Leptomeningeal Myelomatosis: A Rare but Devastating Manifestation of Multiple Myeloma Diagnosed Using Cytology, Flow Cytometry, and Fluorescent in situ Hybridization

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Keywords

Leptomeningeal myelomatosis · Neoplastic meningitis · Cytology · Cerebrospinal fluid · Multiple myeloma · Flow cytometry · Fluorescence in situ hybridization

Abstract

Introduction: Involvement of the central nervous system in patients with multiple myeloma is a rare event. We evaluated the diagnostic workup and prognosis of patients with leptomeningeal myelomatosis (LMM). **Methods:** Between April 2005 and April 2016, we identified 16 cases with LMM. The involvement was diagnosed by magnetic resonance imaging (MRI), cerebrospinal fluid (CSF) cytology as well as flow cytometry. Fluorescence in situ hybridization (FISH) was used in 8/16 cases. In 1 case, genome-wide screening for genetic alterations using single-nucleotide polymorphism (SNP) array analysis was performed. **Results:** The median time from initial diagnosis until the occurrence of LMM was 434 days. At diagnosis, the median age was 60 years. The median cell count was 21/μL (range 1–1,333/μL). All CSF samples showed malignant pleocytosis, confirmed by flow cytometry in 12/16 patients. FISH revealed high-risk features

in the majority of samples. Treatment for LMM consisted of intrathecal chemotherapy and radiation therapy. Genome-wide screening assays revealed different subclones. The outcome was dismal with a median overall survival after the diagnosis of LMM of 82 days. **Conclusion:** By combining several technical procedures, it is possible to identify most patients with LMM. Management of affected patients is challenging and the survival short after a diagnosis of LMM.

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Introduction

In contrast to other malignancies, the frequency of leptomeningeal myelomatosis (LMM) is as low as 1% [1]. Only a few comprehensive overviews with >10 patients have been published [1–7]. LMM is defined as the presence of clonal plasma cells in the cerebrospinal fluid

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Table 1. Baseline characteristics and clinical course of 16 patients with multiple myeloma suffering from leptomeningeal myelomatosis

Pt No.	Age, years	Gender	Initial stage (D/S)	Stage at CNS diagnosis (CRAB)	Heavy chain	Light chain	Radiology imaging	Results	Symptoms	EMD other than CNS	Survival, days	Time from diagnosis to CNS, days	Treatment after CNS diagnosis		Cell count/ μ L	CSF FCM	CSF cytogenetics
													systemic	i.th.			
1	56	M	IIIA	isolated CNS relapse	IgD	κ	skull, CT skull	intra-cerebral masses	left-arm palsy	no	69	172	HD-ARA-C	none	30 Gy WBRT	43	CD38+ CD138+ kappa+
2	59	M	IIIA	isolated CNS relapse	IgA	λ	PET-CT	none	neuropathy	no	1,223	1,068	Dex/ lenalidomide	liposomal ARA-C	36 Gy WBRT	3	CD38+ CD138+ t(4;14)
3	42	F	IIIB	multiple active bone lesions (PET)	n.s.	n.s.	PET-CT, MRI skull	multiple bone lesions, meningeal enhancement, mass N. VII	paraparesis, brachiofacial palsy	no	223	142	MEL/BUS + Auto-PRBCT	liposomal ARA-C, MTX, Dex, ARA-C	30 Gy WBRT	6	CD38+ kappa+ n.a.
4	60	F	IIIA	isolated CNS relapse	IgG	κ	MRI spine	mass S1, cauda equina	paraparesis, radicular pain	no	50	344	none	liposomal ARA-C, MTX, Dex	none	1,333	CD38+ CD138+ CD56+ kappa+
5	74	F	IIIA	anemia, hyper-calcemia	IgG	κ	MRI skull, CT skeleton	meningeal enhancement, spinal osteolysis	confusion, weakness	no	95	1,977	Dex	liposomal ARA-C	none	12	CD38+ CD138+ kappa+ t(1;14)
6	59	M	IIIB	renal failure, anemia	IgG	λ	MRI skull	subdural hematoma	confusion	no	7	357	bortezomib/ Dex	none	none	8	n.d. t(4;14)
7	51	F	IIIB	anemia	none	λ	MRI skull	none	right-arm palsy, diplopia	no	40	463	none	liposomal ARA-C	14 Gy WBRT	24	CD38+ CD138+ lambda+
8	65	M	IIIA	anemia, renal failure, bone lesions	IgG	κ	PET-CT, MRI skull	tentorial mass, meningeal enhancement, testicular mass, bone lesions	nausea	testis	52	1,308	Dex	liposomal ARA-C	none	200	CD38+ CD138+ kappa+ n.a.
9	64	M	IIIA	anemia	IgG	λ	MRI skull, CT skull	left frontal mass	aphasia	no	35	3,357	none	liposomal ARA-C	30 Gy WBRT	1	CD38+, CD138+, lambda+
10	68	M	IIIA	renal failure, anemia	IgG	κ	not done	none	nausea	liver	42	1,186	Dex	liposomal ARA-C	none	6	CD38+ CD138+ kappa+ n.a.
11	52	F	IIIA	anemia	IgG	κ	MRI spine, MRI skull	cervical + thoracic intradural mass	paraparesis	stomach	318	69	none	none	none	20	n.d. del13q1, del17p1, t(1;14)
12	61	M	IIIB	anemia	IgG	λ	MRI skull	none	confusion	no	663	89	none	none	none	23	n.d. normal
13	66	M	IIIA	anemia	IgG	κ	MRI spine	intraspinal tumor S2	cauda equine syndrome	no	404	906	bendamustin, bortezomib, Dex, Cyclo, trofosamide, MEL/lenalidomide	none	20 Gy spinal axis	22	CD38+ CD138+ n.a.
14	71	F	IIIB	anemia	none	λ	MRI skull	multiple intra-cerebral tumors	nervus recurrens palsy	no	4	405	none	none	none	n.a.	CD38+ CD138+ lambda+ CD56+
15	59	F	IIIA	bone lesions	IgA	κ	MRI skull	hydrocephalus malresorptivus, tumor foramen monroi	emesis, nausea	sinus maxillaris	229	883	VTD-PACE, lenalidomide/ Dex	liposomal ARA-C	WBRT	24	CD38+ CD138+ kappa+ CD56+ t(8;14), +1q25
16	62	M	IIIB	bone lesions	IgA	κ	PET-CT	soft tissue tumor T8, S2/3, left musculus rectus lateralis	diplopia	no	313	321	carfilzomib lenalidomide/ Dex	liposomal ARA-C	none	67	CD38+ CD138+ CD56+ kappa+ t(4;14), +1q25

Pt No., patient number; ARA-C, cytarabine; Auto-PRBCT, autologous peripheral blood stem cell transplantation; BUS, busulfan; CNS, central nervous system; CSF, cerebrospinal fluid; CT, computed tomography scan; Cyclo, cyclophosphamide; Dex, dexamethasone; D/S, Durie-Salmon; EMD, extramedullary disease; FCM, flow cytometry; i.th., intrathecal; MEL, melphalan; MRI, magnetic resonance imaging; MTX, methotrexate; n.a., not available; n.d., not determined; N. VII, facial nerve; PET, positron emission tomography; VTD-PACE, bortezomib, dexamethasone, thalidomide, cisplatin, doxorubicin, cyclophosphamide, etoposide; WBRT, whole-brain radiation therapy.

Fig. 1. Flow cytometry of cerebrospinal fluid from patient No. 3. Plasma cells were gated according to standard protocols and showed light chain κ expression.

(CSF). To establish a diagnosis of LMM, either a typical leptomeningeal enhancement on magnetic resonance imaging (MRI) and/or evidence of clonal plasma cells in the CSF is necessary. CSF cytology in malignancies other than myeloma has a limited sensitivity of around 50–60%, but excellent specificity (>95%) when done by an experienced cytologist [8]. Fluorescence-activated cell sorting (FACS) can increase the sensitivity of the detection of tumor cells in patients with aggressive lymphoma at a high risk of central nervous system (CNS) involvement [9, 10], and patients with atypical or suspicious CSF morphology. Therefore, adding flow cytometry may also improve the diagnostic sensitivity and specificity in patients with multiple myeloma (MM). Risk factors for leptomeningeal disease in MM are plasmoblastic morphology, extramedullary disease, elevated lactate dehydrogenase (LDH) and high-risk cytogenetics such as the presence of a translocation t(4;14) and/or a deletion (del) of the chromosomal band 13q14 [2, 3].

Materials and Methods

Patients

Between 2004 and 2016, we identified 16 myeloma patients with leptomeningeal disease treated at the University Hospital of

Ulm in Germany. All of them were suffering from symptomatic MM and CSF analysis was triggered by CNS-related symptoms. CSF samples for cytology, FACS, and cytogenetic studies were drawn by lumbar puncture during standard clinical workup. All patients gave their written informed consent for the procedure.

This retrospective study was conducted in accordance with the principles of the Helsinki Declaration. The local ethics committee approved the evaluation protocol.

CSF Analysis

CSF was obtained by lumbar puncture. Precautions were taken to minimize contamination by peripheral blood.

The fresh specimens were processed following standard procedures as previously published [11, 12]. All smears were analyzed by M.B. Additional FACS analysis was done within a few hours of sampling using standard procedures. CSF volume ranged from 1.5 to 6 mL. The results were analyzed by M.F.-B. and M.B. Samples were judged to be malignant only if a distinct cell population with the disease specific immune-phenotype, i.e., CD38+, CD138+, or a light chain restriction, could be detected.

Fluorescence in situ hybridization (FISH) in combination with immunofluorescent detection of light chain-restricted plasma cells (cIg-FISH) was performed according to standardized protocols using commercially available probes (Abbott Molecular; MetaSystems).

Single-nucleotide polymorphism (SNP) chip analysis was performed from total genomic DNA (500 ng), digested and ligated to adaptors. A generic primer that recognizes the adaptor sequence was used to amplify adaptor-ligated DNA fragments. Then, the amplified DNA was fragmented, labeled, and hybridized to the hu-

Fig. 2. a–d Cytomorphology of 4 representative cases of patients with multiple myeloma affected by leptomeningeal myelomatosis.

man SNP array 6.0 (Affymetrix) for 16 h, followed by automated washing, staining, and scanning. Data analysis was done with a standard software Affymetrix genotyping console™.

For statistical analysis, GraphPad Prism (La Jolla, CA, USA) software was used.

Results

All patients had symptomatic disease with very variable neurologic symptoms. Most of them suffered from central nerve palsy, pain, or changes of mental state. Radiographic studies (MRI and CT scan) revealed pathology in 12 of 16 patients, but only 2 showed meningeal enhancement. The median cell count was $21/\mu\text{L}$ with a wide range ($1\text{--}1,333/\mu\text{L}$). FACS could be performed in 13 out of 16 patients. All samples showed clonal disease with the

expression of CD38 and also CD138 or κ/λ light chain restriction. In 4 samples (patient Nos. 4, 14, 15, and 16) additional expression of CD56 was found (Table 1). Due to low cell counts in some patients (Nos. 2, 3, 9, and 10), FACS added important information for the interpretation of CSF cytology (Fig. 1). In 1 case (patient No. 6), FACS could not be performed because of the small amount of CSF available for analysis, but cytomorphology was unambiguous (Fig. 2).

The median time from a diagnosis of MM to the diagnosis of leptomeningeal spread was 434 days (range 69–3,357 days). All patients finally died (range 7–1,233 days after the diagnosis of LMM). The majority of patients received a specific treatment, except for those with a clearly dismal prognosis, i.e., a life expectancy of less than a few days (i.e., patient No. 6). The median survival time was 82

Fig. 3. Overall survival of 16 patients with multiple myeloma suffering from leptomeningeal myelomatosis (LMM) with a median survival of 82 days after the diagnosis of LMM.

Fig. 4. Characterization of tumor material from patient No. 15. At the time of leptomeningeal myelomatosis presentation, tumor material from different locations was available for analysis. **a** Copy number alterations of chromosome 8. Plasma cells from the CSF present with a gain of 8q and a small biallelic gain. In contrast, the plasma cells in the BM present with a loss of 8p. **b** cIg-FISH of plasma cells from CSF demonstrating a t(8;14).GC, germline control; CSF, cerebrospinal fluid; BM, bone marrow.

days; the Kaplan-Maier estimated survival is presented in Figure 3. An overview of the clinical data and laboratory tests is given in Table 1.

In 1 patient, a more detailed molecular characterization of the disease was possible. In contrast to the presenting bone marrow (BM) 18 months earlier, at the time of progression, a translocation t(8;14) was detectable in the CSF as well as in the BM (Fig. 4); despite the shared acquired IgH translocation, copy number alteration analysis revealed different subclones at these 2 clinical sites.

Discussion

The diagnosis of LMM is based upon MRI and cytological examination of the CSF. MRI is of limited use in leukemia and lymphoma, as previously described [13]; in patients with multiple myeloma, however, MRI adds important diagnostic information. In a large case study published by Paludo et al. [6], only 17% of the patients had no pathologic finding on MRI. Most of them had meningeal enhancement (70%) consistent with LMM, and MRI also

Fig. 5. Proposed algorithm for the identification of leptomeningeal myelomatosis.

revealed intraparenchymal lesions (26%) or dural disease (35%). Imaging of the skull and spine are required for a complete workup.

Cytological analysis of CSF is the gold standard for the diagnosis of neoplastic meningitis. In patients with a low

CSF cell count or doubtful morphology, the addition of flow cytometry is very useful to discriminate reactive from malignant cells and to identify LMM rapidly [14]. In our case study, flow cytometry was able to add diagnostic reliability despite the low cell count (in patient Nos. 2, 3, 9, and

10). Leptomeningeal disease underlies most malignancies of unfavorable prognosis. Despite CNS-targeted treatment, such as the intrathecal application of liposomal ARA-C, methotrexate, or radiotherapy, the prognosis of LMM remains very poor with a median survival of 2 months.

Our data indicate that, in LMM, the median survival is also <3 months. This is in line with 2 larger series that recently reported on the outcome of patients with CNS involvement of MM [6, 7]. Paludo et al. [6] found an incidence of for myelomatous involvement in 0.7% of a large cohort of >4,000 patients diagnosed and treated at the Mayo Clinic; in this group of 29 patients, the median overall survival was only 3.4 months. There was no specific therapeutic intervention identified that could positively influence the natural cause of the disease. In a multi-institutional study reported by Jurczynski et al. [7], the median overall survival for the entire group of 172 patients was 7 months from the onset of CNS involvement. However, when they included the presence of >1 cytogenetic abnormality detected by FISH and at least 1 prior therapy, the median survival of this group of patients was only 2 months. This dismal prognosis may have been caused by the fact that most of the drugs used in myeloma do not penetrate the blood-brain barrier if used for systemic treatment [15]. Newer drugs such as carfilzomib, marizomib, and pomalidomide may penetrate the blood-brain barrier and so may be used as therapeutic options in such situations [5, 16–18]. We observed 1 long-term survivor with LMM (patient No. 2), who was diagnosed with CNS involvement after allogeneic stem cell transplantation. He was treated with intrathecal liposomal ARA-C, CNS irradiation, and lenalidomide/dexamethasone (Table 1) as previously described elsewhere [19]. Nevertheless, he eventually died >3 years after diagnosis of LMM due to a systemic relapse. In most of the malignancies, except for acute lymphoblastic leukemia and Burkitt's lymphoma, spread to the CSF is a late event during the course of disease. Our data show that this is also true for patients with MM; the median time for the diagnosis of LMM after the initial diagnosis is >2 years. Novel drugs, such as bortezomib, lenalidomide, or thalidomide allow long-term control of myeloma. If this also accounts for a higher rate of extramedullary disease presentations, including CNS myeloma is a matter for discussion and further investigation [20]. Therapeutic strategies, including drugs with a higher ability to penetrate the blood-brain barrier such as pomalidomide or newer proteasome inhibitors such as carfilzomib, may improve the prognosis.

From a biological point of view, however, it will eventually be important to target all subclones of the disease,

if not up front then at least at the time of relapse or progression. This was demonstrated in 1 patient for whom we were able to perform whole-genome analysis of the disease at the same time point at different sites of presentation. Whereas the dominant CSF clone was characterized by hyperdiploidy like the clone at first presentation, the clone in the BM had lost this characteristic. Apart from this, copy number alterations, like gains at 8q, were detected only in the CSF sample. This clonal evolution at different sites, particularly between BM and extramedullary disease might partially explain why patients may respond to treatment at one location but not at another. In a landmark paper, Keats et al. [21] were able to analyze the genomic landscape of a high-risk myeloma patient at 7 different time points, showing a dramatic linear evolution. This is a common phenomenon, recently demonstrated by a large systematic study of 51 patients in whom >75% showed spatial genomic heterogeneity [22].

Conclusion

LMM occurs as a rare complication late in the course of MM. Up to now, no reliable clinical or cytogenetic parameters have been available to predict LMM occurrence. Known incidences may increase during the next decade, possibly due to more efficient systemic treatment options for advanced myeloma. A diagnosis can be made using a combination of MRI and CSF cytology and flow cytometry (Fig. 5). Even though no standard treatment for this clinical situation has been described or established, the potential therapeutic measures include combinations of radiotherapy, intrathecal treatment using methotrexate and liposomal ARA-C, and systemic treatment. Further studies to evaluate the effect of new drugs such as pomalidomide, carfilzomib, and marizomib on CNS disease in MM are warranted. Ultimately, the novel technical possibilities in detecting even subclonal disease might help to identify and tailor more effective treatment modalities.

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Disclosure Statement

The authors declare no conflicts of interest.

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