Article

Chimerism Assessment is not a Reliable Surrogate for Disease Monitoring after Stem Cell Transplantation

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Abstract:
Aims: There is a paucity of evidence-based guidelines supporting test selection after allogeneic stem cell transplantation for the assessment of residual disease and bone marrow chimerism, although common clinical practice typically includes some measures of both. This study aims to evaluate the accuracy of chimerism studies to predict residual disease and clinical outcome.
Methods: We retrospectively reviewed both chimerism and residual disease studies associated with 334 bone marrow samples from 143 patients with a range of hematologic malignancies, with a mean of over 2 years of additional follow-up to assess relapse rate.
Results: Of 334 cases, 266 showed no histologic evidence of disease. Ancillary tests for residual disease were available in 260/266 cases, with negative results in 87% (227/260) of cases. Mixed chimerism (MC) was found in 48% (108/227) of these cases. In addition, 30.3% of cases with residual disease by ancillary testing demonstrated complete engraftment (CE). No significant difference in relapse rates between MC (19.5%) and CE (16.3%) patients was found in patients who received reduced intensity chemotherapy conditioning regimens (RIC) (p=0.69). Similarly, relapse rates of MC and CE in patients who received myeloablative conditioning (MAC) were 11.1% and 17.5%, respectively (p=0.47).
Conclusions: Bone marrow engraftment analysis did not reliably correlate with residual disease status as measured by a number of different methods in the setting of typical preparative regimens (classified as either RIC or MAC). In addition, we found no uniform association between chimerism status and relapse. This study raises concerns about the value of chimerism studies to predict clinical outcomes related to disease status.

Keywords: engraftment, chimerism, residual disease monitoring, stem cell transplantation, hematopoietic neoplasms

Introduction

Allogeneic stem cell transplant (SCT) is a potentially curative therapy in multiple hematopoietic disorders, with cures rates exceeding 85% in conditions such as aplastic anemia, thalassemia and chronic myelogenous leukemia [1]. However, in other diseases such as acute lymphoblastic leukemia or acute myeloid leukemia, cure rates with allogeneic SCT are substantially lower, especially in cases of refractory or

advanced disease. Along with transplant-related mortality, relapse remains a major cause of death in these patients. For this reason, patients are routinely monitored indefinitely post-SCT for the presence of their disease.

Post-transplantation bone marrow (BM) biopsies are routinely evaluated for residual disease (RD) by multiple methods including morphology, immunophenotypic studies (including flow cytometry, FC), molecular assays, fluorescence in-situ hybridization (FISH), and cytogenetic studies. Often, the morphologic evaluation is reported out first, with subsequent reports for other studies. FC, FISH, and molecular testing often have sufficient analytic sensitivity to detect RD not evident by morphology. Tests with the highest analytic sensitivity for RD are termed minimal residual disease (MRD) assessments, defined in this study as the detection of less than 0.1% neoplastic cells (typically to a limit of 0.01%). MRD assessment has proven to be a reliable method to predict relapse in multiple hematologic conditions, including chronic myeloid leukemia (CML, by quantitative reverse transcription polymerase chain reaction) and precursor B lymphoblastic leukemia (ALL, typically by FC). To a lesser extent, MRD may be detected in chronic lymphocytic leukemia (CLL) [2] and acute myeloid leukemia (AML) by FC [3–5], but the presence of leukemia-associated markers in this latter disease is inconsistent, and many laboratories achieve levels of analytical sensitivity sufficient for only RD testing. In theory, the analytical sensitivity of many molecular tests may exceed 0.01%. FISH, which typically assesses several hundreds of cells, is sufficiently sensitive only for RD testing (greater than 0.1% neoplastic cells, typically 1-5%). While not all ancillary testing meets criteria for MRD detection, hematopathologists and clinicians still rely upon these tests for RD assessment.

Allogeneic post-transplantation marrows are also assessed by chimerism studies (also known as engraftment studies) to determine the success of the procedure, as well as to evaluate graft-versus-host disease (GVHD), prophylactic regimens, and cellular therapy to promote engraftment and graft-versus-leukemia (GVL) activity [6]. While engraftment studies assess only the chimeric status of the patient, some studies have evaluated the effectiveness of mixed chimerism to predict disease outcome, with discrepant results [5, 7–11].

In an attempt to demonstrate the reliability of BM engraftment status as a surrogate of RD and to predict relapse, we conducted a retrospective analysis of chimerism and RD studies in 334 post-allogeneic stem cell transplant bone marrow biopsies.

Materials and Methods

Patient Population

This study was conducted with approval from the Institutional Review Board at Vanderbilt University Medical Center (VUMC). Due to the study size and its retrospective nature, consent was waived for review of medical records. A REDCap database [12] of adult BM biopsies and associated ancillary tests performed at VUMC was searched between August 2010 and December 2012. A total of 334 post-allogeneic SCT BM biopsies from 143 adult patients with a range of hematologic disorders were identified and retrospectively reviewed. The diseases were categorized according to the standardized testing protocols employed at VUMC beginning in 2011 [13]. These categories are: myelodysplastic syndromes and acute myeloid leukemias (MDS/AML), precursor lymphoblastic leukemias/lymphomas (ALL), chronic myeloid leukemia (CML), non-CML myeloproliferative neoplasms (MPN), MDS/MPN, non-Hodgkin lymphoma (NHL), and plasma cell myeloma (PCM). All patients had undergone matched related or unrelated SCT with either reduced intensity (RIC) or myeloablative (MAC) conditioning. Eligibility criteria included BM biopsies with correlative engraftment studies (short tandem repeat [STR] analysis, 334/334), with or without associated RD tests including FC (317/334), FISH (99/334), and/or molecular

studies targeting disease specific-associated aberrations (152/334). Characteristics of the patients from whom the 334 samples were obtained are summarized in Table 1. The mean follow up was 29.3 months (range 0.1-53.2) by December 2014. Relapse was defined as overt morphologic BM relapse by H&E, with or without additional immunophenotyping studies (immunohistochemistry or FC, except ALL cases where FC was treated as MRD testing).

Bone Marrow Histologic Analysis

Three histologic categories were used to classify bone marrows: overt disease, suspicious for disease, and no overt disease. Categorization was based upon H&E review with or without ancillary immunophenotyping studies (immunohistochemistry and/or FC). Overt disease was defined as clear BM involvement by H&E morphology, immunohistochemistry or FC (except ALL cases as explained above); no overt disease included cases with no histologic or immunophenotypic evidence of disease (except ALL cases); and the suspicious category included cases in which a low level of disease could not be completely excluded by morphology and immunophenotypic studies.

Chimerism & RD Analysis

Chimerism analysis was performed using PCR amplification for 9 highly polymorphic loci (AmpFISTR Profiler Plus ID PCR amplification kit Applied Biosystems, Foster City, CA) and 1 gender-specific locus. The analysis included BM aspirates, peripheral blood (PB), and CD33+ and CD3+ sorted cells, at a sensitivity of 1% recipient cells. The sorted cells were separated by fluorescence activated cell sorting with lineage-specific antibodies. Complete engraftment (CE) was defined as > 99% of donor DNA and mixed chimerism (MC) as $\leq$ 99% donor DNA. MRD/RD studies were performed as per protocols used for post-SCT BM biopsies (listed in Table 2). Molecular and cytogenetic analyses performed and corresponding analytical sensitivities are listed in

Table 1: Patient characteristics of the 334 samples.

		Conditioning Regimens		
	Patients	**RIC**	**Myeloablative**	**Total**
Gender	Female	81	78	159
	Male	103	72	175
Age	Years	25-68	20-62	n/a
	Median	57.50	43.50	n/a
Diagnosis	ALL	8	34	42
	MDS/AML	96	81	177
	CML	7	9	16
	Non-CML MPN	19	5	24
	MDS/MPN	5	3	8
	NHL	33	8	41
	PCM	14	1	15
	Other	2	9	11
Donor	Matched related	85	71	156
	Matched unrelated	99	79	178
Follow-up	Months	0.1-53.2	0.7-52.7	n/a
	mean	28.7	30.1	n/a
	Total	184	150	n/a

Table 3.

Statistics

Categorical variables were summarized as frequency counts and percentages. Continuous variables were reported as median values and ranges. A combination of chi square tests and t-tests were used to compare the type of preparatory regimen with chimerism status, histologic disease burden, and MRD/RD status. Fisher's exact tests were performed instead of chi square tests in disease specific analyses where case numbers were less than 5 per category. Chimerism in BM, unfractionated PB and CD3/CD33 sorted cells samples was compared with t-tests. Comparison of relapse rates between groups was calculated using the chi square test. All events were analyzed from time of biopsy. Relapse rate and time to relapse were calculated separately for myeloablative and reduced intensity chemotherapy SCT from those patients who did not have morphologic or flow cytometric evidence of disease. For overall tests, $p<0.05$ was used to indicate statistical significance.

Results

Correlation of Histologic Disease Burden with RD Status by Molecular Studies, FISH, and Flow Cytometry

BM biopsies (n = 334) included in this study were obtained at various times after SCT. Cases were first categorized based on histologic level of disease. The majority of cases (n = 266, 79.6%) demonstrated no overt disease by histology. In twelve cases (3.6%), the initial findings were considered suspicious, but not definitive, for low level of involvement. The remaining cases (n = 56, 16.8%) presented definite histologic evidence of disease. Ancillary RD/MRD testing was available in 323 of 334 cases (96.7%). RD studies were performed in 260 of 266 morphologically negative marrows. RD was found in 33 cases (12.7%) spanning different diseases (MDS/AML, CML, ALL, and CLL) with RD detected by both FISH and molecular methods, emphasizing the relatively decreased sensitivity of morphology alone. Of the twelve cases deemed suspicious, ten had available RD/MRD testing and four (40%) had confirmed RD by FC or molecular studies. Notably, many of these suspicious cases carried an initial diagnosis of primary myelofibrosis (8/12, 67%), including three of the four cases with proven RD. These four cases had received RIC SCTs and demonstrated MC (see below). The correlation between morphologically overt disease and RD testing was excellent (p <0.001), with 53 of 56 cases (94.6%) positive by FC, FISH and/or molecular studies. The remaining 3 cases of morphologically overt disease with negative ancillary studies were cases of primary myelofibrosis for which inadequate aspirate specimens may have been obtained.

Correlation of Histologic Disease Burden and Type of Preparative Regimen with BM Chimerism Status

Mixed chimerism was detected in 94.6% (53/56), 58.3% (7/12), and 51.1% (136/266) of cases with morphologically overt, suspicious, and no overt disease, respectively. The mean percentage of recipient DNA was 32.1%, 13.8%, and 3.3%, respectively [Figure 1A]. Cases were further divided by type of preparative regimen, either RIC (n = 184) or MAC (n = 150). As expected, there was a statistically significant difference between the total number of cases demonstrating MC in patients with RIC versus MAC SCTs (66.3% (122/184) versus 49.3% (74/150), p=0.002). However, no correlation was found between type of preparative regimen and histologic disease status (p=0.148) [Figure 1B]. RIC regimens were associated with 35/56 (62.5%) of overt cases, 9/12 (75%) of suspicious cases, and 140/266 (52.6%) of cases with no overt disease, whereas MAC regimens were performed in 21/56 (37.5%), 3/12 (25%), and 126/266 (47.4%) of cases with overt, suspicious, and no overt disease, respectively.

RD Status & Chimerism Status in Histologically Negative BMs

Ancillary RD studies were available for 260 of 266 bone marrows with no histologic evidence of disease. Of these, 227 cases (87%) were negative by specific RD testing, with 108 cases (47.5% of RD negative cases, 41.5% of all 260 cases) displaying MC (range 1-66% recipient DNA, mean 6.4%) and 119 cases CE (Table 4). Ten of thirty-three cases (30.3%, 3.8% of all 260 cases) with RD by disease-specific ancillary testing demonstrated CE. Thus, the overall concordance rate of chimerism and RD studies was 54.6% in morphologically negative biopsies (p=0.017). Since chimerism status is dependent initially upon the type of conditioning regimen, the percentages of cases that were RIC versus MAC SCTs are also shown. The concordance between chimerism and RD states is 50% and 60% for cases with RIC and MAC regimens, respectively (p=0.093).

RD Status & Chimerism Status in Histologically Negative BMs by Hematologic Disease

We further analyzed engraftment status and RD/MRD in histologically negative bone marrows by disease. Frequencies of histologic disease burden and chimerism status are shown in Figure 2. Comparing percentages of cases demonstrating CE and cases without morphologic overt disease, the correlation coefficient was 0.48 (95%CI, -0.21 to 0.85), indicating no clear correlation between chimerism and disease status.

Among cases with no overt disease, we compared chimerism status separately in RD+ and RD- cases per test type. Table 5 illustrates the percentage of cases that are disconcordant, i.e. either RD+/CE or RD-/MC. These data demonstrate significant concordance across testing methods between RD and chimerism status only for the MDS/AML category. FISH studies were significantly concordant with RD status in ALL, and molecular with RD status in CML. In all other cases there was no statistical concordance between disease and chimerism states. Significantly there were numerous cases of MC with no evidence of RD across all testing modalities and disease types. Similarly, some cases of CE showed evidence of RD, indicating the superior sensitivity of RD tests than chimerism studies. However, these data should be dependent upon the type of SCT conditioning regimen since many cases of RD-/MC are due to RIC SCTs.

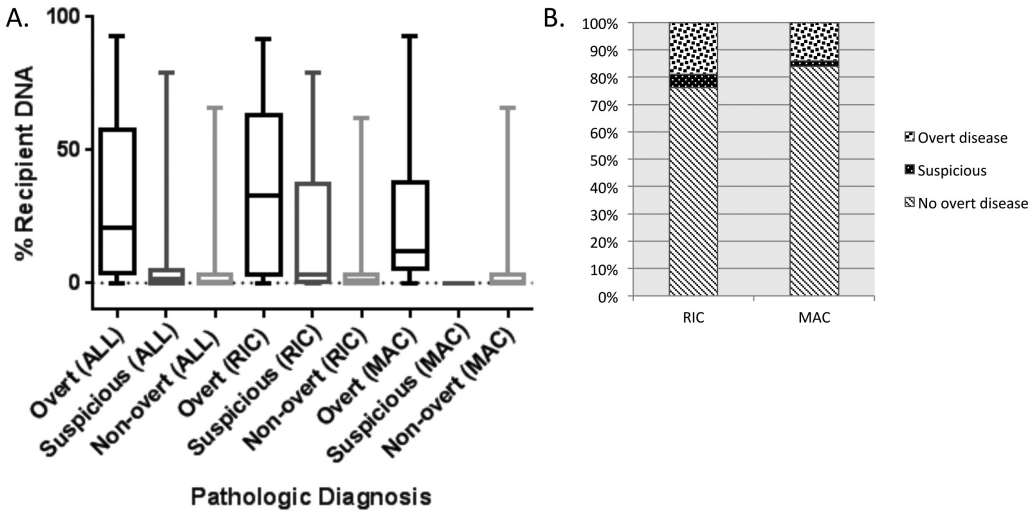
Prognostic Significance of BM Engraftment Status in Negative RD

To determine if chimerism status had any effect on relapse rates independent of disease status, we analyzed relapse rates in 151 patients who had negative

Table 2: Post-transplantation minimal residual disease/residual disease studies performed per protocol at our institution [13]

	Minimal residual disease /Residual disease studies post-SCT			
Diagnosis	**Flow cytometry**	**Karyotype**	**FISH**	**Molecular**
AML	Yes	Yes	Yes, if any previous +	Yes, if any previous +
MDS	Yes	Yes	Yes, if any previous +	No
BM failure	Yes	Yes	Yes, if any previous +	No
PCM	Yes	No	No*	No*
MPN	Yes	Yes	No	Yes, if previous +
NHL	Yes	Yes	No	Yes, if BM previously + **
ALL	Yes	No	No	Yes, if previous +

Note: *: unless overt histologic involvement;
**: IgH and BCL2 rearrangement for FL and DLBCL, IgH only for other B-cell lymphomas, TCR rearrangement for T-cell lymphomas.



	All Cases			RIC Cases			MAC Cases		
	Overt	Suspicious	Non-overt	Overt	Suspicious	Non-overt	Overt	Suspicious	Non-overt
mean	32.1	13.8	3.3	36.3	18.3	4.0	25.1	0.0	2.6
SD	30.7	28.5	9.6	32.0	32.0	10.2	27.7	0.0	8.8
p values									
Overt	NA			NA			NA		
Suspicious	0.003	NA		0.082	NA		0.003	NA	
Non-overt	<0.0001	0.29	NA	<0.0001	0.089	NA	<0.0001	0.27	NA

Figure 1: A. Percentage of recipient DNA for all cases, cases with RIC conditioning, and cases with MAC conditioning separated by the bone marrow histological diagnoses of overt, suspicious, and no overt disease. Tabular statistics are provided including p values for the differences between groups using a Mann Whitney two-tailed comparison. B. Percentage of total RIC or MAC cases with overt, suspicious, and no overt histologic diagnosis. No overt morphologic disease was more frequently associated with MAC regimen (p = 0.148). Abbreviations: RIC, reduced intensity chemotherapy; MAC, myeloablative chemotherapy; SD, standard deviation.

biopsies with no evidence of RD by molecular, cytogenetic or FC studies. Relapse rates in CE and MC were analyzed separately for patients who received RIC (n=84) and MAC regimens (n=67). In RIC patients, no significant difference in relapse rates between MC (8/41, 19.5%; median 310 days, range 68-878) and CE (7/43, 16.3%, median 183 days, range 91-432) patients was found (p=0.69). Similar findings were seen with MAC SCT, where relapse rates of MC and CE patients were 11.1% (3/27; median 140 days, range 127-175) and 17.5% (7/40; median 553 days, range 98-700), respectively (p=0.47). In addition, for all cases of CE and MC, there was no association between preparative regimens and relapse (p=0.88 and p=0.35, respectively, Figure 3).

Comparison of Chimerism Status in BM, PB, & Lineage-specific Compartments (CD3, CD33)

Since previous studies have suggested the enhanced sensitivities of chimerism studies on specific CD3+ or CD33+ fractions of PB over unfractionated whole blood [6, 14, 15], we examined the concordance of chimerism results from these PB fractions and un-

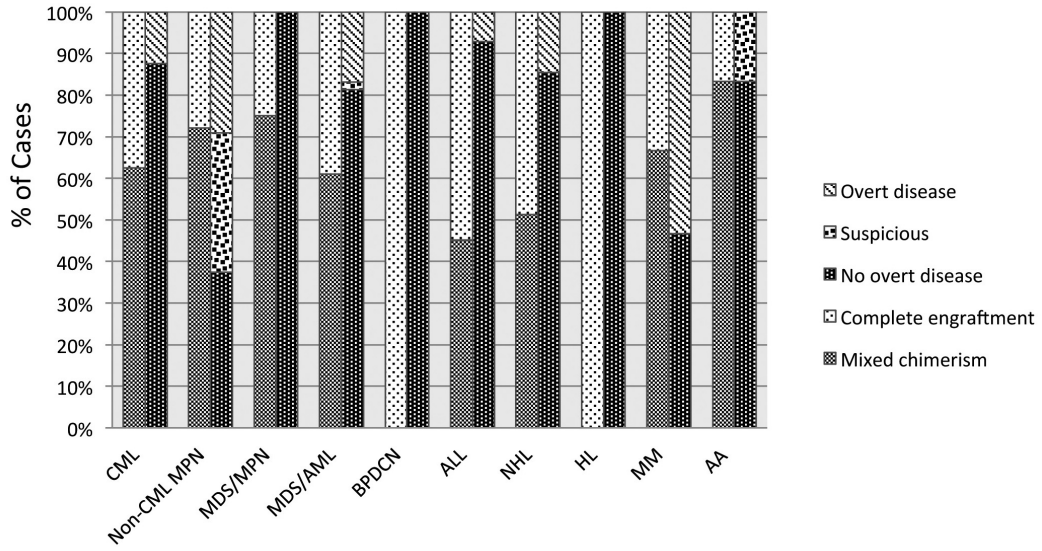


Figure 2: Percentages of cases classified by histologic disease burden and chimerism status per hematologic disease. The correlation coefficient for cases with complete engraftment with cases with no histologic evidence of disease is 0.48. Abbreviations: CML, chronic myeloid leukemia; MPN, myeloproliferative neoplasm; MDS, myelodysplastic syndrome; AML, acute myeloid leukemia; BPDCN, blastic plasmacytoid dendritic cell neoplasm; ALL, precursor lymphoblastic leukemia; NHL, non-Hodgkin lymphoma; HL, classical Hodgkin lymphoma; PCM, plasma cell myeloma; AA, aplastic anemia.

fractionated PB or BM. There were 75 cases with tandem (defined as within 7 days) BM and unfractionated PB chimerism studies. In addition, 135 BM cases had concomitant chimerism studies on the BM and fractionated CD3+ PB, while 139 cases had tandem BM and fractionated CD33+ PB studies. Analysis of results by paired t-test showed a higher detection rate in BM compared to unfractionated PB (mean recipient DNA BM=9.9%, mean recipient DNA PB=6.5%, p=0.036), and BM compared to fractionated CD33+ PB (mean recipient DNA BM=9.7%, mean recipient DNA CD33+=4%, p<0.001). However, fractionated CD3+ PB was more sensitive than BM to detect chimerism (mean recipient DNA BM=10.4%, mean recipient DNA CD3+=18.5%, p=0.001). For myeloid diseases, the patients with MC of their CD33-positive cellular compartment were nearly equally split between those that relapsed (20 of 38, 53%, including 15 RIC SCTs) and those that did not (18 of 38, 47%, including 15 RIC SCTs).

Discussion

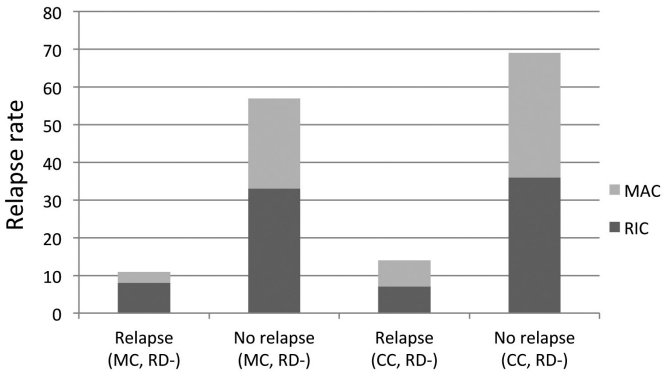
Bone marrow engraftment studies have become an important tool in the post-transplant setting for establishment of chimerism status and surveillance for impending graft rejection [14]. Since patients with no histologic evidence of disease can still harbor submorphologic levels of tumor leading to recurrence [16], it has been suggested that chimerism status can be used as a surrogate of residual disease, as well as a method to predict relapse [17–19].

In this study, we evaluated the correlation between chimerism status and RD burden by ancillary tests, some of which are used for MRD assessment. We analyzed the utility of engraftment status as a surrogate to predict relapse in a cohort of adult patients with diverse hematologic disorders. Overt histologic

Table 3: Analytical sensitivities per testing method/target.

Methods	Targets	Sensitivity
FISH-PCM	t(11;14) IGH-CCND1	1%
	t(4;14) IGH-FGFR3	1%
	17q13 TP53	5%
	13q14 RB1	3%
FISH-MPN	PDGFRA	1%
	PDGFRB	1%
	FGFR1	1%
Molecular	STR	1%
	FLT3	<1%
	NPM1	<1%
	KIT	<1%
	JAK2	<1%
	IGH	5%
	TCR	5%
	CEBPA	10-20%
	BCR/ABL1	0.001%
	IGH/BCL2	0.01%
FC	Blasts (AML, MDS, MPN)	0.1%
	ALL (B cell)#	0.01%
	PCM[&]	0.001%
	NHL*	NA

Note: #: Of the flow cytometry assessments, only that for B-ALL has an analytical sensitivity sufficient to be considered MRD. Although the limit of detection for PCM is even lower, it is well known that flow cytometry underestimates the percentage of plasma cells in most bone marrow aspirate specimens. &: The analytical sensitivity for PCM was validated after the time period of this study, but the number is presented here for a rough reference. *: There are too many potential diagnostic entities within NHL to determine a single limit of detection. FC: Flow cytometry



	RIC	MAC	all
Relapse (MC, RD-)	8	3	11
No relapse (MC, RD-)	33	24	57
Relapse (CE, RD-)	7	7	14
No relapse (CE, RD-)	36	33	69
p values (Chi-square)			
MC,RD- vs CE,RD- relapse rates	0.69	0.47	0.88

Figure 3: Prognostic significance of chimerism status in different types of preparative regimens. No significance difference is identified between relapse rate and chimerism status among patients receiving RIC or MAC conditioning regimens. Abbreviations: RIC, reduced intensity chemotherapy; MAC, myeloablative chemotherapy; CE, complete engraftment; MC, mixed chimerism.

BM involvement almost always correlated with MC (94.6%, 53/54). The rare instances of discordance included two CE cases of PCM, where heterogenous bone marrow involvement may cause sampling differences [20, 21], and a third CLL case with low level of involvement by FC for which PB contamination cannot be excluded. On the other hand, 51.1% of cases with no overt histologic disease demonstrated MC.

One potential limitation of the study is the combined analysis of multiple different disease entities and testing modalities with different analytical sensitivities. This issue merits additional discussion. The analysis of MC is complicated since it is dependent not only on the sensitivity of the chimerism assay, but also on the type of conditioning regimen, time since transplantation (especially for RIC SCTs), the use of T-cell depletion and the number of stem cells infused [16, 22] . In concordance with previous studies [16, 22, 23], MC was more commonly found in RIC cases. As expected, no correlation was found between type of preparative regimen and histologic disease burden, since both regimens are designed to eliminate the patient's underlying disease, albeit by different mechanisms. Myeloablative regimens use high doses of radiation and/or chemotherapy to eradicate the recipient's disease (as well as the immune system), whereas RIC procedures rely on

Table 4: Residual disease status and chimerism status in histologically negative bone marrows.

		Chimerism		
		MC	CE	total
Residual Disease Status	positive	23 (13/10)	10 (3/7)	33 (16/17)
	negative	108 (65/43)	119 (54/65)	227 (119/108)
	total	131 (78/53)	129 (57/72)	260 (135/125)

Note: The overall concordance rate is 54.6% (p value = 0.017 by Chi-square test). The ratio of preparative regimens is provided (number of case RIC/number of cases MAC) below each number. Abbreviations: MC, mixed chimerism; CE, complete engraftment; RIC, reduced intensity chemotherapy; MAC, myeloablative chemotherapy.

graft-versus-tumor-effect.

When we analyzed only histologically negative bone marrows, we found a 54.6% concordance rate between RD test results (*e.g.* molecular studies, FISH, or FC) and chimerism status. Of these, 41.5% of cases were MC/RD-, since patients may be incompletely engrafted without any overt disease (especially in cases of RIC SCTs) or since some RD testing modalities, such as some types of FISH or clonality studies, have lower sensitivities than STR studies. Conversely, the increased analytical sensitivity of MRD studies, such as flow cytometry for ALL, may account for the rare CE/RD+ cases (3.8%). Therefore, we believe that the decision to start disease-targeted therapy (as opposed to GVHD-targeted therapy) should not be solely based on the state of chimerism.

This study also raises questions regarding the util-

Table 5: Residual disease and chimerism correlation in cases with no overt histologic disease by test type.

Method	Diagnosis	n	RD+/CE (%)	RD-/MC (%)	*p*-value
Molecular	AML	30	16.7	40.0	0.6944
	MDS/AML	64	3.1	34.4	**0.0039**
	NHL	29	6.9	41.4	1
	CML	16	0.0	25.0	**0.0338**
	non-CML MPN	13	0.0	53.8	0.528
FISH	ALL	11	0.0	9.1	**0.0242**
	MDS/AML	99	3.0	43.4	**0.0072**
	NHL	12	0.0	25.0	0.1818
	CML	9	0.0	33.3	0.4444
	non-CML MPN	10	0.0	60.0	1
	PCM	8	25.0	37.0	0.4643
	MDS/MPN	4	0.0	50.0	1
Flow Cytometry	ALL	42	2.4	35.7	0.158
	MDS/AML	177	0.0	44.1	**<0.0001**
	NHL	41	2.4	39.0	0.1836
	CML	12	0.0	41.7	0.4697
	non-CML MPN	19	0.0	42.1	0.128
	PCM	12	16.7	16.7	0.5475
	MDS/MPN	7	0.0	71.4	1

Note: P-values are calculated by a Fisher's Exact test due to low cohort numbers in some categories. Abbreviations: ALL, precursor lymphoblastic leukemia; MDS, myelodysplastic syndrome; AML, acute myeloid leukemia; NHL, non-Hodgkin lymphoma; CML, chronic myeloid leukemia; MPN, myeloproliferative neoplasm; PCM, plasma cell myeloma.

ity of chimerism surveillance to predict relapse outside of the MDS/AML disease category. The role of chimerism analysis as a prognostic indicator after transplantation is still controversial in both settings of RIC and MAC regimens. In studies of outcome in MAC patients with a variety of diagnoses, Lamba *et al*. [24] reported higher relapse rates in patients with MC 90 days after transplantation, whereas Mossallan *et al*. [25] found no correlation between low donor chimerism and clinical outcome. Disparities are also found in outcome reports of RIC patients. Koreth *et al*. [26] have reported low donor chimerism at day 30 and day 100 as independent risk factors for relapse and impaired survival. On the other hand, Mickelson *et al*. [9] found no association between total leukocyte or T cell chimerism in PB and relapse or mortality rates. However, these studies utilized different methods for chimerism detection with a wide range of sensitivities (0.1-5%). Some studies used either bone marrow or unfractionated PB at some time-points for comparison, with no analysis of their relative performances to detect chimerism. Our study found that there was no association between BM aspirate chimerism and relapse rates in either RIC or MAC preparative regimens.

In concordance with previous reports [19, 27], we found that BM samples are more sensitive in detecting recipient DNA compared to unfractionated PB. However, BM samples were less sensitive than fractionated CD3+ PB, which is likely related to the lower engraftment rate of T lymphocytes compared to other lineages. In contrast, BM was more sensitive than fractionated CD33+ PB, which could reflect survival of host hematopoiesis in non-myeloid lineages.

To our knowledge, this is the first study to compare lineage-specific PB cells with unfractionated BM for chimerism analysis. Despite the differences in sensitivities with BM samples, lineage-specific testing can be useful in understanding the dynamics of engraftment and predicting GVL and GVHD, allowing assessment of potential complications at a higher sensitivity [28]. Specifically, CD3+ T-cell full donor chimerism has been associated with a higher incidence of GVHD [19, 29]. Furthermore, cell-subset specific analysis can potentially provide information on recurrence of the original clonal disease.

This retrospective study represents a diversity of hematologic disorders, range of disease states at time of transplant, timing of post-transplant samples, variability in preparative regimens, with lack of data on interventions that may have been prompted by chimerism studies results and affected patient outcomes. While this breadth allows global conclusions about STR and RD, as was our intent in this study, at the same time it precludes recommendations for specific diseases or testing modalities. The RD/MRD tests utilized in this study have different analytical sensitivities, and there are different methods of chimerism detection than those used here, some newer ones with higher sensitivity [14, 22]. For instance, in the case of both adult and pediatric ALL patients, it has been shown that increasing MC is associated with higher relapse rates, whereas low level or decreasing MC had no effect on outcome [5, 30, 31]. In addition, due to wide range of diseases and testing modalities, our study was not able to correlate the level and kinetics of chimerism with outcomes, particularly time to relapse.

In conclusion, given the importance of monitoring post-SCT patients for relapse and early intervention [32], multiple efforts have been made to identify reliable predictive tools [17, 18]. Although chimerism studies may be critical for engraftment surveillance, the present study provides evidence that chimerism status cannot be used as a reliable surrogate of residual disease and has little prognostic value to predict relapse outside of the MDS/AML disease category.

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