Stem Cell Transplantation

Clinical and practical value of metaphase fluorescence *in situ* hybridization in follow-up after allogeneic stem cell transplantation in chronic myeloid leukemia

Metaphase-fluorescence *in situ* hybridization analyses, based on as many as 1000 cells were performed on 361 bone marrow specimens to monitor the level of residual disease after allogeneic stem cell transplantation in 102 patients with chronic myeloid leukemia. Residual cells were detected in 33 patients. No spontaneous eradication of residual cells was observed when the frequency of the cells exceeded 1% at any time during the follow-up.

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We used metaphase fluorescence *in situ* hybridization (metaphase-FISH) to study the amount and clinical significance of Philadelphia chromosome (Ph)-positive residual cells in patients with chronic myeloid leukemia (CML) after allogeneic stem cell transplantation (SCT). The study was conducted on a total of 102 consecutive adult patients treated for CML with allogeneic SCT from an HLA-identical sibling donor or an HLA-matched unrelated donor at the Helsinki University Central Hospital between 1992-2002. Table 1 shows the characteristics of the patients and donors. Bone marrow samples for cytogenetic studies were scheduled to be taken 2, 4, and 12 months after SCT, if not otherwise clinically indicated.

Altogether 361 bone marrow samples were analyzed by metaphase-FISH. The method has been described earlier in detail.[1,2] As many as 1000 metaphases can be analyzed using metaphase-FISH without false positive results in leukemia-specific translocations.[3]

The overall follow-up of the 102 patients ranged from 2 to 111 months (median 28 months) after transplantation. Figure 1 indicates the time points of the studies, number of cells studied and the results of the analyses. The patients were studied in two groups depending on whether minimal residual cells were seen during the first months after SCT or not.

Residual cells were detected in single or consecutive samples during the first four months after SCT in 18 patients (17.6%) (Figure 1A). In 13 patients <1% of residual cells were detected. During the first year after transplantation the residual cells became undetectable without intervention in eight patients (3, 36, 52, 60, 63, 92, 101, and 102). This finding agrees with the results of other FISH and polymerase chain reaction (PCR) studies.[1,4-5] Low and falling frequencies of residual cells do not necessarily indicate forthcoming relapse, since they may disappear from the bone marrow as a result of the graft-versus-leukemia effect. However, follow-up is recommended.

In five patients (1, 24, 39, 67, and 90) Ph^+ cells were first observed at frequencies below 1%, but after four months the proportion of abnormal cells exceeded 1% indicating metaphase-FISH relapse. This finding supports the need for continuation of follow-up studies, especially after a positive result, and emphasizes the importance of rapid response from the cytogenetic laboratory. Results from metaphase-FISH can be obtained in two days. Five of the patients (94, 19, 59, 76, and 85) had more than 1% of Ph^+ cells when these cells were first detected.

Most patients (n= 69, 67%) remained in clinical remission and were Ph negative in all the follow-up samples studied. This is in contrast with the results of several reverse transcription (RT)-PCR studies, in which 50 to 70% of the patients expressed BCR-ABL transcripts during the first months after transplantation while in remission.[6-8] This contradiction is most probably due to the greater sensitivity of the RT-PCR technique. Residual positive cells emerging later than four months after transplantation were detected in 15 patients (14.7%) (Figure 1B). Four patients (34, 38, 51, and 71) showed <1% of Ph positive cells at 12 months after SCT in one or more samples. In these patients the residual positive cells vanished when immunosuppression was discontinued as routinely scheduled, and the patients are in complete remission 27-111 months after SCT. In nine patients no residual cells were detected in samples analyzed prior to the FISH or cytogenetic relapse, which occurred within one year after SCT. Two patients relapsed more than four years after SCT.

Our study did not show spontaneous eradication of malignant cells by the immune system in any patient at any time when the frequency of Ph^+ cells was >1%. On the contrary,

Table 1. The characteristics of the CML patients and donors.

	Ph positive at 4 months after SCT	*Ph negative at 4 months after SCT*
Number of patients	18	84
Patients, sex female/male	3/15	39/45
Age at SCT, median (range)	36.5 (18-58)	44 (18-57)
Number of patients, 1 year or more from diagnosis to SCT	6	35
Status of disease at SCT		
CP I	13 (72%)	79 (94%)
AP I	2 (11%)	3 (3.6%)
≥ BC	3 (16.7%)	2 (2.4%)
Donors		
HLA-identical sibling	8 (44%)	51 (61%)
Sibling, 1 A-locus mismatch	1 (5.5%)	1 (1.2%)
Matched unrelated	9 (50%)	32 (38%)
Female / patient male	7 (39%)	22 (26%)
Conditioning		
Cyclophosphamide	18 (100%)	84 (100%)
TBI	17 (94%)	83 (99%)
Busulfan	1 (6%)	1 (1%)
ATG (unrelated SCT)		
Thymoglobulin 12 mg/kg	6 (33%)	12 (14%)
Thymoglobulin 6 mg/kg	2 (11%)	2 (2%)
Atgam 60 mg/kg	0	15 (18%)
None	1 (6%)	2 (2%)
Graft		
Bone marrow	14 (78%)	76 (90%)
Peripheral blood	4 (22%)	8 (10%)
GVHD		
Acute	6 (33%)	40 (48%)
Chronic	5 of 16 (31%)	35 of 80 (44%)
Follow-up time (months)		
Median/range	11.5/2 - 107	29/ 2 -109

SCT: stem cell transplantation; CP: chronic phase; AP: accelerated phase; BC: blast crisis; TBI: total body irradiation; ATG: anti-thymocyte globulin; GVHD: graft-versus-host disease.

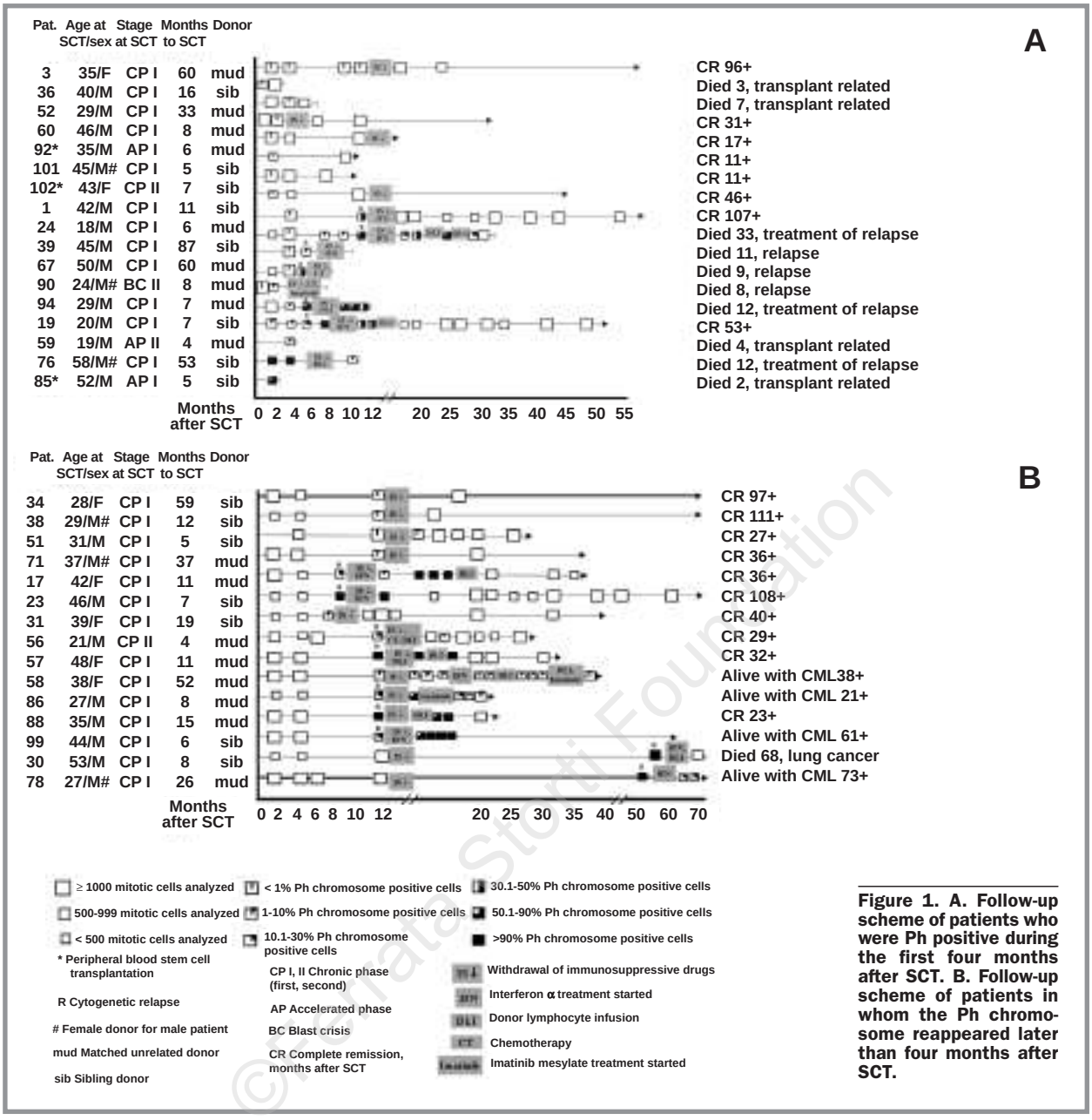

Figure 1. A. Follow-up scheme of patients who were Ph positive during the first four months after SCT. B. Follow-up scheme of patients in whom the Ph chromosome reappeared later than four months after SCT.

the number of Ph+ cells continued to increase in these cases. Therefore the amount and kinetics of Ph+ cells seem to be important factors in predicting possible relapse in CML. If the amount of Ph+ cells starts to rise in consecutive samples exceeding the level of 1%, this should be interpreted as a serious warning signal of relapse. The importance of detecting accumulation of Ph+ cells has also been documented in various other studies.[1,4,9,10]

We conclude that metaphase-FISH is an efficient and reliable way to monitor CML patients after allogeneic stem cell transplantation. Low and decreasing levels of aberrant cells seen by metaphase-FISH during the first months after transplantation in CML do not necessarily forecast imminent relapse, since these cells may disappear from the future samples. However, if the frequency of Ph+ cells increases beyond 1%, this is a clear warning of relapse. Furthermore, it should be noted that normal results obtained during the first months after SCT are not always an indication of continuing remission in later months. Follow-up helps to reveal the patients with increased risk of relapse and in these cases early intervention may prevent an overt relapse.

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References

1. El-Rifai W, Ruutu T, Vettenranta K, Temtamy S, Knuutila S. Minimal residual disease after allogeneic bone marrow transplantation for chronic myeloid leukaemia: a metaphase-FISH study. Br J Haematol 1996;92:365-9.
2. El-Rifai W, Ruutu T, Elonen E, Volin L, Knuutila S. Prognostic value of metaphase-fluorescence in situ hybridization in follow-up of patients with acute myeloid leukemia in remission. Blood 1997;89:3330-4.
3. Nylund SJ, Ruutu T, Saarinen U, Knuutila S. Metaphase fluorescence in situ hybridization (FISH) in the follow-up of 60 patients with haemopoietic malignancies. Br J Haematol 1994;88:778-83.
4. Seong CM, Giralt S, Kantarjian H, Xu J, Swantkowski J, Hayes K, et al. Early detection of relapse by hypermetaphase fluorescence in situ hybridization after allogeneic bone marrow transplantation for chronic myeloid leukemia. J Clin Oncol 2000;18:1831-6.
5. Branford S, Hughes TP, Rudzki Z. Monitoring chronic myeloid leukaemia therapy by real-time quantitative PCR in blood is a reliable alternative to bone marrow cytogenetics. Br J Haematol 1999;107: 587-99.
6. Miyamura K, Tahara T, Tanimoto M, Morishita Y, Kawashima K, Morishima Y, et al. Long persistent bcr-abl positive transcript detected by polymerase chain reaction after marrow transplant for chronic myelogenous leukemia without clinical relapse: a study of 64 patients. Blood 1993;81:1089-93.
7. Gaiger A, Lion T, Kalhs P, Mitterbauer G, Henn T, Haas O, et al. Frequent detection of BCR-ABL specific mRNA in patients with chronic myeloid leukemia (CML) following allogeneic and syngeneic bone marrow transplantation (BMT). Leukemia 1993;7:1766-72.
8. Olavarria E, Kanfer E, Szydlo R, Kaeda J, Rezvani K, Cwynarski K, et al. Early detection of BCR-ABL transcripts by quantitative reverse transcriptase-polymerase chain reaction predicts outcome after allogeneic stem cell transplantation for chronic myeloid leukemia. Blood 2001; 97:1560-5.
9. Lin F, van Rhee F, Goldman JM, Cross NC. Kinetics of increasing BCR-ABL transcript numbers in chronic myeloid leukemia patients who relapse after bone marrow transplantation. Blood 1996;87:4473-8.
10. Radich JP, Gooley T, Bryant E, Chauncey T, Clift R, Beppu L, et al. The significance of bcr-abl molecular detection in chronic myeloid leukemia patients "late," 18 months or more after transplantation. Blood 2001;98:1701-7.

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Heparin-based anticoagulation during peripheral blood stem cell collection may increase the CD34+ cell yield

Heparin combined with acid citrate dextrose (ACD) has been used in children as anticoagulation to diminish secondary effects during leukapheresis. We have found that those procedures performed with heparin and ACD yielded higher numbers of CD34+ cells than those in which the children were anticoagulated only with citrate. The biological explanation of this finding could be found in the events underlying mobilization.

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Heparin is added to the anticoagulation solution in children undergoing leukepheresis to decrease the acid citrate dextrose (ACD) infusion ratio and diminish secondary effects related to citrate chelation of calcium.[1] To the best of our knowledge the influence of the anticoagulation schedule on leukapheresis yield has not been previously studied. We decided to analyze this variable because several recent papers have described some influence of glycosaminoglycan-derived oligosaccharides on the stromal cell-derived factor 1 (SDF-1)-dependent chemotactic effect on peripheral blood progenitor cells (PBPC).[2]

We retrospectively analyzed 257 procedures performed in 224 children diagnosed with a hematologic malignancy (n=88) or solid tumor (n=136), and 33 healthy donors who underwent their first PBPC collection in our unit over the last 10 years. We analyzed the characteristics of all these children, and the procedures (Table 1) in order to evaluate the influence of each variable on the yield. Priming was performed with different cytokines-based schedules (granulocyte colony-stimulating factor, G-CSF; granulocyte-macrophage colony-stimulating factor, GM-CSF). Based on previous studies performed by our group we divided the various schedules into three groups: A (n=32) donors (G-CSF 10 μg/kg/day or G-CSF 5 μg/kg/12 hours subcutaneously), B (n=119). Regular doses of G-CSF (12 μg/kg/day or G-CSF 12 μg/kg/day plus GM-CSF 5 μg/kg/day), and C (n=106); high doses of G-CSF 12 μg/kg/12 hours or 10 μg/kg/12 hours.[3,4] Leukapheresis was performed by the oncology nursing staff on the fifth day of priming as previously described.[5] Initially ACD was the only anticoagulation (ratio between 1:12 to 1:15). Children usually received oral or intravenous calcium supplementation. Since February 1998, in accordance with the experience of Prather *et al.*, anticoagulation with heparin and citrate infused at a higher ratio (30:1) was used.[6] For this solution 5000 units of heparin were added to 500 mL of ACD.

Variables related to the yield were analyzed as previously reported.[3] Data on CD34+ cell count before the apheresis were available in 171 cases so recruitment (ratio of the total CD34+ cells collected to the amount of CD34+ cells estimated to be in peripheral blood prior to the procedure) was analyzed only in these cases.

One hundred and sixty-one children (63%) underwent leukapheresis using anticoagulation with ACD and heparin, as described above, and ninety-six only with ACD. The general leukapheresis characteristics in these two groups were not different (Table 1). PBPC yield did differ between the two groups, as shown in Table 2. Several variables were related to the yield in the multivariate study: prior chemotherapy, previous radiotherapy, child's body weight, and platelet count before the procedure (*data not shown*). Children anticoagulated with heparin plus ACD at a 30:1 ratio had a 2.46 times higher probability of achieving the target CD34+ cell dose than did those anticoagulated with the higher dose of citrate alone ($p = 0.011$-stepwise logistic regression model).

Data on adverse events related to citrate administration were available in 231 patients. Of the children anticoagulated only with ACD, 15 (20%) suffered secondary effects of citrate infusion whereas six children (4%) did so in the other group ($p < 0.0001$). However, adverse hemorragic events were more frequent in the latter group: hematoma occurred at the vascular access site in 1 ACD-anticoagulated patient but in 9 anticoagulated with ACD and heparin and mild hemorrhage after catheter was removed and occurred in 8 patients anticoagulated with heparin plus ACD but in none anticoagulated only with ACD). Bleeding was controlled with local measures. Recruitment was adequate in 111 cases (65%). However, only 25% (3/12) of patient anticoagulated with ACD had