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Cell-free DNA characteristics and chimerism analysis in patients after allogeneic cell transplantation

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ABSTRACT

Cell-free DNA (cfDNA) isolated from plasma or serum has received increasing interest for diagnostic applications in pregnancy, solid tumors and solid organ transplantation. The reported clinical usefulness of cfDNA obtained from plasma or serum in patients undergoing allogeneic cell transplantation (alloHSCT) is scarce.

Objective: To analyze the potential clinical utility of cfDNA chimerism analysis after alloHSCT.

Design and methods: A total of 196 samples obtained from 110 patients were investigated for their chimeric status both in peripheral blood and plasma using standard PCR for microsatellite amplification. Plasma DNA size distribution was analyzed using capillary electrophoresis.

Results: The mean cfDNA concentration in the transplanted patients was 469 ng/ml (range: 50–10,700 ng/ml). The size range of almost 80% of the analyzed fragments was between 80 and 200 bp. In 41 out of the 110 patients included in the study a mixture of donor and recipient plasma cfDNA was detected. There was a statistically significant difference in the percentage of plasma mixed chimerism between the patients without transplant related complications and the patients with either GvHD ($p < 0.05$) or relapse ($p < 0.01$). In those patients who showed improvement of GvHD also displayed a decrease in the observable percentage of recipient cfDNA during GvHD treatment. In patients without improvement or even with worsening of acute GvHD, stable or increasing levels of recipient cfDNA were detected.

Conclusions: cfDNA in combination with peripheral blood and bone marrow cell chimerism analysis might improve its utility in the clinic in particular in those patients with clinical complications after alloHSCT.

1. Introduction

There has been an increase in interest in the use of cell-free DNA (cfDNA) isolated from plasma or serum for diagnostic applications in pregnancy [1], solid tumors [2] and solid organ transplantation [3]. cfDNA has also been found in small amounts and characterized in healthy individuals [4]. In solid organ transplantation, the release of donor cfDNA into the recipient circulation has been associated with graft rejection [5]. DNA mutations, changes in methylation status and loss of heterozygosity among other DNA alterations have been detected in cfDNA from patients with various forms of cancer [6]. Plasma or serum DNA can be obtained easily and without the need for invasive procedures. This makes it a valuable source of genetic material particularly in those cases in which more invasive procedures are not feasible. Furthermore, tissue sampling after solid organ transplantation or in cancer patients is limited by the number and the size of specimens that a pathologist can analyze [7,8].

Donor-recipient chimerism constitutes a standard procedure in the follow-up of patients after allogeneic cell transplantation (alloHSCT) and is mainly performed in peripheral blood, bone marrow or both. However, the reported clinical usefulness of cfDNA obtained from plasma or serum in patients undergoing alloHSCT is scarce [9,10]. In addition, in these studies there is no patient follow-up and they are focused either on relapse after transplantation or on identifying the tissue of origin of the cfDNA. Since graft-versus-host disease (GvHD), relapse and infections among other complications after alloHSCT may potentially release cfDNA into the recipient's circulation we analyzed the detection, its size distribution and the cfDNA chimerism status in patients with complications after alloHSCT.

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Table 1
Patients clinical characteristic.

Gender Male/Female	65/45
Mean age at transplant (range)	50 (22–76)
Donor type	
Related	32
Non related	78
Conditioning regimen	
Standard	35
Reduced toxicity	75
Initial diagnosis	
Acute myeloid leukemia	58
Acute lymphoblastic leukemia	14
Non-Hodgkin lymphoma	4
Chronic myeloid leukemia	3
Multiple myeloma	10
Myelodyplastic syndrome	8
Others	13

2. Patients and methods

2.1. Patient samples

A total of 196 samples obtained from 110 patients were investigated for their chimeric status both in peripheral blood and plasma. Two 10 ml peripheral blood aliquots were collected in EDTA tubes. One of them was used for routine hematopoietic chimerism analysis. The second sample was centrifuged twice for 10 min. The isolated plasma was frozen first in liquid nitrogen and afterwards was stored at −80 °C until further analysis. The plasma isolation procedure was performed within 2 to 4 h after blood was drawn. To compare the concentration and integrity of plasma cfDNA from transplanted patients a control group of 21 healthy blood donors was included. The transplantation procedure and GvHD prophylaxis were performed as previously described [11]. The clinical characteristics of the patients included in the study are listed in Table 1.

Post-transplant events such as relapse, infections, GvHD and viral reactivation among others were defined based on standard clinical and laboratory criteria. The GvHD onset day was defined as the time of starting immunosupressive therapy.

Mixed chimerism (MC) in plasma and peripheral blood was defined as the detection of both donor and recipient DNA in a sample. Patients were routinely investigated for chimerism at days + 30, + 100 and + 365 post-transplant. After the first year patients were monitored for chimerism at 4–6 month intervals. Additional samples for chimerism testing were performed based on the presence of high-risk factors and on clinical grounds namely transplant related complications such as GvHD, relapse, infections among others.

This study was approved by the Ethics Committee of the Albert Ludwigs University, Freiburg, Germany. Written informed consent was obtained from the patients in accordance with the declaration of Helsinki.

2.2. DNA isolation and chimerism analysis

DNA was extracted from the samples using the QIAmp mini blood kit and the QIAmp circulating nucleic acid kit (Qiagen GmbH, Hilden, Germany). DNA concentration was quantified using the QuBit dsDNA assay kit and the QuBit 2.0 fluorometer following the manufacturer's instructions (Thermo Fischer Scientific GmbH, Dreieich, Germany). Size distribution and integrity of the DNA isolated from plasma was assessed using the Fragment Analyzer capillary electrophoresis system (Advanced Analytical Technologies GmbH, Heidelberg, Germany), and the chromatograms were analyzed with the ProSize software (Advanced Analytical Technologies GmbH, Heidelberg, Germany).

Hematopoietic chimerism was performed simultaneously in the peripheral blood and plasma from the same sample using a panel of seven microsatellites (SE33, THO1, D1S80, D14S120, D7S1517, YNZ22, D11S544). The amplification procedure of the microsatellite panel (STR-PCR) has been previously reported [12]. Chimerism testing using STR-PCR, data interpretation and reporting were performed following previously established guidelines and recommendations [13].

Recipient chimerism percentage was calculated according to Thiede et al. [14]. The chimeric status in the follow-up samples was divided into decreasing MC, stable MC and increasing MC which is defined as an increase of at least 5% between two consecutive samples. FLT3-ITD mutational analysis was performed as previously described [15].

2.3. Statistics

Common statistical parameters were calculated using GraphPad version 4.0 software (La Jolla, CA, USA). Parameter comparison correlation coefficients were calculated by the Spearman rank correlation coefficient analysis. For the comparison of qualitative or quantitative variables without a normal distribution the Mann-Whitney, Wilcoxon signed-rank test or Kruskal-Wallis tests were used. Quantitative variables were analyzed with the Student paired *t*-test or Fisher's exact test in the case of small numbers. All tests were 2-sided, and $p \leq 0.05$ was accepted as indicating a statistically significant difference.

3. Results

3.1. Circulating cfDNA characteristics in transplanted patients

The mean cfDNA concentration (ng/ml plasma) in the transplanted patients was 469 ng/ml (range: 50–10,700 ng/ml) while in the healthy donor control group the mean cfDNA concentration was 119 ng/ml (range: 75–180 ng/ml). The cfDNA concentration difference between both groups reached statistical significance ($p = 0.0197$). We next compared the concentrations of cfDNA in patients with and without relapse or GvHD after transplant. There was no significant difference in the cfDNA concentration between patients with either relapse or GvHD and patients without these complications (Table 2).

When analyzing cfDNA from transplanted patients, we could not detect DNA fragments larger than 400 bp when using capillary electrophoresis and the size range of almost 80% of the analyzed fragments was between 80 and 200 bp (Fig. 1).

Plasma cfDNA size distribution in transplanted patients showed a similar pattern when compared with cfDNA from healthy donors. When we compared cfDNA size distribution in patients with and without relapse or GvHD after transplant we found no difference in the cfDNA size distribution between the different groups.

3.2. Chimerism analysis of circulating cfDNA in transplanted patients

After transplantation acute and chronic GvHD was detected in 15 (14%) and 42 (38%) patients, respectively. Relapse of the underlying disease was detected in 6 (5%) patients, three of them with an isolated

Table 2
Plasma DNA concentration in patients with and without complications after alloHSCT.

	Plasma DNA concentration[a] (range)	*p* value
Healthy donors	119 (75–180)	0.0197[b]
No GvHD/relapse	306 (50–1944)	
Acute GvHD	408 (116–758)	0.055
Chronic GvHD	304 (53–1416)	0.302
Relapse	449 (111–944)	0.076

The other *p* values results from the comparison of patients with and without complications after transplant.

[a] Concentrations are given in ng/ml plasma.

[b] This *p* value results from the comparison of healthy donors with the whole cohort of transplanted patients.

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