Neoplastic meningitis in patients with acute myeloid leukemia scheduled for allogeneic hematopoietic stem cell transplantation

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ABSTRACT

We analyzed the frequency of neoplastic meningitis in patients with acute myeloid leukemia prior to allogeneic hematopoietic stem cell transplantation at our institution. Between 1996 and 2009, cerebrospinal fluid samples of 204 adult patients were examined during pre-transplant work-up for cell counts and, if abnormal, morphologically. We found blasts in cerebrospinal fluid samples of 17 patients with either persistent (n=9) or newly diagnosed (n=8) neoplastic meningitis. All patients proceeded to transplant. The proportion of patients with central nervous system involvement was significantly higher in patients with refractory disease at the time of transplantation compared with patients responding to prior systemic therapy (19% *vs.* 4.6%; *P*=0.003). Since most of the patients with central nervous system involvement were asymptomatic, cerebrospinal fluid evaluation should be considered at least in patients with refractory acute myeloid leukemia.

Key words: acute myeloid leukemia, stem cell transplantation, central nervous system, refractory disease.

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Introduction

Central nervous system (CNS) involvement in acute myeloid leukemia (AML) is a rare event with an estimated incidence below 5% at diagnosis. In the recently published guidelines for diagnosis and management of AML in adults, lumbar puncture is not recommended as a routine diagnostic procedure in patients without CNS symptoms, although it may be considered in special situations (e.g. hyperleukocytosis).[1] CNS targeted prophylaxis in AML has been abandoned based on the results of previous studies; for example, an MRC trial published in 1986.[2] Similar considerations apply to patients in the transplant setting. The probability of developing CNS relapse after hematopoietic stem cell transplantation (HSCT) for patients with AML transplanted in remission is low.[3] Furthermore, patients transplanted in the last 20 years received conditioning regimens with either total body irradiation (TBI) or high-dose busulfan, both of which penetrate the blood-brain barrier. A survey of the European Group for Blood and Bone Marrow Transplantation (EBMT) published in 2005 concluded that a lumbar puncture and intrathecal prophylaxis is not indicated in AML patients transplanted in remission.[4] These conclusions, however, only apply to patients receiving myeloablative conditioning with TBI or high-dose busulfan.

In the last decade, the indications for allogeneic HSCT have changed considerably with more patients with refractory disease being treated.[5,6] Furthermore, many patients are now receiving radiation-free, fludarabine-based reduced-intensity conditioning (RIC) regimens.[7] This type of conditioning does not reach the cerebrospinal fluid (CSF) compartment and is potentially associated with an increased risk of CNS relapse. Indeed, Davies *et al.* reported CNS relapse after reduced-intensity conditioning.[8]

Over the last years, lumbar puncture (LP) and intrathecal prophylaxis were part of our standard pre-transplant work-up for patients with AML. In this study, we analyzed frequency of CNS involvement and its prognostic impact.

Design and Methods

Between 1996 and 2009, 250 adult patients with AML underwent allogeneic HSCT. Eleven patients had two transplants, one patient was transplanted three times, resulting in a total of 263 transplant procedures. CSF evaluation by lumbar puncture ("index LP") and the prophylactic intrathecal application of 12 mg methotrexate were carried out within a median interval of 16 days prior to transplant. Of the 250 patients, 204 patients (81.6%) underwent lumbar puncture, 46 patients were not evaluated for different reasons, such as high blast counts in the peripheral blood, coagulation disorder or anatomical obstacles. All patients gave their written informed consent for the procedure: 2-3 mL of CSF were collected by lumbar puncture and evalu-

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ated by cell counting and in cases with cell counts above 2/μL by cytological analysis as well. Precautions were taken to avoid peripheral blood contamination. Cell counts were performed by diluting 20 μL of CSF with trypan blue stain according to the expected cell count. The suspension was transferred into a *Neubauer* modified chamber, the 4 edge squares were analyzed and the viable cells were counted using a x40 high power microscope. For cytological analysis, CSF was diluted to an expected cell count of 50-100 cells/μl. We then transferred 200 μl of cell suspension into a Shandon cytospin device (GMI, Inc, Ramsey, Minnesota, USA). Centrifugation was performed at 1,500 rpm and low acceleration for five minutes. Films were dried and stained with May-Gruenwald-Giemsa. The results of morphological analysis were available for 85 patients. Flow cytometry was used only in cases with an ambiguous morphological result. All films were evaluated by an experienced hematologist. All samples with obvious blood contamination were judged to be non-diagnostic and excluded from this analysis.

The statistical analysis (comprising contingency tables with two-sided Fisher's exact test and Kaplan-Maier plots with the log rank test) were performed by means of the GraphPad Prism version 4.00 for Windows (GraphPad Software, San Diego California USA, *www.graphpad.com*).

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

Results and Discussion

The overall frequency of neoplastic meningitis (NM) throughout the whole treatment period including primary diagnosis, all cytoreductive chemotherapy cycles and also posttransplant course was as high as 15% (31/204). Eighteen patients were diagnosed to have NM prior to the index LP. Of these 18 patients, 9 were successfully treated before HSCT documented by a negative CSF cytology at index LP, whereas the other 9 had persistent disease with a positive cytology at index LP. In addition, 8 patients with previously unknown NM were newly diagnosed by the index LP, resulting in 17 (8%) patients with active CNS disease entering the transplant procedure (Table 1 and *Online Supplementary Figure S1*).

We observed CNS involvement also in 8 patients with CSF cell counts less or equal than 3 cells/μL. In these patients, involvement could only be detected by morphological CSF examination. All patients underwent intrathecal chemotherapy (methotrexate alone, methotrexate in combination with cytarabine or liposomal cytarabine). Three patients received additional radiotherapy with a cerebrospinal boost prior to transplantation, 2 patients were irradiated posttransplant. The conditioning regimens for patients with CNS involvement were heterogeneous. The frequency of CNS involvement for patients scheduled for RIC was

Table 1. Acute myeloid leukemia patients with central nervous system disease at index puncture: disease characteristics and outcome after treatment.

UPN	Age	Fab	Cytogenetics	Molecular genetics	Transplant-regimen	Remission status	History of EML	Radiation	CNS disease intrathecal therapy	Cell count	Outcome
706	52	M1	t(8;21), -X	n.d.	Haplo; TT, TBI, CY, ATG	CR2	no	no	MTX	3	dead, NRM
887	34	M5	normal karyotype	FLT3-ITD	MRD, BU/CY/RIT	RD	CNS	no	MTX, ARA-C	20	dead, relapse
908	49	M1	normal karyotype	n.d.	MUD, BU/CY/RIT	PR	CNS	no	MTX, ARA-C	3	dead, relapse
924	53	M0	normal karyotype	n.d.	MUD, BU/CY/RIT	RD	no	no	MTX	5	dead, NRM
1079	53	M1	add(19)(p13)	n.d.	MUD, BU/CY/RIT	CR2	testis, CNS	prior TX 25.2 Gy	ARA-C, lip.ARA-C	2	dead, relapse
1101	31	M1	46,XX,abn(11q23)	n.d.	MUD; Flamsa	RD	CNS	no	ARA-C, MTX, dexamethasone	2	dead, NRM
1129	50	M4	normal karyotype	n.d.	MUD; Flamsa	CR1	CNS	post TX 24Gy (63 days after HSCT)	MTX	2	dead, relapse
1371	38	M0	normal karyotype	n.d.	MUD; Flamsa	RD	CNS, skin, pleura	no	MTX, ARA-C	13	dead, relapse
1392	47	M3	t(15;17)	n.d.	MUD,BU/CY/ RIT	CR2	no	no	MTX, ARA-C	3	alive
1407	43	M1	normal karyotype	n.d.	MUD; Flamsa	RD	no	no	MTX, ARA-C, lip.ARA-C	317	dead, relapse
1475	56	NS	48,XX,+8,+19	n.d.	MUD; Flamsa	RD	no	no	MTX, lip.ARA-C	2	alive
1522	52	M4	normal karyotype	FLT3 WT, NPM WT, CEBPA mutated	Haplo, RIT, TT, FLU, TBI	CR2	CNS	post TX 18 Gy (49 days after HSCT)	MTX, lip.ARA-C	8	dead, NRM
1584	36	M4	normal karyotype	FLT3 WT, NPM WT	MUD; Flamsa	RD	CNS	prior TX 25.2 Gy	ARA-C, lip.ARA-C	5	dead, relapse
1638	22	NS	complex karyotype	FLT3 WT, NPM WT	MUD; Flamsa	RD	no	no	MTX	6	dead, NRM
1705	49	M4	normal karyotype	FLT3-ITD mut, NPM mut	MUD; Flamsa	RD	CNS	prior TX 25.2 Gy	ARA-C, MTX, lip.ARA-C	499	dead, relapse
1728	52	M4	normal karyotype	FLT3-ITD mut, NPM mut	MUD; Flamsa	RD	skin	no	MTX, lip.ARA-C	404	dead, relapse
1740	47	M5	normal karyotype	FLT3-ITD mut, NPM mut	MRD,TBI; CY	CR1	no	no	MTX, lip.ARA-C	3	dead, NRM

Haplo: haploidentical donor, MUD: matched unrelated donor, MRD: matched related donor, RIT: radioimmunotherapy, TBI: total body irradiation, CY: cyclophosphamide, FLU: fludarabine, TT: thiotepa, ATG: anti thymocyte globuline, Flamsa: fludarabine, ARA-C, amsacrine, ATG, CR: complete remission, RD: refractory disease, CR2: second complete remission, n.d.: not done, NRM: non-relapse mortality, NS: not specified, MTX: methotrexate 12 or 15 mg, ARA-C: cytarabine 40 mg, dexamethasone: 4 mg; lip.ARA-C: liposomal cytarabine (DepoCyte®) 50 mg, EML: extramedullary leukemia.

10.7% (9/84). After HSCT, 3 patients (3/17, 18%) were found to have a positive CSF cytology, suffering either from relapse (n=1) or persistence (n=2). In addition, 5 patients had *de novo* CNS disease. All 8 patients with CNS manifestation after transplant also suffered from systemic relapse (*Online Supplementary Figure S1* and *Online Supplementary Table S1*). We analyzed several risk factors for CNS disease, such as age, karyotype, meningeosis at diagnosis and remission status. The only two risk factors with a significant association to CNS disease at the time of the index LP were remission status and previously diagnosed neoplastic meningitis. CNS involvement was strongly associated with refractory disease at the time of index puncture: 10 of 53 (19%) patients with refractory disease had CNS involvement compared with 7 of 151 (4.6%) who had achieved at least partial remission (P=0.003). Furthermore, 9 of 18 (50%) patients with a previous history of CNS disease compared with 8 of 178 (4.5%) without a previous history suffered from CNS disease (P<0.0001).

We are confident that our data on the frequency of CNS involvement are reliable since we took great care to prevent contamination of our samples by peripheral blood. It is likely that even by CSF morphological analysis we underestimated the real frequency of CNS involvement, given that the sensitivity of CSF cytology is only between 50-60%.[9] Additional flow cytometry may improve diagnostic reliability, as has been shown for aggressive lymphoma.[10] The risk factors for CNS involvement in AML (CNS involvement at diagnosis, refractory disease) in our study are similar to those reported in retrospective registry studies.

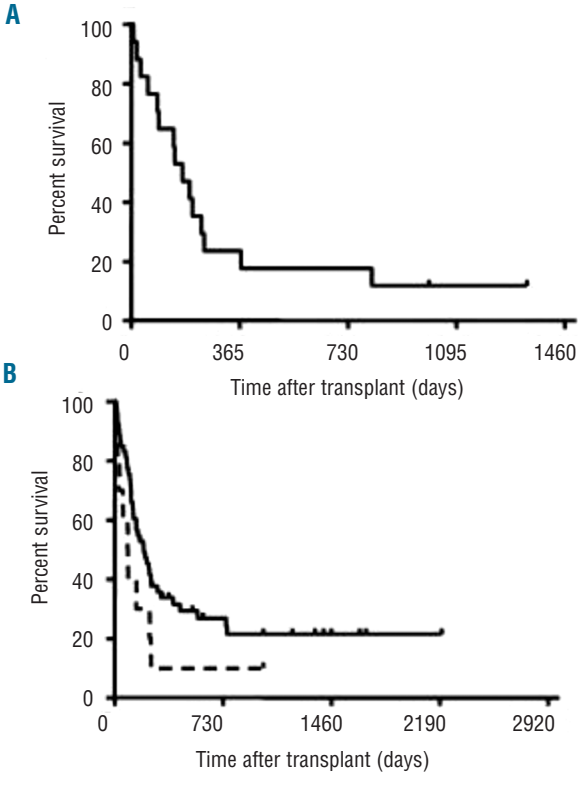

Figure 1. Probability of overall survival in acute myeloid leukemia patients with CNS involvement prior to transplantation. (A) The probability of overall survival in all 17 patients with central nervous system involvement. (B) Only patients with refractory disease: the patients without central nervous system involvement are shown with the solid line; patients with central nervous system involvement are shown with the dotted line.

Our data suggest that CNS-directed therapy is effective in terms of blast clearance in the CNS but we cannot identify the optimal agent or combination of agents in our data set. A recent Spanish survey included 17 patients with AML treated either at the time of diagnosis or at relapse for CNS disease. The investigators employed the same cytotoxic drugs as were used in our study.[11] The main alternative to intrathecal chemotherapy is radiation therapy to the cranium and the spine. Although it has been shown that such an approach is efficient in eradicating the disease from the CNS, the study from Sanders *et al.* failed to show that this was associated with a survival benefit for the patients, mainly because of their very unfavorable prognosis.[12] Unfortunately, successful treatment of CNS-disease did not appear to improve the dismal long-term prognosis of our patients (Figure 1A). The majority of our refractory patients were treated within the FLAMSA-protocol which is considered to be a reduced intensity conditioning regimen. As published by Schmid *et al.*, the patients with CNS involvement did not benefit from this approach, although it uses low-dose TBI (4 Gy) and intermediate-dose cytarabine (4 x 2 g/m^2) (Figure 1B).[6] Our current approach to CNS disease seems not to be effective in terms of cure rate or survival. There are several potential reasons for this very poor outcome of patients with CNS involvement. In our series, some patients had other sites of extramedullary involvement (skin, testis, pleural effusion). Relapse patterns after donor lymphocyte infusions have also clearly demonstrated that extramedullary disease is a poor target for the graft-*versus*-leukemia effect.[13] Alternatively, CNS-involvement may simply be a surrogate marker for AML which are resistant to chemo- and/or radiotherapy. We were unable to analyze the frequency of CNS relapse and the prognostic impact of CNS involvement prior to transplant in the subgroup of patients scheduled for RIC separately because this analysis was confounded by the fact that the majority of RIC patients received FLAMSA and had refractory disease. Since we performed LP outside the transplant setting (before and after HSCT) only in patients with history of NM or with clinical signs of neoplastic meningitis, our frequency of CNS disease at diagnosis of AML or at relapse after HSCT is probably too low.

Our own data and a review of the literature indicate that an LP and careful morphological evaluation of CSF should be performed prior to transplant in patients with a history of CNS involvement, refractory disease and with involvement of other extramedullary sites.[14-17] Lumbar puncture may also be considered in patients scheduled for RIC[8] and in those with risk factors for CNS involvement, such as FAB M4/M5 morphology, and CD56 expression on the leukemic blasts.[18] Perhaps new imaging techniques, such as 18F-fluorodeoxythymidin-positron emission tomography (FLT-PET) may help to identify unknown extramedullary leukemia allowing additional radiotherapy to involved sites.[19]

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