CLINICAL ASPECTS

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 leukemia, chronic lymphoblastic leukemia 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 leukemia, chronic lymphocytic leukemia, multiple myelomas.

Rezumat: Descoperirea anticorpilor monoclonali a făcut posibilă investigarea imunologică a hemopatiilor maligne, fapt care a ușurat diagnosticul pozitiv. Prezentăm câteva cazuri de leucemii acute, leucemii limfatice cronice și 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 immunologic, flowcytometrie, leucemie acută, leucemie limfatică cronice, mielom multiplu

INTRODUCTION

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

CLINICO-BIOLOGICO-HISTOLOGICO-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 ecchimoses, paleness, effort dyspnoea, inappetence, weight loss. Objective – fever, intense paleness, ecchimoses 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 Gumpecht nuclear shadows; cellular series were hypoplastic marked, frequently free nuclei, possible of blastic etiology. Conclusion: possible acute leukemia. 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, slgM, CD20. Thus, the diagnosis of medullar proliferation on B lymphoid line was established: acute lymphoblastic leukemia (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 mucotegumentary paleness, weight loss (2 kilos in two months), hollow cough. He came to the hospital with fever (38°C), 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-myeloperoxidase 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-myeloperoxidazo 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 +/- nuclei (lymphoblasts); rare granulocytary precursors; presence of granular and hyperlobate megakarocytes, small and medium sized thrombocytes groups. Conclusion: ALL aspect. Peroxydases reaction – negative - peroxydases 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 leucocytosis (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. Peroxydases reaction (orthotoluidin method): negative peroxydazo-negative. Flowcytometric analysis identified a majority cellular population (92%), based on the expression of antigen 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/mm³, 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/mm³. 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 myeloperoxidazo-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. Immunohistochemcy 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 negatives. Hemoleucogramme proved a moderate anemia
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development up to the myelocyte. Presence of
anulor granulocytes, polyploids; medullar aspect:
40% blasts of type I and II with Auer bodies, some of
them with bilobed nucleus. The cytotoxic 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 myelodysplastic syndrome.

The patient CA aged 64, accused initially polakuria, 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, with the diameter of maximum 3 cm, without intra-abdominal adenopathies. The patient was hospitalized in order to continue the investigations and the treatment, having a relatively good clinical state, but presenting mobile, flexible, painful laterocervical, subclavicle, submandibulary 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 radiography – 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 fludarabine therapy.

The patient BN, aged 67, 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, painful laterocervical, subclavicle, submandibulary 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
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(AMT, tome II, no. 2, 2008, page 179)
Inquinal 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, normocytic 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%), slgM 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 hydroxurea 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 cholecystitis and needed hospitalization in the gastroenterology section. Subsequently, the patient returned to hospital because of pains in the right hypochondrium and fever, without adenopathies, without hepatosplenomegaly, leukocytosis up to 15640/mm³, 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 1 cm 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 plasmocitary infiltrate with myelomatous aspect of 10-12%, with preserved haematopenies 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 -), comon 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 preB 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 leukemias. (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 granulocytic 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).

THANKS
This article makes part of the Excellence Research Grant, 179/2006, financed by the Ministry of Research and Education, through the Academy of Medical Sciences and VIASAN, whom we wish to thank on this occasion.

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