Assessment of hematopoetic stem cell molecular engraftment based on STR analysis

Saniya A. Abdrakhmanova, Zhulduz Zh. Zhanzakova, Aida A. Turganbekova, Zhazira K. Saduakas

Scientific Production Center of Transfusiology, Ministry of Healthcare, Nur-Sultan, Republic of Kazakhstan

Contact: Dr. Saniya A. Abdrakhmanova
E-mail: A.saniya@mail.ru

Introduction

Donor chimerism monitoring is the main way to control the process of hematopoietic engraftment. Assessment of the engraftment course in oncohematological patients is important for the choice of treatment strategy and further management of the patient.

Patients and methods

In order to assess engraftment, 38 patients with oncohematologic diseases were analyzed who underwent 38 hematopoietic stem cell transplantations (HSCT) from 2017 to 2019, including 13 women (34%) and 25 men (66%). The median age at the time of transplantation was 33 (2–47) years. The gender distribution among donors was 19 women (50%), and 19 males (50%). The patients were divided into two groups: 26 patients (68%) after allogeneic compatible HSCT (allo-HSCT), and 12 patients (32%), after haploidentical HSCT (haplo-HSCT). The donor chimerism was determined by polymerase chain reaction of short tandem repeats (STR-PCR) in peripheral blood on days +30, +60, and +100 after HSCT. The study was conducted more frequently in cases of mixed chimerism detection. For amplification of STR markers, the commercial AmpFlSTR® Identifiler® Plus Kit (UK) kit was used; PCR products were separated by capillary electrophoresis on a 3500 Genetic Analyzer (HITACHI Applied Biosystems, Japan). The alleles were identified using the Chimer Marker v3.1.0 software

Results

Concerning ratios of donor/recipient pairs, the female donor/male recipient combinations (15 pairs), and male donor/male recipient transplants (13 pairs) were more common than male donor/female recipient and female donor/female recipient combinations. When assessing the stem cell engraftment, all markers were informative for the studied donor/recipient pairs. According to the degree of significance, the loci in the allo-HSCT pairs were distributed as follows: D13S317 / D18S51> D5S818 / D16S539 / D21S11 / D7S820> TH01 / AMEL / FGA / D8s1179 / D2S1338> CSF1PO / D3S1358 / TPOX> D19S433 / W19A. The numbers of informative genetic loci in the donor/recipient pairs varied from 4 to 13. For haplo-HSCT pairs, the distribution of loci was as follows: D13S317 / D7S820 / AMEL> D16S539 / D2S1338 / D18S51> D5S818 / FGA>D8s1179 / D21S11 / CSF1PO / D3S1358 / VWA> D19S433> TH01 / TPOX, with 1 to 8 informative loci. According to the results of our analysis, complete donor chimerism (99-100%) in haplo-HSCT was found in 2 patients (18%) on the day +30 after HSCT, according to HLA matching degree of 5/10 and 6/10; the remaining ten cases showed mixed chimerism. On day +100, 2 out of 10 reached full donor chimerism. Complete chimerism was revealed in 11 pairs with allo-HSCT, among them, the HLA matching degree was 10/10 in 9 pairs, and 5/10, in two pairs. By 100 days, 3 patients developed a transition from mixed to complete chimerism.

Conclusion

The analysis showed an association between HLA typing results, and the type of performed HSCT (allo- or haplo-HSCT). Chimerism monitoring after transplantation is an integral part of more effective prognosis for relapse and factor of improved survival for the patients after HSCT.

Keywords

Chimerism, engraftment, hematopoietic stem cell transplantation, STR loci.

Оценка молекулярного приживления гемопоэтических стволовых клеток на основе анализа STR

Сания А. Абдрахманова, Жулдуз Ж. Жанзакова, Аида А. Турганбекова, Жазира К. Садуакас

Научно-производственный центр трансфузиологии Министерства здравоохранения, Нур-Султан, Республика Казахстан

Введение

Мониторинг химеризма – основной способ контроля процесса приживления трансплантата. Оценка динамики приживления у онкогематологических больных является важным для выбора тактики лечения и дальнейшего ведения пациента.

Пациенты и методы

С целью оценки приживления было проанализировано 38 пациентов с онкогематологическими заболеваниями, которым с 2017 по 2019 год было проведено 38 трансплантаций гемопоэтических стволовых клеток (ТГСК), из них 13 женщин (34%) и 25 мужчин (66%). Медиана возраста на момент трансплантации составила 33 (диапазон 2-47) года. Среди доноров распределение по полу было 19 женщин (50%) и 19 мужчин (50%). Пациенты были поделены на две группы: пациенты после аллогенной ТГСК (алло-ТГСК) 26 (68%), 12 (32%) пациентов после гаплоидентичной ТГСК (гапло-ТГСК).

Определение химеризма проводилось методом полимеразной цепной реакции коротких тандемных повторов – (STR-ПЦР) в периферической крови на 30, 60 и 100-й дни после ТГСК. При выявлении смешанного химеризма исследование проводили чаще. Для амплификации маркеров использовали коммерческий набор AmpFlSTR® Identifiler® Plus Kit (UK), разделение продуктов ПЦР производили с помощью капиллярного электрофореза на анализаторе 3500 Genetic Analyzer (HITACHI Applied Biosystems, Япония). Идентификацию аллелей осуществляли с использованием программного обеспечения Chimer Marker v3.1.0.

Результаты

По соотношению пар донор/реципиент чаще встречались сочетания донор-женщина/ реципиент-мужчина (15 пар), донор-мужчина/ реципиент-мужчина (13 пар), реже встретились сочетания донор-мужчина/реципиент-женщина и донор-женщина/ реципиент-женщина. При оценке приживления все маркеры были информативны для исследуемых пар донор/реципиент. По степени значимости локусы у пар алло-ТГСК распределялись следующим образом: D13S317/D18S51 >D5S818/D16S539/ D21S11/ D7S820 > TH01/AMEL/FGA/D8s1179/D2S1338 > CSF1PO/D3S1358/TPOX > D19S433/VWA. Количество информативных локусов между парами донор/реципиент составило 4-13. У пар гапло-ТГСК распределение локусов следующее: D13S317/D7S820/ AMEL > D16S539/ D2S1338/D18S51 > D5S818/FGA > D8s1179/D21S11/ CSF1PO/D3S1358/VWA > D19S433 > TH01/TPOX, с количеством информативных локусов 1-8. По результатам анализа полный донорский химеризм (99-100%) у гапло-ТГСК был установлен у 2 (18%) пациентов на 30 день после ТГСК, по степени соответствия 5/10 и 6/10 по HLA системе, у оставшихся 10 наблюдался смешанный химеризм. На 100 сутки 2 из 10 вышли на полный донорский химеризм. У 11 пар с алло-ТГСК был выявлен полный химеризм, среди них степень сочетания HLA у 9 пар – 10/10 и у двух пар – 5/10. К 100 суткам у 3 наблюдался переход со смешанного химеризма к полному.

Выводы

Анализ показал зависимость соответствия HLA системы и источника перенесенной ТГСК (алло- или гапло-ТГСК). Мониторинг химеризма после трансплантации является неотъемлемой частью более эффективного прогнозирования развития рецидива и фактором улучшения выживаемости пациентов после ТГСК.

Ключевые слова

Химеризм, приживление, трансплантация гемопоэтических стволовых клеток, STR- локусы.

Impact of additional chromosomal abnormalities on survival after allo-HSCT in CML patients

Ksenia S. Afanaseva, Elena V. Morozova, Julia J. Vlasova, Maria V. Barabanshikova, Nikolai Y. Tcvetkov, Tatiana L. Gindina, Ildar M. Barkhatov, Sergey N. Bondarenko, Ivan S. Moiseev, Elena I. Darskaya, Boris V. Afanasyev

Raisa Gorbacheva Memorial Research Institute of Pediatric Oncology, Hematology and Transplantation, Chair of Hematology, Transfusiology and Transplantology, Pavlov First Saint Petersburg State Medical University, St. Petersburg, Russia

Contact: Dr. Ksenia S. Afanaseva
E-mail: afanasevaksenya11@gmail.com

Introduction

Widespread use of targeted therapy with 2nd and 3rd generation tyrosine kinase inhibitors (TKIs) and appropriate revision of indications for allogeneic hematopoietic stem cell transplantation (allo-HSCT) allowed to achieve optimal therapeutic responses in the majority of chronic myelogenous leukemia (CML) patients. Nevertheless, inadequate therapeutic response and relapses, which are in most cases associated with additional chromosomal aberrations (ACAs) and mutations in BCR-ABL kinase domain (BCR-ABL KD), still remain a problem leading to decreased overall survival (OS) in patients. Moreover, there is still no comprehensive concept delineating ACAs role and prognostic value on therapy responses. There are scarce data on the role of ACAs in allo-HSCT outcomes. The aim of our study was to evaluate the ACAs impact upon long-term OS in allo-HSCT recipients.

Patients and methods

This study included retrospective data on the cohort of 101 CML patients with median age of 38 years (range, 19-61) undergoing allo-HSCT from HLA-matched sibling (n=26), haploidentical donor (n=14), or unrelated donor (n=61) in the R. M. Gorbacheva Memorial Institute between 2010 and 2019. By the time of allo-HSCT, 11 of these patients (11%) were in chronic phase 1 (CP1); 58 (57%), in CP>1; 23, in acceleration phase (AP, 23%), and 9 (9%) were in blast crisis (BC). All the patients received 1st, 2nd or 3rd generation TKIs prior to allo-HSCT. 39 patients (39% of the total) had BCR-ABL KD mutations, whereas T315I mutation was found in 15 of them (15%). All the patients were divided into prognostic groups, depending on ACAs, according to revision of ACAs prognostic value on therapy results. 34 patients (34%) had any ACAs in Ph+ cells at any given moment starting from diagnosis, 22 (22%) of these patients had high risk group ACAs (single i(17)(q10), -7/del7q or 3q26.2 or as a component of complex ACAs and complex ACAs without these three chromosomal abnormalities). Sixteen patients (16%) had both BCR-ABL KD mutations and ACAs. A cytogenetic study of bone marrow was carried out according to standard cytogenetic procedure, mutation analysis was performed by Sanger sequencing. OS were estimated by Kaplan-Meier (long-rank test).