Correlation between peripheral blood and bone marrow regarding FLT3-ITD and NPM1 mutational status in patients with acute myeloid leukemia

It is more convenient to use peripheral blood (PB) than bone marrow (BM) for various laboratory studies if the former can serve as well as the latter. In patients with newly diagnosed acute myeloid leukemia (AML) and more than 30% blasts in PB there is considerable concordance between PB and BM in results of flow cytometric and cytogenetic analyses.[1] At present, however, information about mutations in the NPM1 gene and internal tandem duplications (ITD) in the FLT3 gene are assuming as much significance as cytogenetics in decisions about therapy.[2,3] For example, patients with NPM1 mutations and no FLT3- ITD are felt to have such low relapse rates that allogeneic transplant is not justified, while the opposite applies in patients with FLT3-ITD. Patients with NPM1 mutations and/or FLT3-ITD often present with high white blood cell (WBC) and PB blast counts.[4] Here we investigate the concordance between PB and BM in detecting NPM1 mutations and FLT3-ITD.

We reviewed 1016 patients with newly diagnosed or relapsed/refractory AML treated at Seattle Cancer Care Alliance/University of Washington, USA, from January 2008 to December 2013. Newly diagnosed AML required 20% or more blasts in blood or marrow while the criterion for relapsed AML was more than 5% blasts in BM or PB unrelated to recovery of normal hematopoiesis. In 30 of these patients, samples for FLT3-ITD mutation analysis and in 18 of these patients, samples for NPM1 determination were sent from BM and PB with at most three days separating the PB and BM samples. We compared the clinical characteristics of these patients, as well as the correlation for FLT3-ITD allelic ratio in both PB and BM using Spearman correlation analysis. The study was approved by the institutional review board (IRB) at the University of Washington and Fred Hutchinson Cancer Research Center. These data were also independently reviewed by a hematopathologist.

The median WBC count was 13x10^9/L (range 0.170-117.47x10^9/L) and median absolute blood blasts were 1.535 (range 0-113.06x10^9/L) in the 30 patients in whom FLT3-ITD mutation was performed in both PB and BM. Corresponding values were 21 (range 1.19-97x10^9/L) and 5.2 (range 230-62x10^9/L) in the 18 patients in whom NPM1 mutation was performed in both PB and BM, respectively. There was complete concordance between PB and BM in detecting FLT3-ITD mutation: 8 had FLT3-ITD detected in both PB and BM while 22 had no mutation detected. In 2 patients FLT3-ITD were detected in PB despite very low WBC counts (0.4x10^9/L and 0.17x10^9/L) and no circulating blasts on the smear differentials, with the finding confirmed by the presence of an FLT3-ITD in BM (Table 1). Allelic ratios were highly correlated in PB and BM with correlation coefficient 0.92 (95%CI: 0.82-0.96; P<0.0001). There was also complete concordance between PB and BM in the 18 patients in whom NPM1 mutations were analyzed in both: 4 had mutations in both PB and BM and 14 were NPM1 wild type in both PB and BM. All of the patients with NPM1 mutations had elevated WBC counts and high percentage of circulating blasts.

Next, we looked at the 227 patients who had FLT3-ITD mutational status evaluated in the PB. These included the 30 patients described previously who had FLT3-ITD mutation performed in both PB and BM. Of these, 61 patients were positive for FLT3-ITD and the rest were negative, and most of these patients had no corresponding BM study performed concurrently. While the median WBC and absolute blasts were 30.41x10^9/L and 20.5x10^9/L for the 61 patients who were FLT3-ITD positive, and the values were 8.4x10^9/L and 1.9x10^9/L for patients who were negative. Out of these 61 patients, 13 patients had absolute blast counts less than 2x10^9/L (Table 2). We also looked at the 152 patients who had NPM1 mutational status evaluated in the PB. These included the 18 patients described above. NPM1 mutation was detected in 37 of the 152 patients. The median WBC and absolute blasts were 30x10^9/L and 26.9x10^9/L for the 37 patients who were NPM1 positive, and the values were 18.6x10^9/L and 6.5x10^9/L for patients who were negative. Out of these 37 patients, 13 patients had absolute blast counts less than 2x10^9/L.

The presence of tumor specific DNA in plasma of patients with solid tumors has been reported in numerous previous studies, including breast, lung, colorectal and prostate cancers.[5,6] Conventional cytogenetic, FISH or molecular techniques using either PB or BM samples have been commonly used for detecting possible genetic and molecular abnormalities in various hematologic malignancies. In patients with chronic myelogenous leukemia[7] or

Table 1. FLT3-ITD mutation in peripheral blood and bone marrow.

WBC (x10^9/L)	Blast %	Abs. blast (x10^9/L)	Allele ratio	
			PB	BM
0.22	7	0.01	0	0
0.33	0	0	0	0
0.78	0	0	0	0
0.87	10	0.09	0	0
1.19	1	1.19	0	0
1.31	5	0.07	0	0
1.75	0	0	0	0
1.88	12	0.23	0	0
2.96	52	1.54	0	0
5.82	0	0	0	0
6.02	0	0	0	0
8.29	78	6.47	0	0
9.8	3	0.26	0	0
19.85	27	5.36	0	0
25.47	26	6.72	0	0
27.1	73	20.19	0	0
37.71	1	0.36	0	0
42.04	46	19.97	0	0
53.79	48.74	26.22	0	0
65.11	65	42.32	0	0
96.6	82	79.83	0	0
97.29	63	61.78	0	0
0.17	0	0	0.49	1.44
0.4	0	0	6.9	2.86
16.38	63	9.16	0.57	0.42
18	82	14.76	0.4	0.59
22.6	41	9.26	0.11	0.09
28.87	14	3.98	0.08	0.14
30.57	5	1.53	0.03	0.04
117.47	96	113.06	0.54	0.5

Table 2. FLT3-ITD mutation in patients with absolute blast less than $2x10^9$/L.

WBC ($x10^9$/L)	Blast %	Abs. blast ($x10^9$/L)	Allele ratio in PB
0.94	5	0.05	0.01
41.39	4	1.66	0.01
30.57	5	1.53	0.03
2.86	9	0.26	0.15
2.18	37	0.81	0.23
2.18	6	0.13	0.29
1.23	0	0	0.41
7.39	26	1.92	0.42
0.17	0	0	0.49
1.37	8	0.11	0.6
4.94	4	0.2	0.67
0.66	63	0.42	4.07
0.64	0	0	6.9

acute promyelocytic leukemia,[8] cytogenetics and molecular studies for BCR-ABl or PML-RARA are frequently carried out using PB, and are sometimes preferred over BM to monitor disease status during treatment. It is also more convenient for the patients since this does not involve more invasive bone marrow aspiration and biopsy. Jilani *et al.* reported that FLT3-ITD were more readily detected in DNA extracted from PB plasma than from BM, reflecting PB plasma is enriched in leukemic cell DNA compared with normal DNA.[9] Hussein *et al.* studied the feasibility of using PB in place of BM for cytogenetic studies during the evaluation of hematologic malignancies (42 of 242 were AML patients).[10] They found cytogenetic studies were successfully performed using PB in 58% of the cases, and this was 76% in AML patients. On multivariate analysis, only progenitor cells/blast and BM chromosome abnormality predicted success in obtaining metaphases from PB. The success rate improved as the number of circulating progenitor cells increased.

Despite the small patient numbers, our study suggests high sensitivity and specificity of PB for detection of FLT3-ITD and NPM1 mutations when the absolute blast count is more than $2x10^9$/L. Given the greater rapidity with which PB can be obtained, PB might be used *in lieu* of BM for detection of FLT3-ITD and NPM1 mutations in these patients at the time of initial diagnosis or following treatment response. In some cases, FLT3-ITD can be detected in PB even when the absolute blast count is less than $2x10^9$/L, although further work is needed to examine concordance with BM and clinical significance in such cases.

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