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The parallel between CD45 expression and extra-medullary evolution in aggressive myeloma with high serum lactate dehydrogenase

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Case Report

A 65-year-old female was admitted to our Hospital in March 1996 due to cephalgia, diplopy and chin paresthesias. Physical examination revealed paralysis of the left third cranial nerve and of the trigeminus inferior branch. Computed tomographic (CT) cranial scan revealed diffuse skull and sphenoid osteolysis with a stricture of the inferior orbital fissure. Laboratory examinations showed: leukocytes 5.7×10^9/L, hemoglobin 9.5 g/dL, platelets 111×10^9/L, monoclonal serum IgGκ, no Bence-Jones proteinuria, IgG 3232 mg/dL (normal: 800-1800 mg/dL), IgA 31 mg/dL (normal: 90-450 mg/dL), IgM 29 mg/dL (normal: 60-340 mg/dL), creatinine 1.3 mg/dL, C-reactive protein 1.4 mg/dL (normal: < 1.2 mg/dL). Calcium, β_2-microglobulin and lactate dehydrogenase (LDH) were increased to 13.3 mg/dL (normal: 8.3-10.5 mg/dL), 4.4 mg/L (normal: 1-3 mg/L) and 527 U/L (normal: 230-460 U/L), respectively. Bone marrow (BM) biopsy and aspirate showed a 70% infiltration of immature plasma cells (Figure 1). Immunohistochemical analysis showed that plasma cells were negative for CD20, CD56, CD45, CD45RA and CD45RO. No additional osteolysis was detected by skeletal survey. Chest x-ray and abdomen ultrasound were normal.

The presence of serum paraprotein, high BM plasma cell infiltration, osteolityc lesions and hypercalcemia with normal renal function were consistent with a diagnosis of stage III A multiple myeloma. Treatment and clinical behavior of the patient are summarized in Figure 2. The patient was treated with four cycles of melphalan and prednisone. Multiple scalp swellings, a left maxillary mass and bilateral exophthalmus developed in late August. CT cranial scan showed masses in both orbits, in the left frontal sinus and in the ethmoid sinus with osteolytic lesions in the adjacent bone structures.

The presence of soft tissue masses grossly confined to adjoining para-skeletal tissues in a MM patient strongly indicated extra-medullary spread by direct continuity of the myelomatous process.[1] However, histological demonstration was needed for a definitive diagnosis.

Bioptic examination of the maxillary mass demonstrated sheets of plasmablasts. At that time, a restaging of MM revealed no reduction of LDH, β_2-microglobulin and paraprotein as compared to values detected at diagnosis; BM smear showed 80% plasma cell infiltration; additive rib lesions were detected by systemic X-ray.

Serum LDH, β_2-microglobulin and paraprotein profiles, high BM plasma cell infiltration, worsening of skeletal picture and development of extra-medullary plasmacytomas suggested MM progression.

The patient was started on three combined regimens of vincristine, adriamycin and dexamethasone (VAD). Three days after the start of the first cycle, the patient developed dyspnea and was admitted to the Hospital. Upon entry, temperarure was 36°C, pulse was 80 and blood pressure was 160/90. On physical examination, the heart, abdomen and extremities were normal, except for ungual cyanosis. Diminished breath sounds were present over the lower lung fields. Laboratory examinations showed: leukocytes 7.6×10^9/L, hemoglobin 9.0 gr/dL, platelets 55×10^9/L, creatinine 1.3 mg/dL, proteins 7.4 gr/dL, albumin 3.5 g/dL, aspartate aminotransferase 29 U/L, LDH 708 U/L and C-reactive protein 3.7 mg/dL. Fibrin degradation products were absent. Analysis of arterial blood gases showed: partial pressure of oxygen 60 mm Hg, partial pressure of carbon dioxide 52 mm Hg and pH 7.4. A chest radiograph showed a bilateral pleural effusion, and the upper lungs appeared clear (Figure 3). A tuberculine test was negative. No increase of serum anti-Aspergillus antibody titer was detected. Electrocardiography and cardiac ultrasound were normal. Pulmonary perfusion and ventilation scan were negative.

Physical examination, laboratory findings and echocardiography ruled out the presence of a trasudate due to congestive heart failure, liver cirrhosis or uremia. Thus, the most likely hypothesis was an exudative pleural effusion due to infection or extra-medullary progression of the MM process. Infection is the most common cause of pulmonary abnormalities in an immunocompromized host. Indeed, the polyclonal humoral immune suppression and the

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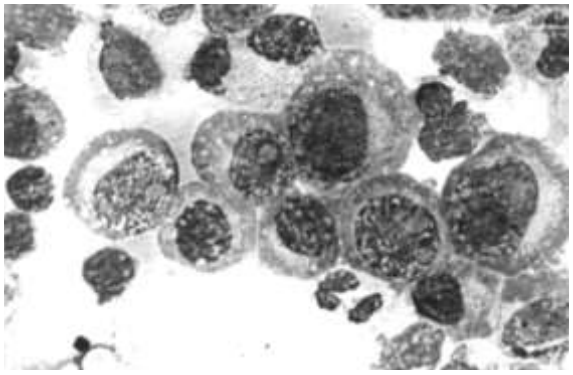

Figure 1. Bone marrow smear at diagnosis. Majority of plasma cells showed immature morphology. May-Grünwald Giemsa staining. Original magnification x 160.

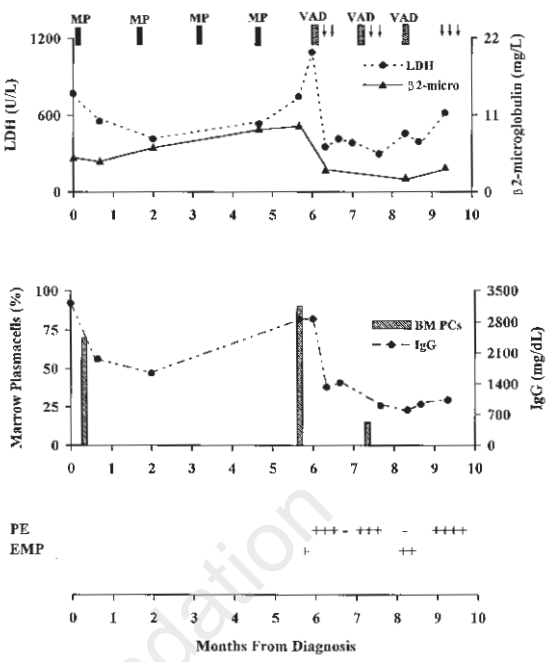

Figure 2. Therapy and clinical parameters of the patient. During VAD courses, a drastic decrease of LDH and β2-microglobulin was synchronous with the reduction of serum paraprotein and bone marrow plasma cells, while malignant pleural effusion recurred and extra-medullary plasmacytomas arose and persisted. Each arrow indicates therapeutic thoracentesis.
MP, melphalan, prednisone; VAD, vincristine, adriamycin, dexamethasone; BM, bone marrow; PCs, plasma cells; PE, pleural effusion; EMP, extra-medullary plasmacytomas.

treatment with corticosteroids predispose MM patients to infections with both encapsulated organisms and fungi.[2,3] The absence of fever, pleuritic pain and productive cough, normal blood leukocyte counts as well as the lack of risk factors for a fungal infection of the pleural cavity[4] and undetectable anti-Aspergillus antibodies in the serum likely excluded either para-pneumonic or fungal effusion. Although anergy to tubercolin test may occur in immunosuppressed individuals with tubercolosis, the old age of the patient, the absence of tuberculous risk factors and the characteristics of the pleural effusion (i.e., large-sided and bilateral) likely excluded a tuberculous pleuritis.[5]

High LDH levels and plasma cell anaplasia made likely an invasion of the pleural cavity by the myelomatous process.[6-9] Although myelomatous pleural effusion may be due to invasion of the pleural space from adjacent tumors or lymphatic obstruction due to mediastinal lymphonode infiltration, direct involvement of the pleura may occur as a result of the hematogenous spread of MM plasma cells to sites distant from the skeleton.[1,10] The diagnostic procedure of choice is the research of both paraprotein and malignant plasma cells in the pleural fluid (PF); flow cytometric analysis supports the cytologic diagnosis and better characterizes MM plasma cells.

Thoracentesis was performed and revealed serosanguineous fluid. Laboratory analysis of the PF showed: specific gravity 1020; pH 7.4; proteins 3.5 g/dL; glucose 60 mg/dL; LDH 198 U/L; monoclonal IgGκ; IgG 639 mg/dL; IgA 8 mg/dL; IgM 7 mg/dL; Ig light chain κ and λ 181 and 13 mg/dL, respectively, and leukocytes 1×10^9/L. PF cultures were negative for bacteria and fungi.

Cytologic examination showed that cellularity was almost totally represented by plasmablasts (Figure 4). Mononuclear cells (MNCs) obtained from PF sample were incubated with the combination of fluorescein-isothiocyanate (FITC)- and phycoerythrin (PE)-conjugated monoclonal antibodies (MoAbs) as follows: CD19 FITC/CD38 PE; CD20 FITC/CD38 PE; CD45 FITC/CD38 PE; CD45RO FITC/CD38 PE; CD45RA FITC/CD38 PE; CD10 FITC/CD38 PE; CD38 FITC/CD56 PE and CD38 FITC/CD34 PE (CD38 FITC: Landerdiagnostico, Madrid, Spain; CD45RO: Dakopatts, Glostrup, Denmark; the other MoAbs: Becton Dickinson, Mountain View, CA, USA). Each fluorescence analysis included an isotypic double negative control.

PF plasma cells, identified by the $CD38^{++}$ fraction, constituted 100% of total MNCs and were negative for CD19, CD20, CD10, CD34 and CD56. Interestingly, CD45, CD45RA and CD45RO were detected on 95%, 38% and 40% of PF plasma cells, respectively.

Clinical and phenotypic features of the patient consisted with a picture of aggressive myeloma with high serum LDH (AMHL), a rare clinical entity that is characterized by resistance to conventional chemotherapy, wide-spread disease, poor prognosis and fulminant course.[7-9,11,12]

After thoracentesis, respiratory symptoms disappeared and chest X-ray on discharge showed a drastic reduction of the pleural effusion. In October, the patient was readmitted to the hospital. Physical examination revealed a slight improvement of both

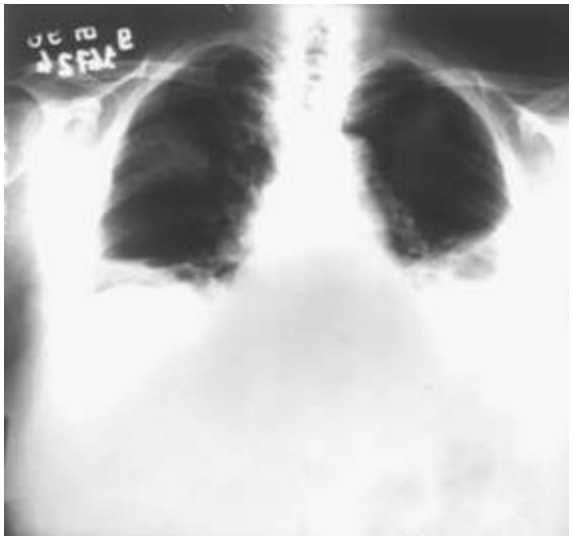

Figure 3. Radiograph of the chest showing bilateral pleural effusion.

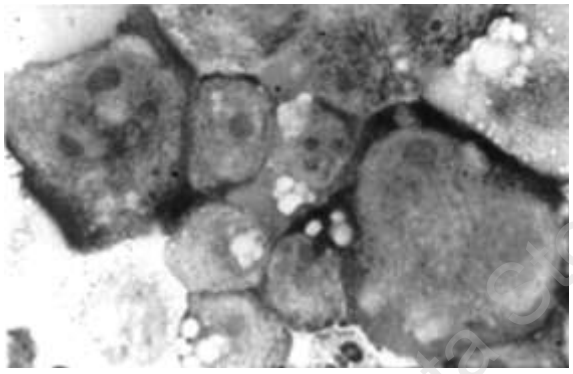

Figure 4. Cytologic examination of the pleural fluid (cytospin slide). Almost total cells were plasmablasts. May-Grünwald Giemsa staining. Original magnification ×160.

scalp swellings and exophthalmus. A restaging of MM showed a reduction of LDH, β_2-microglobulin and paraprotein and a drastic decrease of BM plasma cells that showed the same morphologic and phenotypic pattern detected on PF plasma cells. The second VAD course was performed. During hospitalization, myelomatous pleural effusion recurred. CT scan demonstrated a slight reduction of the previously observed cranial masses, but additive swellings in the right maxilla and a tumor in the left chest wall were newly detected.

Disappearance of serum paraprotein associated with extra-skeletal evolution has been suggested to reflect dedifferentiation of the neoplastic clone.[8,9,12,13] However, the decreases of LDH, β_2-microglobulin and BM infiltration, well known clinical markers of disease activity,[7,8,9,14] were recorded. This finding likely indicated the presence of a less aggressive BM plasma cell population that substantially attained response to VAD regimen.

In November, a third VAD cycle was performed without any complication. Patient's conditions improved transiently, but then worsened one month later, when massive pleural effusion recurred and the patient died due to respiratory and acute renal failure. At that time, elevation of serum LDH levels was recorded.

Discussion

Newly diagnosed MM patients with high LDH levels are known to experience chemotherapy resistance and clinical aggressiveness.[7-9] In such group of patients, early tumor cells with *LDH phenotype* seem to expand preferentially during disease evolution as a result of drug resistance and/or proliferative advantage.

The correlation between plasma cell anaplasia and disease aggressiveness as well as the co-existence of plasma cells with different clinical behavior and drug sensitivity have been previously observed in cases of aggressive MM.[6,13,15] Accordingly, the extraskeletal manifestations of our patient were paralleled by a worsening of plasma cell morphology and persisted during VAD courses despite the medullary remission. Although the clinical refractoriness to VAD regimen has been linked to the multi-drug resistant phenotype (mdr) of MM plasma cells,[16,17] a variety of mechanisms other than mdr are likely to regulate resistance to treatment in AMHL patients.[11,18] Moreover, despite alternative therapeutic approaches now available for refractory MM,[19,20] no treatment has been demonstrated effective for patients in the aggressive phase.[18] Thus, further characterization of AMHL cells is needed to provide clues about the pathogenesis and the appropriate treatment of this clinical entity.

Recently, a pathophysiological role of surface antigens in the biology of MM has been suggested and typical phenotype characteristics have been associated with disease progression.

Adhesion molecules such as CD56 likely play a role in the contact between myeloma cells and the BM microenvironment.[21] Indeed, whereas during the chronic MM phase neoplastic plasma cells are known to over-express CD56, extra-medullary spreading has been associated with a dramatic down-regulation of CD56 on BM and peripheral blood plasma cells.[21] The suggested correlation between lack of CD56 on MM cells and BM homing disruption was confirmed in our case. However, the early detection of CD56 down-expression during the medullary phase confirms the hypothesis that the acquisition of further aggressive properties is likely implicated in extra-skeletal progression.[18]

CD45 molecule is a trans-membrane tyrosine-phosphatasis that is implicated in a number of intracellular signalling pathways and regulates various cell functions, such as adhesive interactions, proliferation and differentiation.[22,23] According to the progressive decrease of CD45 expression at the later stages of B-

cell differentiation, plasmacytic malignances usually display the $CD45^-$ phenotype pattern that was observed in our patient at diagnosis.[24] Expression of CD45 on malignant plasma cells has been correlated with the earlier stages of putative B-cell differentiation.[25] Evidence has been provided that primitive $CD45^{++}$ plasma cells with high labelling index (LI) and aggressive behavior may be associated with the less aggressive $CD45^-$ plasma cells in the same patient.[25] Whether plasma cells of disease evolution were clonally related to the original BM tumor cells of diagnosis in our case was not investigated. However, extra-skeletal progression was related to the emergence of a dedifferentiated $CD45^{++}$ plasma cell population with selective advantage of migration to extra-medullary sites and primary VAD resistance. Moreover, the treatment-mediated reduction of residual, sensitive $CD45^-$ marrow plasma cells responsible for paraprotein synthesis might explain BM remission during disease dissemination.

A high plasma cell proliferative potential has been invariably associated with the escape from the *plateau* phase and poor prognosis.[18] Higher LI of extra-medullary plasma cells compared with the BM population[15] suggests that highly proliferating plasma cells may display, besides drug resistance,[14,25] a greater tendency to spread. Recently, CD45 molecule has been detected to mediate both suppression of apoptosis and cellular adhesion in mouse malignant lymphoma.[26] By analyzing the plasma cell phenotype of our AMHL case during both medullary phase and disease dissemination, we found that extra-skeletal evolution was paralleled by a strong over-expression of CD45 molecule on myelomatous plasma cells. Whether CD45 plays an actual role in either disease aggressiveness or dissemination in AMHL is still unknown. However, our findings suggest that studies on signaling pathways through CD45 should be performed to better understand the pathogenesis of AMHL and to develop new therapeutic approaches.

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