

동종 조혈모세포 이식 후 세포 분획별 STR 키메라즘 분석에서 서로 다른 결과를 보인 4예

고리영¹ · 조성숙¹ · 송유정¹ · 허 경¹ · 오성용² · 김성현² · 권혁찬² · 김효진² · 한진영¹

동아대학교 의과대학 진단검사의학교실¹ · 내과학교실²

Clinical Utility of Chimerism Status Assessed by Lineage-Specific Short Tandem Repeat Analysis: Experience from Four Cases of Allogeneic Stem Cell Transplantation

Ri-Young Goh, M.S.¹, Sung-Suk Cho, M.T.¹, Yoo-Jeong Song, M.S.¹, Kyeong Heo, M.S.¹, Sung-Yong Oh, M.D.², Sung-Hyun Kim, M.D.², Hyeok-Chan Kwon, M.D.², Hyo-Jin Kim, M.D.², and Jin-Yeong Han, M.D.¹

Departments of Laboratory Medicine¹ and Internal Medicine², Dong-A University College of Medicine, Busan, Korea

Chimerism testing permits early prediction and documentation of successful engraftment, and also facilitates detection of impending graft rejection. In this study, we serially monitored chimerism status by short tandem repeat-based PCR in nucleated cells (NC), T cells and natural killer (NK) cells after myeloablative allogeneic stem cell transplantation (SCT). Four patients with myeloid malignancies showed discrepant chimerism results among those three fractions. Three patients had mixed chimerism (MC) of donor/host T cells at a time point around the onset of chronic graft-versus-host disease (GVHD). In two patients with disease relapse, MC of NK cells preceded a morphological relapse or NK cells showed a higher percentage of patient cells compared to NC. Therefore, our study shows that chimerism analysis in lineage-specific cells might be useful in predicting clinical outcome after allogeneic SCT in certain patients. (*Korean J Lab Med* 2009;29:277-81)

Key Words : Lineage-specific chimerism, Myeloablative stem cell transplantation, Myeloid malignancy, T cells, NK cells

INTRODUCTION

Allogeneic hematopoietic stem cell transplantation (HSCT) has become a well-established treatment modality for many malignant and nonmalignant diseases [1].

The existence of donor cells in the peripheral blood (PB) of patients (chimerism) in the post-transplant period is an important prognostic indicator of rejection, relapse or graft-versus-host diseases (GVHD) [2]. The goal of HSCT is to create a 100% donor origin, because any decrement raises the possibility that the patient may be at risk for relapse of the malignancy [3]. In few reports, patients can show discrepant chimerism results among different cell fractions. They reveal complete chimerism (CC) in one cell compartment, whereas others could be totally or in part recipient-derived, constituting so called 'split chimerism' [4].

Received : November 10, 2008

Manuscript No : KJLM2195

Revision received : May 24, 2009

Accepted : July 20, 2009

Corresponding author : Jin-Yeong Han, M.D.

Department of Laboratory Medicine, Dong-A University College of Medicine, 1 Dongdaesin-dong 3-ga, Seo-gu, Busan 602-715, Korea
Tel : +82-51-240-5323, Fax : +82-51-255-9366
E-mail : jyhan@dau.ac.kr

*This study was supported by a grant of the Korea Health 21 R&D Project, Ministry of Health & Welfare, Republic of Korea (A050099).

For chimerism analysis, methods that are time consuming and/or have low sensitivity, such as based on analyses of erythrocyte antigens, leukocyte isoenzymes or conventional cytogenetics, have been replaced in recent years by DNA technologies. PCR-based analysis of short tandem repeats (STR) or variable number of tandem repeats (VNTR) polymorphisms is one of the most widely used techniques [5].

There have been a few reports about split chimerism or discrepant results among various cell lineages after myeloablative HSCT. In this study, we observed that chimerism status of different cell lineages such as nucleated cells (NC), T cells and natural killer (NK) cells varied in four cases with myeloid malignancies.

CASE REPORTS

1. Chimerism analysis

T cells (CD3+) and NK cells (CD56+) were separated from PB using RosetteSep antibody cocktail (Stem Cell Technologies, Vancouver, BC, Canada) according to the manufacturer's recommendation with minor modifications. NC were isolated on density gradient centrifugation using Ficoll-Paque (d=1.077 g/mL, Pharmacia, Sweden). Genomic DNA was extracted from NC, CD3+ and CD56+ cells using QIAamp Blood Kit (Qiagen, Chatsworth, CA, USA). Chimerism studies were performed using the AmpFISTR Identifiler PCR Amplification Kit (Applied Biosystems, Foster City, CA, USA). PCR products were then analyzed using ABI 310 Genetic Analyzer (Applied Biosystems). Quantification of the fluorescence and calculation of the precise DNA fragment size were made with the genotyper software (Applied Biosystems). The percentage of donor and recipient DNA was calculated from individual proportions of donor and recipient peak areas in relation to the summation of all signals from each pair of informative STR markers.

Clinical and medical records of the patients were carefully reviewed. All of the four patients received myeloablative condition regimen (busulfan 130 mg/m² plus flu-

darabin 40 mg/m²). Written informed consent was obtained from all patients.

2. Case 1

A 32-yr-old male was diagnosed as AML without maturation in September 2004. Karyotyping of 20 bone marrow (BM) cells revealed a 46,XY pattern. Immunofluorescence typing of gated leukemic blasts showed positivity for CD7 (82.8%), CD33 (61.3%) and MPO (92.7%). The patient received PBSCT (CD34+ cells, 6×10^6 /kg) from an HLA-identical sibling donor on March 2005. Eight informative STR loci were found between the donor and recipient. He engrafted on day 21. On day 248, chimerism analysis of NC, T cells and NK cells showed all CC. However, on day 371, NC showed 100% donor origin, while 46.2% of T cells were recipient cells. NK cells could not be obtained because of small volume of samples. On day 553, chimerism monitoring showed that NC and T cells were 35.2% and 100% donor origin, respectively, while NK cells were host origin (Fig. 1A, arrows). On the same day, BM smear revealed 40% of myeloblasts. Chronic GVHD was observed continuously since day 247 and he died of relapse on day 571.

3. Case 2

A 29-yr-old male was diagnosed with chronic phase of CML in May 2005. All 20 analyzed metaphase using G-banding method had 46,XY,t(9;22)(q34;q11.2). FISH analysis using the LIS BCR/ABL dual color, dual fusion probe (Vysis, Downers Grove, IL, USA) showed 99.4% of gene fusions. The patient was treated with Imatinib mesylate (STI571: Glivec or Gleevec), but only a minor cytogenetic response was seen. Thus, he underwent PBSCT (CD34+ cells, 7.58×10^6 /kg) from an HLA-identical sibling donor on January 2006. Eight informative STR loci were found between the donor and recipient. On day 291 post-transplant, both NC and NK cells showed CC, but T cells were 27.5% host origin, and chronic GVHD was observed on the same day (Fig. 1B, arrow). All chimerism analysis thereafter showed donor cells.

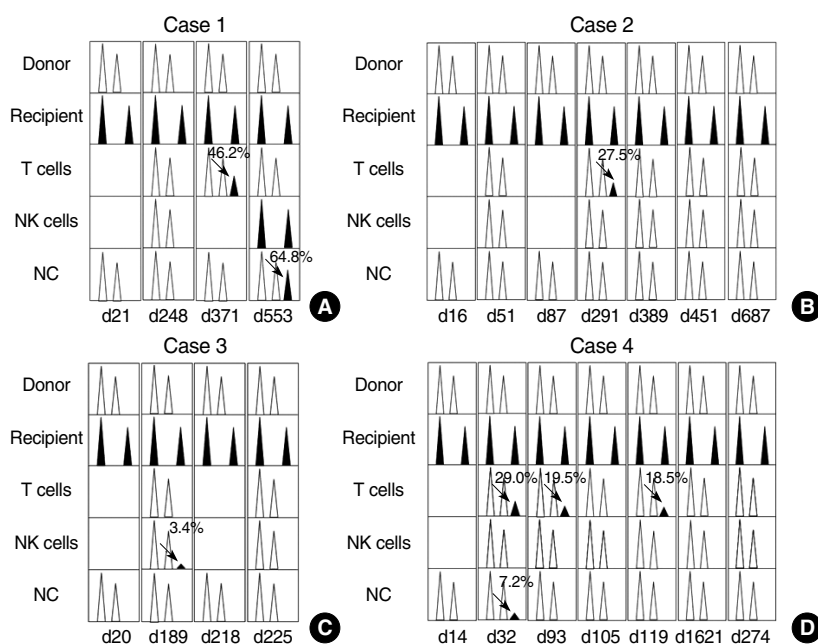


Fig. 1. Schematic presentation of serial lineage-specific chimerism data in four patients. (A) 46.2% MC of T cells on day 371 and 100% patient cells of NK cells and 64.8% MC of NC on day 553, respectively (arrows). (B) MC of T cells alone on day 291 (arrow). (C) On day 189, only NK cells showed 3.4% MC (arrow). (D) MC of T cells showing 29.0%, 19.5%, and 18.5% of patient cells on day 32, 93, and 119, respectively. NC showed 7.2% of patient cells briefly at day 32 (arrows). Abbreviations: MC, mixed chimerism; NC, nucleated cells; NK, natural killer.

4. Case 3

A 19-yr-old male was admitted on September 2005 with complaints of fever, cough, easy bruising and gum bleeding. He was diagnosed with AML with 11q23 abnormalities. Immunophenotyping of BM aspirates showed expression of CD13 (82.8%), CD33 (61.3%), CD34 (30.1%) and MPO (92.7%). Chromosome study showed an abnormal karyotype: 46,XY,del(11)(q23) in all 20 cells analyzed. FISH studies were done using the LSI MLL dual color, break apart rearrangement probe (Vysis) and revealed a single MLL gene rearrangement (one fusion signal plus one split signal) in 96.6% of interphase cells. The patient underwent unrelated BMT with $3.02 \times 10^6/\text{kg}$ CD34+ cells on March 2006. The donor was a 29-yr-old male and had one HLA (Cw)-mismatch. Fifteen informative STR loci were found between the donor and recipient. NC and T cells achieved full donor type, but NK cells were 3.4% recipient type on day 189 (Fig. 1C, arrow). On day 190, blasts appeared in BM (54.5% of all NC), whereas PB blasts were not observed. He died of pneumonia on day 237.

5. Case 4

A 47-yr-old male was diagnosed as MDS, refractory

cytopenia with multilineage dysplasia in August 2006. Karyotyping analysis of 20 cells from synchronized BM cultures was normal. He underwent allogeneic PBSCT (CD34+ cells, $5.32 \times 10^6/\text{kg}$) from his 60-yr-old brother in November 2006. Pre-transplant engraft monitoring showed nine informative STR markers. Serial monitoring of chimerism showed that NK cells were continuously 100% donor origin, but T cells showed 29.0%, 19.5%, and 18.5% recipient origin on day 32, 93, and 119, respectively (Fig. 1D, arrows). NC also showed 7.2% recipient origin briefly on day 32. Chronic GVHD was observed continuously from day 130 to 220. He died of cytomegalovirus (CMV) pneumonia about 15 months after PBSCT.

DISCUSSION

Chimerism analysis is now routinely performed and considered as an indicator for the recurrence of malignant or nonmalignant diseases [6]. Several studies have been performed during the last 20 yrs, and the consensus is that mixed chimerism (MC) is more associated with graft rejection than CC [7, 8]. The aim of this study was to analyze lineage-specific cell chimerism by multiplex STR amplification, thus evaluating the status of engraftment and predicting the outcome of allogeneic HSCT. In the present

study, we could confirm the usefulness as well as discrepancy of lineage-specific chimerism in at least four cases of myeloid malignancies.

In one AML case, 54.5% of blasts appeared in BM, whereas PB showed no blasts. In this patient, NK cell chimerism preceded a morphological relapse. Zeiser et al. [9] reported that conventional chimerism analysis had lower sensitivity of less than 3% compared to less than 0.1% of lineage-specific chimerism analysis. They also found that chimerism status in CD34+ and CD3+ cells was comparatively sensitive and specific for relapse at mean interval of 24 days in patients with myeloid malignancies. Miura et al. [10] reported similar results that MC in T cells and NK cells could be frequently observed in patients with a disease relapse.

Another AML patient with lineage discrepancy in chimerism, where 64.8% of NC and 100% of NK cells of host origin and 100% of T cells of donor origin were observed, was consistent with a disease relapse. Baron and Sandmaier [5] found that low levels of donor T cell and NK cell chimerism on day 14 were associated with increased probabilities of graft rejection and that earlier establishment of donor NK cell chimerism was associated with an improved progression free survival in patients who had undergone non-myeloablative SCT. Similar results were reported by Mattes-Martin et al. [11], suggesting that the chimerism status in NK cells on day 28 might be able to identify patients at high risk for late graft rejection. It is not clear why MC of NK cell fractions preceded a morphological relapse in these AML patients. Those myeloblasts did not show CD56 positivity at diagnosis in either of cases. However, we could not totally exclude the possibility that the blasts expressed a low level of aberrant CD56 from the onset, or acquired the aberrant marker during treatment or relapse. Specific enrichment of myeloid cell fractions would render the assay more sensitive to detect a disease progression.

Data from Michallet et al. [12] showed a significant correlation between chimerism status and overall survival as well as the incidences of acute and chronic GVHD. Matteson et al. [13] reported that about 82% of their patients

had MC for donor/host T cells at the onset of acute GVHD. Acute GVHD was not observed in our study and three cases had MC of T cells at a time point around the onset of chronic GVHD, while NC and/or NK cells showing CC. Types of conditioning regimens and post-transplant immunosuppression, methods used to collect cellular subsets and sensitivity of chimerism analysis all appear to affect the status of MC after SCT. Our results supplement additional data on this controversial subject.

In the previous studies, there have been quite few reports about split chimerism or discrepant results among various cell lineages. In this study, we observed that chimerism status of different cell lineages such as NC, T cells and NK cells varied in the four cases with myeloid malignancies. Chimerism analysis on fractionated cellular subpopulations could address the contributions of the different cell lineages in the engraftment and relapse processes. It is obvious that analysis of leukocyte subsets is time consuming and can not be performed routinely. However, in high risk patients or in situations lacking other suitable disease markers, sequential chimerism analysis in leukocyte subsets might be useful for the early detection of relapse, thus facilitating the monitoring of therapeutic intervention.

요 약

키메리즘 분석은 조기에 성공적인 생착을 예측하거나 확인할 수 있게 하며, 이식 실패를 예측하는 데 또한 유용하다. 이 연구에서는 골수억제 조혈모세포 이식 후 정기적으로 유핵세포, T 세포, NK 세포에서 short tandem repeat-PCR법을 이용하여 키메리즘 상태를 분석하였다. 4명의 골수계종양 환자에서 세포 분획별로 상이한 키메리즘 결과를 나타내었다. 3명의 환자에서 만성이식편대숙주질환이 관찰될 즈음에 T 세포 혼합키메리즘이 관찰되었다. 재발을 보인 2명의 환자에서는 NK 세포 혼합키메리즘이 재발에 선행하여 나타나거나 NK 세포에서 유핵세포에 비해 환자형 세포의 백분율이 높게 관찰되었다. 따라서, 이 연구를 통해 조혈모세포이식 후 세포 분획별 키메리즘 분석이 임상적 결과를 예측하는 유용한 인자가 될 수 있음을 확인하였다.

REFERENCES

1. Weng L, Dyson J, Dazzi F. Low-intensity transplant regimens facilitate recruitment of donor-specific regulatory T cells that promote hematopoietic engraftment. *Proc Natl Acad Sci USA* 2007;104:8415-20.
2. Lobashevsky AL, Senkbeil RW, Townsend JE, Mink CA, Thomas JM. Quantitative analysis of chimerism using a short tandem repeat method on a fluorescent automated DNA sequencer. *Clin Lab Haematol* 2006;28:40-9.
3. Lassaletta A, Ramírez M, Montero JM, González-Vicent M, Balas A, Madero L, et al. Full donor chimerism by day 30 after allogeneic peripheral blood progenitor cell transplantation is associated with a low risk of relapse in pediatric patients with hematological malignancies. *Leukemia* 2005;19:504-6.
4. Bader P, Niethammer D, Willasch A, Kreyenberg H, Klingebiel T. How and when should we monitor chimerism after allogeneic stem cell transplantation? *Bone Marrow Transplant* 2005;35:107-19.
5. Baron F and Sandmaier BM. Chimerism and outcomes after allogeneic hematopoietic cell transplantation following nonmyeloablative conditioning. *Leukemia* 2006;20:1690-700.
6. Fernández-Avilés F, Urbano-Ispizua A, Aymerich M, Colomer D, Rovira M, Martínez C, et al. Serial quantification of lymphoid and myeloid mixed chimerism using multiplex PCR amplification of short tandem repeat-markers predicts graft rejection and relapse, respectively, after allogeneic transplantation of CD34+ selected cells from peripheral blood. *Leukemia* 2003;17:613-20.
7. Bader P, Stoll K, Huber S, Geiselhart A, Handgretinger R, Niemeyer C, et al. Characterization of lineage-specific chimaerism in patients with acute leukaemia and myelodysplastic syndrome after allogeneic stem cell transplantation before and after relapse. *Br J Haematol* 2000;108:761-78.
8. Huisman C, de Weger RA, de Vries L, Tilanus MG, Verdonck LF. Chimerism analysis within 6 months of allogeneic stem cell transplantation predicts relapse in acute myeloid leukemia. *Bone Marrow Transplant* 2007;39:285-91.
9. Zeiser R, Spyridonidis A, Wäsch R, Ihorst G, Grillich C, Bertz H, et al. Evaluation of immunomodulatory treatment based on conventional and lineage-specific chimerism analysis in patients with myeloid malignancies after myeloablative allogeneic hematopoietic cell transplantation. *Leukemia* 2005;19:814-21.
10. Miura Y, Tanaka J, Toubai T, Tsutsumi Y, Kato N, Hirate D, et al. Analysis of donor-type chimerism in lineage-specific cell populations after allogeneic myeloablative and non-myeloablative stem cell transplantation. *Bone Marrow Transplant* 2006;37:837-43.
11. Matthes-Martin S, Lion T, Haas OA, Frommlet F, Daxberger H, König M, et al. Lineage-specific chimaerism after stem cell transplantation in children following reduced intensity conditioning: potential predictive value of NK cell chimaerism for late graft rejection. *Leukemia* 2003;17:1934-42.
12. Michallet AS, Fürst S, Le QH, Dubois V, Paire A, Nicolini F, et al. Impact of chimaerism analysis and kinetics on allogeneic haematopoietic stem cell transplantation outcome after conventional and reduced-intensity conditioning regimens. *Br J Haematol* 2005;128:676-89.
13. Mattsson J, Uzunel M, Brune M, Hentschke P, Barkholt L, Stierner U, et al. Mixed chimaerism is common at the time of acute graft-versus-host disease and disease response in patients receiving non-myeloablative conditioning and allogeneic stem cell transplantation. *Br J Haematol* 2001;115:935-44.