fapt care a uşurat diagnosticul pozitiv. Prezentăm câteva cazuri de leucemii acute, leucemii limfatice cronice şi unul de mielom multiplu la care flowcytometria a fost utilă pentru precizarea diagnosticului. Astăzi, flowcytometria constituie un instrument indispensabil în studiul hemopatiilor maligne.*

Cuvinte cheie: *diagnostic imunologic, flowcytometrie, leucemie acută, leucemie limfatică cronică, mielom multiplu*

INTRODUCTION

The use of monoclonal antibodies in the immunologic study of the malign hemopathies allowed the establishment of precise diagnoses, useful for choosing a proper treatment and for the prognostic formulation.

CLINICO-BIOLOGICO-HYSTOLOGICO-IMMUNOLOGIC CORRELATIONS

Hereby, we present a few cases in which flowcytometry was essential for establishing the diagnosis and for further conduct.

The patient GN, aged 21 was hospitalized in the emergency medical section for asthenia, fever, generalized ecchymoses, paleness, effort dyspnoea, inappetence, weight loss. Objective – fever, intense paleness, ecchymoses at the level of limbs, left laterocervical microadenopathies, bilaterally axillary (diameter maxim 1 cm) and bilaterally inguinally; normal liver, spleen. Hemoleucogramme showed: L 158300/mm³, Hb 6,5g/dl, Ht 18,4%, Tr 22000/mm³. The bone marrow showed an osseous with altered architecture, with a cellularity of about 95-98%, with diffuse infiltration through atypical lymphoid proliferation of lymphoblast of slightly increased sizes and pleomorph aspect, with nucleolated nucleus; the elements of the other medullar lines were quantitatively reduced, disorganized, with reduced maturation; reticulinic fibrosis areas disposed in a network of fine fibres, Myelogramme showed the presence of metaplasia with atypical blastic cells in proportion of almost 73%. The blasts presented the features of the lymphoid series, with the size between 9-18 micrometers, with round nucleus, reticular chromatin, sometimes more dense, 1-2 nucleoli, more or less visible, the cytoplasm was in the majority of cases intensely basophilous, large enough, sometimes reduced, revealing the nucleus; rare atypical mitoses, relatively frequent Gumprecht nuclear shadows; cellular series were hypoplasic marked, frequently free nuclei, possible of blastic etiology. Conclusion: possible acute leukaemia. LAL- L1. Flowcytometry established the following phenotype: CD45+, CD19+, HLA-DR+, cyTdT+ (17%), AC133-1+, cyCD79a+ (25%), CD15+ (45%); negative markers: CD5, CD10, CD34, CD13, CD33, CD7, CD3, CD16, CD56, GlyA, cyCD3, cyMPO, sIgM, CD20. Thus, the diagnosis of medullar proliferation on B lymphoid line was established: acute lymphoblastic leukaemia (ALL) – proB, with partial expression of myeloid markers (CD). This diagnosis allowed making the proper scheme of treatment and the establishment of prognostic (negatively influenced by the presence of myeloid markers).

The patient CI, aged 20, presented for about 2 months an asthenia marked by mucotegumentrary paleness, weight loss (2 kilos in two months), hollow cough. He came to the hospital with fever (38^0C), shiver, marked paleness, hepatosplenomegaly (liver with the anterior edge 4 cm under rebord, spleen with the inferior pole at the level of umbilical cord, with the increased consistency and adenopathies under the right submaxillary of 1 cm, and right axilla of 0,5 cm. Biologically, he had important inflammatory syndrome, severe anemia, moderated leukokeratosis with 65% blasts in periphery (negative-myeloperoxydazo and positive PAS), normal trombocytes. Biochemically – hepatocytolisis and cholestasis syndrome, antigen HBs – positive, anti-VHC antibodies – negatives, HIV –

negative, VDLD – negative. At haematological level: L 27500/mm³, Hb 6,3g/dl, Ht 17,4%, Tr 192000/mm³, reticulocytes 01,%, leukocytary formula: blasts 54%, Mc 2%, N1,S17,E0,B0,L24,M2%, trombocytes in normal groups, erythrocytary morphology: moderated macrocytosis, poikilocytosis (+/-) with drops, eliptocytes, negative-myeloperoxydazo blasts, PAS-negatives, VSH 136 mm/h, fibrinogen 175mg/dl. Myelogramme: PBO of the iliac crista: bone with normal consistency, slightly hypercellular bone marrow with monomorph aspect, made up of blasts in percentage of 95%, most of them small, increased nucleo-cytoplasmatic relation, close to 1; the cytoplasm when it can be seen was without granulations, irregular nuclear shape, sometimes with cytoplasmatic prolongations, denser chromatin +/- nucleoli (lymphoblasts); rare granulocytary precursors; presence of granular and hyperlobate megakariocytes, small and medium sized thrombocytes groups. Conclusion: ALL aspect. Peroxydases reaction – negative - peroxidases blasts. PAS reaction: PAS-positive blasts 45%. Bone marrow was also examined in other university centre, which pleaded for ALL type L2. FAB with the dislocation of the normal haematopoiesis. Immunophenotypation was made, establishing the diagnosis of biphenotypic acute leukaemia: 76% cells were CD 45+, presenting: CD34+, DR-, CD125+, CD33+, CD13+, CD15+/-, MPO+/-, TdT+/-, CD19+, CD79+, CD10-/+, mature lymphocytes T, B, NK = 14%, granulocytes 10%. Conclusion: proliferation of cells being in the early stage of maturation, compatible with the biphenotypic acute leukaemia (myelo and lymphoblastic B). Molecular biology: bcr – abl gene - negative, abl - positive, AF – 6/MLL negative, AF -9/MLL –negative. The patient was not compatible with his two brothers regarding the HLA system.

Patient LC, aged 38 ani, was hospitalized in order to investigate an important leukocytosis (172520/mm³), a severe anemia (Hb 3,8g/dl, Ht 11,9%) and a thrombocytopenia (44000/mm³) ambulatorily detected. The patient accused marked asthenia for almost one month, shivers, weight loss (approximately 5 kg in the last months), profuse transfusions, metrorrhagia for almost one month. Relatively good clinical health state, cutaneous-mucous intense paleness, laterocervical adenopathies, axillary of about 1,5, cm, mobile, not painful; liver with the inferior edge 1 cm under rebord; spleen – with the inferior pole palpable in profound inspiration. Leukocytary formula: blasts 92%, Mt <1%, NN1, NS2, E<1, B<1, Li5%, M<1%, small and medium sized blasts with condensed chromatin nuclei, without visible nucleoli, together with large sized blasts, with finer chromatin, with visible nucleoli, cytoplasm with moderated basophilia, some of them with intracytoplasmatic vacuoles. Myelogramme: hypercellularly marked bone marrow, with monomorph aspect; about 92% of cells were of blastic type, with moderated anisocytosis, together with small and medium sized blasts, with increased nucleo-cytoplasmatic relation, more condense chromatin nucleus, invisible nucleolus, less towards moderated basophile cytoplasm, some of them with vacuoles without granulations; large sized blasts can be found, with finer chromatin and visible nucleoli, better represented cytoplasm, with the same features (lymphoblasts); very rare erythroblasts, some of them with megaloblastic or cariorexis changes, rare polymorphonuclear, rare lymphocytes. Conclusion: hyperplasic intense bone marrow, with ALL type 2 aspect. Peroxidases reaction (orthotoluidin method): negative peroxydazo-negative. Flowcytometric analysis identified a majority cellular population (92%), based on the expression of antigene CD45, of the internal complexity and of the expression of the analyzed markers, as well as a reduced intensity of the cellular population, having the following phenotype: CD34+, TdT+/-, CD79a+, cyCD22+/-, CD19+, DR+, CD38+, CD15+, allowing to conclude that it was about a cellular proliferation of B lymphocytes lines (BI-proB stage), with a co-expression of (myeloid marker).

The female patient MA, aged 21 presented one month before hospitalization, an episode of infection of the upper respiratory ducts with partial amelioration under antibiotics, but with the persistence of the vesperal febricity. Subsequently, the radiological aspect suggested an interstitial pneumonia that did not improve after treatment. Ambulatorily, although the number of leucocytes was of 5200/mm^3, leukocytary formula presented 20% blasts (together with 31% segmented neutrophils, 47% lymphocytes and 1% monocytes); haemoglobin was of 10 g/dl and the number of thrombocytes was of 68000/mm^3. Clinically: discrete paleness, right laterocervical adenopathy with the diameter of 0,5 cm, the inferior pole of the spleen was palpable in profound inspiration. The cytochemical examination of the peripheral blasts showed that there were myeloperoxydazo-negative and PAS-positive (3%). Regarding the immunophenotypation from the peripheral blood, a population of 20% cells of weak CD45 + reduced internal complexity was isolated; HLA-DR+, CD34+; the other tested markers were negative; the result suggested that the cells were very young. Regarding the Myelogramme, 99-100% blasts of variable sizes were found, with round nucleus and dense chromatin, with agranular basophile cytoplasm; rare blasts with handle mirror aspect, aspect that pleads for acute leukaemia with haematopoiesis dislocation. Immunohistochemy made on the biopsied bone fragment revealed the presence of CD34+ diffuse, CD10+ in the isolated cells, TdT+ zone, CD79a+ in frequent tumoral cells, CD3+ in rare small spread lymphocytes, MPOX-, CD68+ isolated; conclusion was: ALL proB (pre-preB).

The patient OI, aged 56, without personal pathological antecedents was initialized hospitalized in another hospital for vesperal fever, shiver; from the radiological point of view – peri and infrahilar drawing accentuated in the right part. Fever seemed to be resistant to amoxicillin + clavulamic acid and gentamicin, then to ciprofloxacin with ceftriaxone and the hemocultures were negative. Hemoleucogramme proved a moderate anemia

(Hb 8,4 g/dl), a slight leukocytosis, with 15 % blasts and easy thrombocytopenia; for this reason the patient was transferred to the Haematology section. Clinically speaking – mucous – tegumentary paleness, teleangiectases on the upper thorax, without organomegalies, without gingival hypertrophies, without hemoragipary syndrome, with fesperal fever of 30,5ºC and afebrile in the mornings. Biologically, the patient presented; moderated anemia, easy thrombocytopenia (97000/mm³), without coagulation disorders (11900/mm³), with 24% blasts in the periphery, with hypogranular granulocytes, polyploids; medullar aspect: 40% blasts of type I and II with Auer bodies, some of them with bilobed nucleus. The cytochemical examination showed the presence of 10% peroxidazo-positive blasts. Diagnosis: myeloblastic acute leukaemia (LAM). HLA grouping established that the patient was 100% compatible with his sister. Immunophenotypation was made, which established the following phenotype: CD45+, CD34+ (75%), CD13+, CD33dim+ (30%), HLA DR+, AC133-1+ (50%), CD117+ (60%), MPOcy+; negative markers CD5, CD10, CD19, CD7, CD3, CD16, CD56, CD41a, CD14, CD64, CD4, CD8, CD20, CD22, IgMs, CD79acy, CD3cy, TdTcy, GLY A. The hystopathologic examination pleaded for promyelocytar-myeloblast acute leukaemia, type M2 FAB, preceded / associated with myelodysplasic syndrome.

The patient CA aged 64, accused initially polakyuria, dysuria, nocturne shiver and lombar pains, reason for which the patient was submitted to a series of analyses, ambulatorily, which emphasized an increased value of glycaemia (227mg/dl), increased LDH (325u/l), important leukocytosis - 95900/mm³ with 92% lymphocytes and presence of nuclear shadows on the smear, negative bacteriological culture of urine, no hypogamaglobunemia. He was hospitalized with the suspicion of chronic lymphoproliferative syndrome, because he presented right submandibullary adenopathies of 2,5 cm, 2 laterocervical adenopathies of 1 cm in diameter and left axillary of 3 cm, which were mobile, unpainful, of slightly increased consistency, liver with the inferior edge 4 cm under the costal arch, palpable spleen in profound inspiration, leucocytosis 110000/mm³, with 93% adult, small lymphocytes and nuclear shadows, bone marrow examination confirmed the diagnosis of chronic lymphatic leukaemia. Myelogramme: bone marrow easily hypercellulary through infiltration with adult, small lymphocytes, in percentage of 80% and rare medium lymphocytes; presence of nuclear shadows. The myeloic series were reduced quantitatively. The erythoid series was represented in a percentage of 80% with normoblasts and rare dyspalsic sideropenic erythroblasts. Present granulocytary series, in percentage of 12% with development up to the myelocyte. Presence of thrombocytogenic megakacyocytes and thrombocytes in normal groups. Conclusion: bone marrow easily hypercellulary. Chronic Lymphoproliferative syndrome. Medullar hemosiderin – in macrophages – highly increased, sideroblasts 14%.

Thorax radioscopy did not emphasize intrathorax adenopathies. Abdominal echography: long axle spleen 14,2 cm, liver with the antero-posterior diameter of the right lobe of 15 cm, without intra-abdominal adenopathies. The hystopathologic examination was in course of development. Immunophenotypation was made, which showed monoclonal proliferation of B mature cells, with score 3. Cells B CD19+ 93%, CD3+ 6%, Th 2,5%, Ts 3,5%, NK 1%; B cells expressed the following phenotype: CD 5+, CD 23+, CD 79 b+, FMC7-, lambda chains++, CD43+, CD20+, CD38-, CD11C-, CD103 -. It is known that within the chronic lymphatic leukaemia, the immunophenotype includes the following positive: CD5, CD11c (weak+), CD19, CD20 (weak+), CD22 (weak+), CD23, CD43, CD79a and surface Ig. (waek+); usually negative markers: CD10, cyclin D1 and FMC7. The patient did not meet the score of 4 or 5, necessary in order to establish the certitude of the chronic lymphatic leukaemia certitude (LLC), but from the researched panel, surface lgM antibody was missing, although the patient presented axillary adenopathies which could be biopsied, he refused the proposed biopsy, so the medullar section was sent for the immunohistochemical examination.

The patient NV aged 47 presented odynophagia for a week, as well as night shivers and certain laterocervical tumoral formations. The analyses made ambulatorily revealed leukocytosis with lymphocytosis. The abdominal echography showed the existence of a hepatosplenomegaly and of retroperitoneal adenopathies. The patient was hospitalized in order to continue the investigations and the treatment, having a relatively good clinical state, but presenting mobile, flexible, unpainful laterocervical, subclavicle, submandibullary and axially adenopathies, with the diameter of maximum 3 cm, liver with the inferior edge 2 cm under the costal arch, spleen with the inferior pole 3 cm under the costal arch, the confirmation of the second abdominal echography. On this occasion, the interaortal-caves, celiac adenopathies were revealed, with the diameter up to 3,5 cm. Biologically: leukocytosis 32200/mm³ with lymphoid elements 70%, nuclear shadows on the smear, LDH, VSH, easily increased fibrinogen, the bone marrow revealed infiltration with adult, small lymphocytes, some of them clivated, in proportion of 78%; the aspect pleads for a chronic lymphoproliferative syndrome, LLC probable or nonHodgkin's malign lymphoma. Histopathologic examination of the bone marrow was in course of development. Pulmonary radioscopy – without intrathorax tumoral formations. Immunophenotypation established the diagnosis of chronic lymphoproliferative with mature B cell CD10-, CD19+, CD20+, CD5 dim+, CD22+, CD23+, HLA–DR+, kappa5-, lambda5-, FMC7-. This diagnosis allowed the application of fludarabin therapy.

The patient BN, aged 67, presented edematous syndrome and weight loss, reason for which the patient was hospitalized. Clinical state good, venectasias at the level of chicks, scleral subicterus, adenopathies tending to merge in blocks, submandibullary, aterocervically,

inquinally and axillary adenopathies, liver with the inferior edge 7 cm under the costal arch, spleen with the inferior pole at the level of the umbilical cord. Presence of leukocytosis - 346900/mm³, with 94% lymphocytes, easy normochromic, normocicytary anemia. Bone marrow examination showed the presence of medullar infiltration with adult, small lymphocytes, 86%. Immunophenotypation showed the presence of a certain atypical population, in proportion of 95% with the following phenotype: CD19+, CD5+, CD20var+, CD22+, CD23+, FMC7dim+ (45%), sIgM weak+, kappa+; negative markers: CD10, CD34, lambda. Conclusion: monoclonal proliferation of the atypical mature B lymphocyte, type ALL with B cells.

The patient BA, aged 64 has been known with chronic granulocytic leukaemia since 2000, for which he was initially treated with hydroxiurea and interferon alpha. Due to the fact that 6 months ago, the gene BCR/ABL to be proved positive, the patient was treated with imatinib mesilate. This spring, the patient suffered an episode of acute cholangitis due to a vesicular microlithiasis and needed hospitalization in the gastroenterology section. Subsequently, the patient returned to hospital because of pains in the right hypocondrium and fever, without adenopathies, without hepatosplenomegaly, leukocytosis up to 15640/mm^3, but with a balanced leukocytary formula and easy thrombocytosis. Hyperproteinemia of 11,4 g/dl was also detected. Mention must be made of the fact that the patient presented for almost a year an increased VSH (erythrocyte sedimentation rate) - (90-100mm/h). More, during the latest hospitalization, the patient had a monoclonal peak in the area of the γ-globulins of 5,2 g/dl; G immunglobulins were 3774 mg/dl, Bence Jones proteins – absent; hyperuricemia; medullar aspiration with aspect of chronic granulocytic leukaemia + infiltration with myelomatous cells in percentage of 16%. Radiography of the skull-cap showed an osteolysis area of 2,5 / 2 cm, in the frontal right region. The abdominal echography revealed a hepatomegaly (right lobe with the craniocaudal diameter of 14,4 cm), one calculus of 1cm in the cholecyst and long axle spleen of 12,3 cm. Thorax radioscopy showed an acute pneumonia of the right upper lobe. Myelogramme emphasized the presence of an interstitial plasmocytary infiltrate with myelomatous aspect of 10-12%, with preserved haematopoiesis and with the presence of all series. Medullar aspiration proved to be compatible with the diagnosis of multiple myeloma of plasmocytic, medullar infiltration, level 1. (below 20%). Cytometry in flux identified o population of 5% of myelomatous cells: CD45+ weak, CD38+, CD138+, CD56+. Flowcytometry proved to be useful because it confirmed the suspicion of multiple myeloma associated to the chronic granulocytic leukaemia, a very rare association.

DISCUSSION

A recently published study concluded that flowcytometry may increase the accuracy of acute leukaemia diagnosis. The authors observed the existence of a concordance of 94,1% between morphology and immunology; 4 cases which were wrongly diagnosticated benefited from a correct immunologic diagnosis. Especially CD13 and CD33 antigens were associated to the myeloid lines (1), inclusively in LAM type 1, where they are strongly expressed. (2); LAM type 3 had frequently CD34 weakly expressed, and HLA-DR was negative (1), while in the type 1 HLA-DR was strongly expressed (2); CD14 was frequently expressed in types 4 and 5 of LAM; the antigens associated to the lymphoid lines (CD7) were easily found in ALL, where antigens of myeloid line were also found (1). In another research, in LAM type 1 antigens CD11b, CD15, MPO, CD117 were weakly expressed and the antigens of the T lines, CD4 and CD7 were strongly expressed. (2)

The casuistics we presented also included 3 patients with ALL B. It was considered that almost all these patients were positive for the establishment of TdT, HLA-DR, CD19, cytoCD79a; usually, CD10 and CD24 markers were also present; occasionally, CD20, cytoCD22 (lines specific) may also be present, CD13, CD33. This type of leukaemia may be divided in the form of B early precursors B (CD10-, CD19+, TdT+, cytoplasmic mu -, surface Ig -), coomon form (CD10+, CD19+, TdT+, cytoplasmic mu -, surface Ig -), forme preB (CD10+, CD19+, TdT+, cytoplasmic mu +, Ig de suprafaţă -) and the form of mature B lymphocytes (CD10+/-, CD19+, TdT-, cytoplasmic mu -, surface Ig +) (3). It is considered that the forms proB and preB of ALL B, ALL T and LAM type1, which are difficult to differentiate morphologically, may be well differentiated through the immunologic phenotype analysis. (2).

There are no characteristic immunologic markers in order to make the difference between LAM type 1 and LAM type 2, but because the positiveness rate of the antigens CD11b, CD15, MPO, CD117 is significantly lower in type 1 as against type 2, these markers may be used as reference indicators for differentiating the two types of leukaemia. It is also observed that CD 117 is predominantly expresses in LAM, being useful for the differential diagnosis regarding ALL (2).

It is essential that lymphoblastic acute leukaemias should be differentiated from those myeloblastic and in special cases, to conclude that they are biphenotypic, as in the case of one of the patients presented by us. A group of researchers suggest that together with the morphologic and cytochemical examination, a panel of monoclonal antibodies towards MPO, cyCD3, cyCD79a, CD13, CD33, CD10, CD19, CD2 and CD117 may be cost-efficient and highly predictive screening for the linear differentiation prediction of the acute leukaemias. (4).

CLL B is easily to be diagnosticated by flowcytometry, which may detect CD5 and CD19 co-expression, as well as that of CD20 and CD23 (5) and HLA-DR (6). The following markers are usually expressed: CD11c (weakly), CD22 (weakly), CD43, CD79a and surface immunoglobulins; CD10, cyclin D1

are FMC7 are usually negative (3). During cell development, the quantity of surface immunoglobulins increases; on the lymphocyte surface, the following occur: the receptor for erythrocytes from mouse emphasized by M rosettes, receptor Fc for IgG and CD21 (receptor for C3d component of the complement and for the Epstein-Barr virus). Regarding the B mature lymphocyte, the following changes may be observed: loss of antigens CD21, CD10, increased loss of lymphocytes production that form the M rosettes and the increase of the surface immunoglobulins. (7).

Flowcytometry proved to be useful in establishing the diagnosis of the chronic granuloctyrary leukaemia too, with a monoclonal peak Ig G occurring in dynamics. Myelomatous plasmocytes frequency expresses cytoplasmatic immunoglobulins (IgG > IgA > others) and not surface immunoglobulins; CD38, CD79a and CD138 are usually expressed; the frequent negative markers are CD19 and CD20 (3). CD79a is an antibody that may identify the antigen of the B cells, from B precursor cell to plasmocyte, while CD138 is a strong marker for the identification of plasmocytes (8).

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BIBLIOGRAPHY

1. Sun XL, Fang MY, Jiang F, Jing Y. Immunologic Classification Used in Typing 68 Cases of Acute Leukemias. Zhoungguo Shi Yan Xue Ye Xue Za Zhi. 2006; 14 (1): 39-41.
2. Zhao F, Chen Y. Immunologic Characteristics and Prognosis of Acute Myeloid Leukemia M1. Zhongguo Shi Yan Xue Ye Xue Za Zhi. 2007; 15 (4): 687-691.
3. Burke JM. Dx/Rx: Leukemia. Jones and Bartlett Publishers, Sudbury, Massachusetts, 2006.
4. Thalhammer-Scherrer R, Mitterbauer G, Simonitsch I, Jaeger U, Lechner K, Schneider B, Fonatsch C, Schwarzinger I. The Immunophenotype of 325 Adult Acute Leukemias. Am J Clin Pathol. 2002; 117 (3): 380-389.
5. Management Strategies for Chronic Lymphocytic Leukemia.www.medscape.com/viewprogram/2417_pn; disponibil în 31.03.2008.
6. Kusenda J, Babusikova O. Antigen Modulation Followed by Quantitative Flow Cytometry of B-Chronic Lymphocytic Leukemia Cells After Treatment. Neoplasma. 2004; 51 (2): 97-102.
7. Ciocoiu AV. Leucemia limfatică cronică. În: Păun R (sub redacţia). Tratat de Medicină Internă. Hematologie. Partea a II-a. Ed Medicală, Bucureşti, 1999: 330-346. (Chonic Lymphatic Leukaemia). Treatise of Internal Medicine. Haematology, Part II.
8. Sun T J. Flow cytometry and molecular genetics for hematologic neoplasms. www.medscape.com/viewprogram/8973_pnt, disponibil în 28.03.2008.