Published in final edited form as:

Hum Immunol. 2018 July ; 79(7): 537–538. doi:10.1016/j.humimm.2018.04.001.

Donor cell chimerism evaluation in cerebrospinal fluid by short tandem repeat analysis following allogeneic hematopoietic stem cell transplantation

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Abstract

Cerebrospinal fluid (CSF) cell count, morphology and flow cytometric evaluations are used to investigate central nervous system (CNS) involvement in leukemia/lymphoma. We performed CSF short tandem repeat (STR) analysis to monitor CSF chimerism status and evaluate for disease involvement in 11 asymptomatic pediatric allogeneic hematopoietic stem cell transplantation (HSCT) recipients with hemato-lymphoid neoplasms and high risk for or history of CNS involvement. Eighteen (64%) of the samples with median CSF cell count of 1/mm³ with 90% lymphocytes gave conclusive STR results, suggesting that this DNA-based method can be used in monitoring CSF chimerism status after HSCT with an acceptable yield.

Keywords

Hematopoietic stem cell transplantation; Donor chimerism; Cerebrospinal fluid; Short tandem repeat analysis

1. Introduction

Sustained full donor chimerism in the bone marrow is associated with relapse-free survival and thought to be a sign of graft-versus-leukemia/lymphoma (GvL) effect in allogeneic hematopoietic stem cell transplantation (HSCT) recipients. The beneficial role of donor-derived immune cells after allogeneic HSCT in metastatic central nervous system (CNS) leukemia or lymphoma has been debated. It is postulated that the GvL effect mediated by donor lymphocytes in the CNS is an operational mechanism of maintenance of the remission as reflected in successful utilization of allogeneic BMT in central nervous system acute

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lymphoblastic leukemia (ALL) [1]. Therefore, monitoring chimerism status in cerebrospinal fluid (CSF) can be a useful tool. Here, we summarize our experience in CSF short tandem repeat (STR) analysis in allogeneic HSCT with emphasis on the feasibility of assessing chimerism status in CSF in the early- and mid-term post-transplant period in the absence of CNS symptoms.

2. Materials and methods

We retrospectively reviewed the available data from our experience to determine if STR analysis could be a feasible method to determine donor chimerism status in CSF cells following allogeneic HSCT from 2007 through 2016. Human Investigation Committee at Wayne State University approval was obtained. Review of medical records was carried out in leukemia or lymphoma patients whose CSF samples were submitted following allogeneic HSCT for STR analysis due to either underlying CNS disease or known risk for CNS involvement. None of the patients had CNS signs or symptoms at the time of evaluation. Samples were obtained as part of the routine post-transplant disease surveillance in high-risk patients treated at our institution.

Ten different loci were studied and the average donor percentage of the informative loci was reported in STR analysis. DNA was extracted using the Qiagen QIAmp DNA extraction KIT (www.qiagen.com). Briefly, CSF cells were digested with proteases and the DNA precipitated and cleaned using ethanol and ethanol containing buffer, respectively. STR testing was carried out using the AmpFLSTR® Profiler Plus® PCR Amplification Kit (ThermoFisher Scientific, USA.) This kit amplifies nine short tandem repeat (STR) loci and the Amelogenin locus in a multiplex fashion. The STR loci amplified were D3S1358, D5S818, D7S820, D8S1179, D13S317, D18S51, D21S11, FGA, and vWA. Conclusive results were obtained when enough DNA was obtained from the cells in CSF. When low DNA yielded no STR profiles or no DNA was obtained, we have reported the results as “no result”. The sensitivity of STR analysis via this method is 5%.

3. Results

Eleven allogeneic HSCT recipients, seven with ALL, one acute myeloid leukemia, one Burkitt lymphoma, one Burkitt-like non-Hodgkin lymphoma, and one blastic plasmacytoid dendritic cell neoplasm were included in the analysis. Seven of the children had CNS disease at diagnosis and four were at high risk of involvement. All children received myeloablative conditioning regimens. Eight patients received tacrolimus and mycophenolate mofetil and three received tacrolimus and methotrexate for graft versus host disease prophylaxis. A total of 28 CSF samples obtained from 11 patients were analyzed. Eighteen (64%) of the 28 samples gave conclusive results from nine of the 11 studied cases. Among all the samples with conclusive results, median CSF cell count was $1/\text{mm}^3$ (0–48) and 90% (50–100) of those cells were lymphocytes per analysis of morphology on Wright-Giemsa-stained cytopsin slides (Table 1). Cerebrospinal fluid samples that did not provide conclusive chimerism results ($N = 10$) via STR had a median CSF cell count of $2/\text{mm}^3$ (1–615).

A total of eight samples from six patients had both STR and flow cytometric analysis with conclusive results. Of those six patients, four had a history of CNS involvement. All of those samples revealed no involvement of CNS disease by flow cytometry and with CSF donor chimerism of greater than 95 to 100%.

The first post-HSCT evaluation was conclusive in eight of eleven patients with a median post-HSCT day of 76 (30–147); CSF donor cell chimerism ranged between 92 and 100%. Bone marrow samples obtained at that time revealed 99% (95–100) and 100% donor cell chimerism in lymphoid and myeloid cells, respectively (Table 1). In one ALL case, CSF continued to show 100% donor cell chimerism at the time of systemic relapse.

4. Discussion

Determining cell count, cell morphology evaluation, and flow cytometric analysis of CSF cells are useful and complementary techniques for the study of CNS relapse in leukemia and lymphoma. Donor chimerism status has been investigated by using sex chromosome fluorescence in-situ hybridization method in HSCT recipients following cross-sex HSCT, but has not been investigated using STR analysis of CSF cells [2]. Recently, Waterhouse et al. reported on the utility of CSF STR analysis in adult patients presenting with neurological complications at different time points following allogeneic HSCT. They reported correlation with disease recurrence between STR and flow cytometry results in some cases [3]. Of note, these symptomatic patients had larger numbers of cells present in the cerebrospinal fluid compared to the asymptomatic pediatric patients presented in this paper.

Eighteen of the 28 samples (64%) gave conclusive results in nine of 11 cases in our study. When evaluating the first post-HSCT CSF STR analysis in individual cases, the samples from eight of eleven patients demonstrated conclusive results. In those cases, the median post-HSCT day was 76 (30–147); median CSF cell count was $2/\text{mm}^3$ with a median of 82% lymphocytes. These results suggest that STR analysis following allogeneic HSCT could be used as an adjunct to monitor CSF donor chimerism status relatively early after HSCT in patients even without neurological symptoms or signs with an acceptable yield despite the small number of cells available in samples.

This DNA-based method, which is less dependent on cell integrity when compared to morphological, cytogenetic, and flow cytometric testing was feasible based on these results. Detection of recipient cells in CSF despite full donor chimerism in the bone marrow by STR analysis without CNS disease-associated symptoms may identify developing isolated CNS disease and indicate the need for closer monitoring and early therapeutic interventions. This method can be used in tracing the allogeneic cells in experimental models as well.

Acknowledgments

Funding sources

This research did not receive any specific grant from funding agencies in the public, commercial, or not-for-profit sectors.

Abbreviations:

GvL	graft versus leukemia/lymphoma
HSCT	hematopoietic stem cell transplantation
CNS	central nervous system
ALL	acute lymphoblastic leukemia
CSF	cerebrospinal fluid
STR	short tandem repeat

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Table 1

Cerebrospinal fluid characteristics and chimerism status.

Case	Test	Diagnosis	Age at BMT (yrs)	Post BMT Date	NC count ($\times 10^9/L$)	RBC count ($\times 10^9/L$)	Lymphocytes (%)	Monocytes (%)	Neutrophils (%)	CSF Donor (%)	BM-L Donor (%)	BM-M Donor (%)
1	1	BPCN	9.7	35	1	0	87	13	0	97	100	100
2	2			119	0	0	93	7	0	94	100	100
2	3	ALL	9.1	30	7	10	64	34	1	100	> 95	100
4	4			98	6	0	96	4	0	100	100	100
3	5	ALL	9.9	271	1	0	80	20	0	100	NE	NE
6	6			650	0	0	79	21	0	> 95	NE	NE
4	7	ALL	1.6	123	8	0	76	24	0	100	NE	NE
5	8	ALL	6.3	147	2	0	100	0	0	100	3 (PB)	85 (PB)
9	9			532	0	0	100	0	0	100	7	85
6	10	AML	1.3	30	0	0	88	12	0	97	100	100
7	11	BL	11.9	101	2	1	50	44	6	100	> 95	100
12	12			354	0	0	93	7	0	100	> 95	> 95
13	13			445	0	0	100	0	0	100	NE	NE
14	14			591	1	0	100	0	0	100	NE	NE
8	15	ALL	9.3	97	7	30	92	8	0	100	100	100
9	16	BL-NHL	17.7	54	0	30	75	23	2	99	100	100
17	17			148	1	0	91	9	0	100	100 (PB)	100 (PB)
18	18			278	2	0	89	9	0	100	NE	NE

Abbreviations: BPCN Blastic plasmacytic dendritic cell neoplasm; ALL acute lymphoblastic leukemia; AML acute myelogenous leukemia; BL Burkitt Lymphoma; BL-NHL Burkitt-like non-Hodgkin lymphoma; BM-L bone marrow lymphoid; BM-M bone marrow myeloid; NE not evaluated; PB peripheral blood (in two cases peripheral blood chimerism status was studied rather than bone marrow).