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Case Study

Myelomatous Meningitis Evaluated by Multiparameter Flow Cytometry : Report of a Case and Review of the Literature

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Central nervous system (CNS) involvement in multiple myeloma (MM) is uncommon. Among its possible presentations, leptomeningeal involvement of MM, also termed central nervous system myelomatosis (CNS-MM) is rare and is characterized by the presence of neoplastic plasma cells in the cerebrospinal fluid (CSF). So far, 187 cases of CNS-MM have been reported : the great majority of them were diagnosed by cytological assays and flow cytometry was used in only eight cases. We describe a case of CNS-MM in a 62-year-old woman, previously treated with chemotherapy (VTD) and autologous peripheral blood hematopoietic stem cell transplantation for stage IIIB IgG-λ MM. After achieving a very good partial response, the patient showed progression of disease, with an extramedullary localization. During administration of second-line therapy, the patient showed severe neurological symptoms. MRI resulted negative. Diagnosis of CNS-MM was made by multiparameter flow cytometry, which showed the presence of $CD56^+$ plasma cells in a CSF sample, in the absence of plasma cell leukemia. In this paper we also present a review of the eight previous cases of CNS-MM diagnosed by flow cytometry. We found that the application of flow cytometry in cases of MM with neurological symptoms allows a rapid diagnosis of CNS-MM and provides useful information about plasma cell phenotype (including CD56 expression). Some cases of CNS-MM are characterized by normal MRI. In addition, some evidences deriving from the review of literature suggest that CSF monitoring by flow cytometry in such cases might be used to evaluate the efficacy of drugs capable of crossing the blood-brain barrier. 〔*J Clin Exp Hematop 54(2) : 129-136, 2014*〕

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INTRODUCTION

Although neurologic manifestations often complicate the course of patients with multiple myeloma (MM),[1,2] central nervous system (CNS) invasion is rare. This presentation of MM can occur as primary parenchymal brain lesion, osteodural or leptomeningeal involvement. The latter CNS localization of MM, also termed myelomatous meningitis or central nervous system myelomatosis (CNS-MM) is very uncommon, accounting for about 1% of patients[3-5] and is characterized by the presence of neoplastic plasma cells in the cerebrospinal fluid (CSF).[5,6] The pathogenesis of such a phenomenon might be due to the haematogenous spread of plasma cells or of their circulating progenitors. CNS-MM is responsible for a variety of neurological symptoms, which are more frequently represented by confusion, limb weakness, headache, visual disturbances, gait disturbances, speech disturbances.[4,7]

We recently observed a case of CNS-MM after chemotherapy and transplantation of autologous peripheral blood hematopoietic stem cells. Diagnosis was made by multiparameter flow cytometry on a CSF sample, in the presence of normal computed tomography (CT) and magnetic resonance imaging (MRI). Due to the low frequency of CNS-MM, a limited number of cases have so far been reported. After an

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extensive search in PubMed we were able to review the available literature. We found that a total number of 187 cases had been reported until 2012.[3-5,8-24] The same case was described in two different instances.[19,23] Therefore, we reviewed review articles and single case reports in order to obtain more information about patients characteristics, the method used to detect plasma cells in the CSF and plasma cell phenotype. The result of our search was that the great majority of cases of CNS-MM were diagnosed by cytological assays, with sporadic use of immunocytochemistry to demonstrate plasma cell clonality. We found only eight cases of CNS-MM diagnosed by means of flow cytometry.[8,15,16,18,20,22-24] Thus, in the current paper we report both our experience and a review of previous cases investigated by flow cytometric methods.

CASE REPORT

A 62-year-old Caucasian female, with a 15-month history of multiple myeloma, presented with confusion, limb weakness and gait and speech disturbances. She had been diagnosed as having a stage IIIB IgG-λ MM in September 2011. At presentation, neoplastic plasma cells resulted positive for the CD56 molecule both at immunohistochemical staining and at flow cytometric analysis. Cytogenetics, carried out on myeloaspirate samples by fluorescence *in situ* hybridization techniques, had shown the presence of 20% metaphases carrying del (17p13). No circulating plasma cells were detected at light microscope examination at diagnosis.

After diagnosis, the patient underwent therapy with the VTD regimen (four courses), obtaining partial remission, according to the International Myeloma Working Group uniform response criteria.[25] Infusion of autologous peripheral blood hematopoietic stem cells was carried out in April 2012 and the patient achieved a very good partial response. However, in August 2012 an extramedullary localization (8 × 8 × 6 cm), in the left antero-lateral paravertebral region (D11-L2), involving aorta, was diagnosed. Samples from a myeloaspirate were subjected to morphology, which showed 2% plasma cells, and to flow cytometry, which was carried out by a 6-color multiparameter method a FacsCanto II cytometer (Becton Dickinson, Buccinasco Milano), equipped with two lasers (488 and 633 nm). Monoclonal antibodies (MoAbs) conjugated with FITC, PE, PerCP-Cy5.5, PE-Cy7, APC and APC-Cy7 (purchased from Becton Dickinson) were assembled to organize diagnostic panels, with a fixed combination of CD138, CD38, CD19 and CD45, and with the addition of other MoAbs useful to study plasma cell phenotype : CD27, CD20, CD117, CD56, and CD28. Plasma cells were identified as $CD138^+/CD38^+$ events, after a first gate, which was set on a FSC/SSC cytogram to include events with low SSC values. Clonality was studied by means of rabbit F $(ab')_2$ polyclonal antibodies directed to human κ and λ immunoglobulin light chains (purchased from Dako, Glostrup, Denmark). Intracytoplasmic detection of κ and λ light chains was obtained by Intrasure permeabilization kit (Becton Dickinson). Five hundred thousands events were acquired for every tube and data were analyzed by FacsDiva software (Becton Dickinson). Flow cytometry yielded a small λ-positive plasma cell population (0.4%) with an abnormal phenotype which was similar to that found at diagnosis (i.e. $CD56^+$). There was no evidence for plasma cell leukemia. A second-line therapy with the VTD-PACE schedule was administered and two courses were completed.

The neurologic symptoms appeared before the scheduled third course of VTD-PACE. Despite normal CT and MRI, the patient experienced a rapid worsening of neurological symptoms and underwent lumbar puncture, which was atraumatic and non-hemorrhagic. Chemistries carried out on a CSF sample showed : total proteins 167 mg/dL (normal value 20-40), glucose 50 mg/dL (normal value 50-60), and chloride 113 mEq/L (normal value 121-133). White blood cell count of CSF, carried out by ADVIA 2120 system, was 0.349 × 10^9/L.

CSF was subjected to flow cytometric analysis, following the method described by Benevolo *et al*.[26] and using the same MoAbs panels as for bone marrow and peripheral blood samples. CSF examination by flow cytometry yielded more than 30,000 events positive for CD138, CD38, CD56, CD28 and cytoplasmic λ chains, negative for CD19, CD45 and CD27 (Fig. 1). Thus, CNS-MM was diagnosed. Unfortunately, a cytospin of the CSF sample was not carried out.

Morphologic examination of peripheral blood samples was negative for plasma cell leukemia. A simultaneous flow cytometric examination of peripheral blood showed the presence of a very small (0.028%, 0.001 × 10^9/L absolute number) population of circulating plasma cells with abnormal phenotype which included positivity for CD56 (Fig. 2). At this time, the monoclonal IgG-λ protein was 1.7 g/dL. Unfortunately, our patient died two days after hospital admission. Permission for autopsy was not obtained.

REVIEW OF CASES STUDIED BY FLOW CYTOMETRY

After the revision of literature, eight previous cases of CNS-MM diagnosed by flow cytometry were found.[8,15,16,18,20,22-24] Non-homogeneous results were available, since cytogenetics was not reported in 4 cases and data about CD56 expression were not reported in 3 cases. Significant neurologic symptoms were described in all cases but one, but in 2 cases MRI was negative. Male to female ratio was 1 : 1 and age was less than 65 years in 7 of the 8 patients. In 3 cases a light chain MM was reported, along with a case of IgD MM. Plasma cell leukemia was observed only in one case. No details were provided about the method used to analyze CSF by means of flow cytometry. New

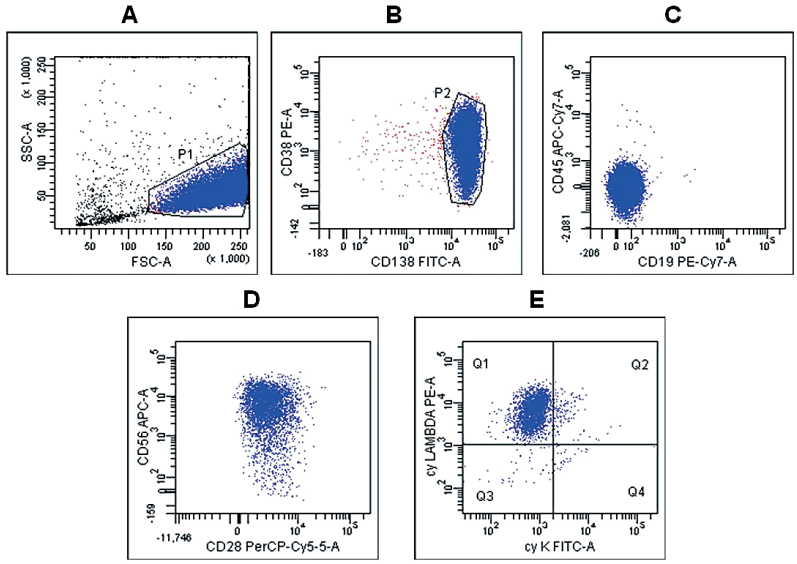

Fig. 1. Flow cytometric analysis of central nervous system. (***1A***) FSC/SSC gating (*P1*). (***1B***) CD138/CD38 gating (*P2*) to include plasma cells. (***1C***) Anomalous plasma cell phenotype ($CD19^-$, $CD45^-$). (***1D***) Positivity for CD56 (and co-expression of CD28). (***1E***) Restriction for cytoplasmic λ light chain.

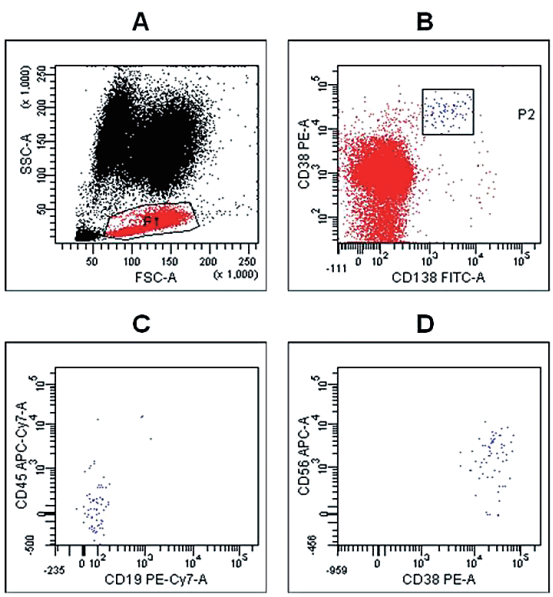

Fig. 2. Plasma cell detection in peripheral blood. (***2A***) FSC/SSC gating (*P1*). (***2B***) CD13/CD38 gating (*P2*) to include plasma cells. (***2C***) Plasma cells are negative for both CD19 and CD45. (***2D***) Positive expression of CD56.

drugs, such as Thalidomide and Bortezomib were administered in all cases before CNS-MM development, and two patients were treated with autologous stem cell transplantation.

Monitoring of CSF showed plasma cell clearance in some cases, either after intrathecal therapy[15,16,18,20] or by administering drugs capable of crossing the blood-brain barrier, such as lenalidomide[23] and pomalidomide.[24] Radiotherapy as adjuvant treatment was given in two cases,[15,22] but it not was possible to established its role in patients' outcome.

Prognosis was very poor and patients died after 9-150 days, with two exceptions.[15,24] The longest follow-up was limited to ten months.[24] Relevant data deriving from the review of literature are reported in Tables 1-3.

DISCUSSION

The current case is representative of an uncommon evolution of MM and highlights the usefulness of multiparameter flow cytometry in detecting neoplastic plasma cells in the CSF. Among the 187 cases of CNS-MM so far reported, almost all were diagnosed by means of morphology using light microscopy. In some instances, immunocytochemical assays were carried out to demonstrate plasma cell clonality.

We used flow cytometry because this method is able to detect both normal and pathologic plasma cells with great sensitivity and specificity.[27,28] Plasma cells are always characterized by CD138/CD38 co-expression, and both clonality and phenotypic aberrations are easily detected. In addition, sensitivity of multiparameter flow cytometry is as high as 10^{-4}.[29] Therefore, this method can be applied to investigate CSF in order to diagnose CNS-MM, giving additional useful information about plasma cell phenotype, which can be compared to the bone marrow and/or peripheral blood counterpart.

After our survey, we found that only eight previous cases

Table 1. Main characteristics of patients with central nervous system myelomatosis diagnosed by means of multiparameter flow cytometry

Patients (age, sex)	Isotype	CD56	Cytogenetics	Plasma cell leukemia	References
Male, 36	IgA-λ	NA	NA	Yes	8
Female, 71	κ	+	+ 1, 13q-, c-myc	No	15
Female, 62	κ	+	t(11;14) (q11;q32)	No	16
Male, 63	IgA-κ	NA	NA	No	18
Male, 64	λ	NA	NA	No	20
Female, 60	IgD-λ	−	Complex hyperdiploid karyotype*	No	22
Female, 59	IgG-κ	+	NA	No	23
Male, 60	IgG-κ	+	Complex karyotype**	NA	24
Female, 62	IgA-κ	+	del (17p13)	No	Current paper

NA, not available ; *, 51 XX, del (1) (q32), + 3, + der (8), t(1;8) (q21;p11), + 9, + 12, + 17, + 19, [18]/46 XX [2] ; **, del (1) (p22), − 2, − 4, − 9, der (13), der (4), − 16

Table 2. Patients with central nervous system (CNS) myelomatosis diagnosed by means of multiparameter flow cytometry : relevant clinical features

Patients (age, sex)	Symptoms	MRI	Previous therapies	Timing of CSF evaluation	References
Male, 36	Headache, disturbance of speech, gait and balance disturbance	Meningeal enhancement	VAD, ASCT	CR	8
Female, 71	Right lumbosacral discomfort	L4 vertebral body lesion	VD	CR	15
Female, 62	Convulsion, confusion	Meningeal enhancement	VTD	At diagnosis and after first line therapy	16
Male, 63	Headache, double vision	No meningeal enhancement	TAD, AST	Relapse	18
Male, 64	Nausea, vomiting, headache	No meningeal enhancement	VAD, VBCMP/VBAD,	PD	20
Female, 60	Bilateral distal weakness in lower extremities, lumbar pain, right facial nerve palsy	Mild and smooth enhancement of the cauda equina region	VAD	NA	22
Female, 59	Vacant episodes, slurred speech, confusion, left facial droop and right arm weakness	Enlarged ventricles, hydrocephalus and meningeal enhancement	VD	CR	23
Male, 60	Absent	NA	VTD, VRDA	VGPR	24
Female, 62	Confusion, limb weakness, gait and speech disturbances	No meningeal enhancement	VTD, ASCT	Relapse	Current paper

MRI, magnetic resonance imaging ; CR, complete remission ; PD, progressive disease ; VGPR, very good partial response ; ASCT, autologous peripheral blood hematopoietic stem cell transplantation ; VTD, Bortezomib, Thalidomide, Dexamethasone ; VCDD, Bortezomib, Cyclophosphamide, Pegylated liposomal doxorubicin ; VAD, Vincristine, Adryamicin, Dexamethasone ; VBCMP, Vincristine, Carmustine, Cyclophosphamide, Melphalan, Prednisone ; VBAD, Vincristine, Carmustine, Adryamicin, Dexamethasone ; VD, Bortezomib, Dexamethasone ; TAD, Thalidomide, Adryamicin, Dexamethasone ; VRDA, Bortezomib, Lenalidomide, Dexamethasone, Adryamicin ; NA, not available

of CNS-MM had been studied by means of flow cytometry.[8,15,16,18,20,22-24] Indeed, the great majority of CNS-MM described in the literature were reported either before the era of flow cytometry or before the consolidated use of such a method in the diagnostic approach to MM and related diseases.

Our case was characterized by the following features : neurological symptoms appeared during a phase of extramedullary localization despite the administration of salvage therapy ; tumor burden was low, with a small bone marrow involvement and mild IgG-λ monoclonal protein (1.7 g/dL) ; CT and MRI were negative and diagnosis was made by flow cytometric analysis of CSF ; CSF plasma cells showed the same immunophenotype as the bone marrow and the circulating counterparts ; the CD56 molecule was expressed ; plasma cell leukemia was not observed and meningeal involvement occurred in the presence of a negligible absolute number of circulating clonal plasma cells ; the course of meningeal involvement was very rapid and our patient died before starting any additional therapy.

The precise pathogenesis of CNS-MM unknown, but hematogeneous spread is most probable mechanism of meningeal

Table 3. Patients with central nervous system myelomatosis (CNS-MM) diagnosed by means of multiparameter flow cytometry : therapy for meningeal involvement and outcome after CNS-MM diagnosis

Patients (age, sex)	Therapy for CNS-MM	Outcome	Authopsy	References
Male, 36	Intrathecal Thiotepa and Hydrocortisone ; intrathecal Methotrexate, Cytosine Arabinoside and Hydrocortisone	Died after 9 days	NA	8
Female, 71	Intrathecal liposomal Cytosine Arabinoside plus cauda equina irradiation	Asymptomatic after 5 months	Confirmative	15
Femal, 62	Intrathecal Cytosine Arabinoside	Died	NA	16
Male, 63	Intrathecal Methotrexate and Prednisone	Died after 3 months	NA	18
Male, 64	Intrathecal Methotrexate and Hydrocortisone	Died after 5 months	NA	20
Female, 60	Lumbar radiation plus intrathecal Methotrexate, Cytosine Arabinoside and Hydrocortisone	Died after 2 months	NA	22
Female, 59	Lenalidomide and Dexamethasone	Died after 40 days	NA	23
Male, 60	Pomalidomide and Dexamethasone	Alive and in CR after ten months	NA	24
Female, 62	None	Died after 2 days	NA	Current paper

NA, not available ; CR, complete remission ;

invasion. It has been argued that CNS-MM mimics leukemic meningitis, in which involvement of the CNS first becomes apparent in the walls of superficial arachnoid veins and surrounding adventitia. With more advanced stages of leukemic infiltration, the arachnoid trabeculae are destroyed and neoplastic cells are able to spill over into the CSF, and are thus detectable on cytological and/or flow cytometric examination.[30]

CNS-MM is rarely observed at first disease presentation and appears to be more frequent in patients with refractory disease (or relapsed disease), with particular involvement of patients with other extramedullary localizations, which can be either synchronous or metachronous. Although it occurs in prevalence in stage III patients,[4] the burden of disease often is not particularly high and a low degree of bone marrow infiltration seems to characterize many cases of SNC localization, as described by Rasche *et al*.[17]

Despite the probable derivation from of circulating neoplastic plasma cells (and/or their precursors), plasma cell leukemia can be detected only in about 5% of cases.[4] SNC-MM can occur even when the absolute number of clonal plasma cells, measured by means of a very sensitive flow cytometric assay, is negligible, as demonstrated by our case report.

The reasons why SNC-MM occurs in a very small subset of patients are unknown. A possible favoring role has been suggested for new drugs, such as Thalidomide, Bortezomib, or Lenalidomide, and/or high dose regimens followed by autologous stem cell transplantation. However, the literature shows that CNS-MM occurred also in times when these drugs were not available. One interesting study involving a high number of cases has confirmed that the incidence of extramedullary MM (including CNS involvement) has increased, but the conclusions were that this particular phenomenon is probably due to more sensitive imaging techniques and to the prolongation of patients' survival which has been obtained with the use of more effective treatment regimens.[31] Indeed, it has been shown that multiple relapses may occur from different subclones of myeloma cells, which are probably selected by more aggressive treatments.[32,33]

The role of adhesion molecules in favoring CNS-MM (as well as other extramedullary localizations of MM) is a debated matter. In a previous clinical study concerning either cases of CNS-MM or other extramedullary sites of localization of MM, immunocytochemical techniques were applied, with the evidence of substantial lack of CD56 expression.[21,34-40] The hypothesis that CD56 down-regulation might favor the migration of MM plasma cells from the bone marrow was put forward.

More recent reports, however, are not in agreement with such a hypothesis. In fact, by using flow cytometry, the CD56 molecule was found on MM plasma cells from additional cases of CNS-MM the patients with CNS-MM[15,16,21,23,40] and in our patient. In addition, Rasche *et al*. reported positivity for CD56 in eight of ten patients with extramedullary relapse of MM, using immunohistochemical assays.[17] Therefore, the role of CD56 down-regulation in favoring the localization of MM plasma cells in the CSF, as well as in other sites, deserves further investigation and more cases should be analyzed by means of flow cytometry not only using samples from the bone marrow, but also measuring CD56 expression on plasma cells obtained from CSF samples.

Similarly, the possible role of another adhesion molecule, such as CD31 is unclear. Some immunohistochemical studies

have shown that CD31 is expressed by marrow MM plasma cells[41] but not by extramedullary localizations of MM.[42] However, other studies ruled-out a role for CD31 in intracranial plasmacytomas.[19] Taken together, the above observations are in agreement with the necessity of further study to establish a possible pathogenetic role of adhesion molecules in the onset of CNS-MM.

Adverse cytogenetics has been hypothesized as a factor favoring the development of SNC-MM.[3,10,17] Several observation are in agreement with a possible role of p53 deletions,[4] high nuclear accumulation of the p53 protein,[34] and del (17p13).[10] An interesting report by Gozzetti *et al*.[16] showed the possible involvement of t(11;14), which seems to be associated with standard risk, but also with high risk in the presence of plasma cell leukemia. However, morphology excluded such a feature and flow cytometry was not used to enumerate circulating plasma cells.

Another point of interest is represented by MRI findings in CNS-MM.[6] As described in other hematologic neoplasms such as leukemia and lymphoma,[43] the diagnostic value of MRI for diagnosing leptomeningeal involvement in MM seems to be limited, since it can be negative in about 10% of patients.[4] In such cases, lumbar puncture becomes mandatory and flow cytometry might show its great usefulness in order to improve diagnosis.

In conclusion, CNS-MM represents an interesting and severe complication in a subset of MM patients. It can be suspected on the basis of the onset of neurological symptoms, which often can occur in patients with non-particularly high burden of disease. MRI is a useful diagnostic tool, but confirmation of CNS-MM is obtained by lumbar puncture. In this setting, multiparameter flow cytometry offers the best way to detect clonal plasma cells and to analyze their phenotype, including the expression of adhesion molecules potentially involved in meningeal localization.

To date, significant evidence has been obtained about the role of flow cytometry in CSF evaluation in non-Hodgkin's lymphomas[25,44] and in diseases characterized by atypical and/or reactive lymphocytes.[43-47]

The use of flow cytometry in detecting CNS-MM has so far been limited to few reported cases, as shown in our review of the literature. We think that the application of flow cytometry in cases of multiple myeloma, suspected for CNS involvement, should be encouraged with the aim of improving the clinical diagnostic strategy and establishing both the most useful MoAb panels and the most specific gating strategy, giving great attention to possible artifacts, as suggested by recent data by Craig *et al*.[48]

Finally, a precocious diagnosis of CNS-MM, especially when MRI results to be negative, might be very important to design therapeutic strategies in this setting of patients. Prognosis of CNS-MM is very poor and median time from diagnosis to death is 2 months (ranging 0.1-25). The most effective treatment schedule for CNS-MM is unknown and previous experiences with intrathecal infusion of cytarabine, thiotepa, methotrexate or steroids did not show significant results.[4] In our case we were not able to start with additional therapy, because of the rapid progression to death. However, we think that great attention should be paid towards drugs capable of penetrating through the blood-brain barrier, such as lenalidomide and pomalidomide.[23,24]

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