*Journal of the Egyptian Nat. Cancer Inst., Vol. 14, No. 4, December: 275-287, 2002*

# Variable Number Tandem Repeat Polymorphism as a Tool of Chimerism Detection in Allogeneic Stem Cell Transplantation

AZZA M. KAMEL, M.D.*; GHADA I. MOSSALLAM, M.D.*; HOSSAM K. MAHMOUD, M.D.**; NAYERA HAMDY, M.D.*; ALAA EL HADDAD, M.D.**; OMAR FAHMY, M.D.*** and AMR A. NASSAR, MS.c.****

*The Departments of Clinical Pathology*, Medical Oncology**, National Cancer Institute, Internal Medecine***, Faculty of Medecine, Cairo University, and Internal Medecine, National Research Center.****

## ABSTRACT

***Background:*** Allogeneic haematopoeitic stem cell transplantation currently represents the only available therapeutic approach in several malignant and non malignant haematologic disorders. Evaluation of post-transplantation chimerism by PCR has become a routine approach to monitor its clinical outcome. Variable number tandem repeat (VNTR) loci are a group of DNA sequences that represents a source of highly polymorphic markers and that have been employed for the evaluation of the state of chimerism.

***Material and Methods:*** In this work, seven VNTR loci (Apo-B, 33.1, 33.4, 33.6, YNZ-22, D1S80 and H-Ras) were studied in 158 donors for the relative distribution of their various alleles and the discriminative power of the different loci was studied in 105 patient/donor pairs. PCR was used to amplify different loci and an informative locus was selected for post-transplantation chimerism detection in each patient. In our series 84 transplanted patients (34 post myeloablative and 50 post nonmyeloablative conditioning regimens) were followed up for the state of chimerism.

***Results:*** Apo-B was the most polymorphic VNTR locus with 12 detected alleles. The discriminative power of different loci; excluding H-Ras, ranged from 46% to 58%. The 6 studied loci were informative in 95% of patient/donor pairs. The incidence of complete chimerism (CC) was 91% post myeloablative and 63% post nonmyeloablative. The incidence of mixed chimerism (MC) was 6% post myeloablative and 20% post nonnmyeloablative transplant. Autologous recovery (AR) occurred in 3% postmyeloablative and 17% post nonmyeloablative transplant.

***Conclusion:*** Assessment of chimerism is an indispensable tool to manipulate patients after nonmyeloablative conditioning. It permits the evaluation of engraftment, differential diagnosis of pancytopenia and early detection of impending relapse or rejection after both myeloablative and nonmyeloablative transplantation.

***Key Words:*** *Allogeneic transplantation - VNTR- Chimerism.*

## INTRODUCTION

Allogeneic stem cell transplantation (ASCT) is the treatment of choice for many haematologic diseases [4,39]. Successful allogeneic transplantation is associated with engraftment of donor cells in the recipient's bone marrow, a condition known as complete chimerism (CC). Engraftment with coexistence of both donor and recipient-derived haemopoeitic cells, the so called mixed chimerism (MC), was considered by several investigators a risk factor for the development of subsequent relapse [5,7].

The need to differentiate between factors that influence the outcome of allogeneic transplantation including graft failure or rejection, graft versus host disease (GVHD), intercurrent infections and disease relapse necessitates post-transplantation follow up of the state of chimerism [19]. The emergence of new approaches such as nonmyeloablative stem cell transplantation which intends to induce a state of MC as a mean to reduce toxicity and as a platform for allogeneic adoptive cellular immunotherapy requires the detection of chimerism as an indispensable tool [42].

Many methods have been used to characterize the state of chimerism after BMT to determine the success or failure of engraftment and to detect recurrence of the disease. Early investigators had to rely on techniques such as red blood cell phenotyping, immunoglobulin isotype analysis and cytogenetics to assess the chimeric state [11,33,53,54]. Conventional cytogenetics for chimerism testing has largely been replaced by

fluorescence in situ hybridization (FISH) technique in sex-mismatched transplants using Y chromosome -specific probes [18]. Limitations of these techniques included limited degree of polymorphism, poor sensitivity and required a donor and recipient that are sex mismatched. A variety of DNA based techniques have been applied for chimerism analysis, including detection of restriction fragment length polymorphism (RFLP) and polymerase chain reaction (PCR) of Y chromosome or polymorphic genetic loci namely variable number of tandem repeats (VNTR) or short tandem repeats (STR) [48].

The use of variable number tandem repeats (VNTRs), also known as minisatellites, which are abundant throughout the human genome allows distinction between the donor and the recipient cells [46]. They provide a rich source of polymorphic markers resulting from variation in the number of copies of tandemly repeated motif at each site or locus [24]. These markers are inherited in a Mendelian fashion and can be very informative when a locus has multiple alleles. Introduction of the PCR as a method for rapid amplification of the human minisatellite regions has provided a powerful tool for assessing chimerism. The main advantage of this method is its enhanced sensitivity [26].

Monitoring of chimerism by PCR has become a routine diagnostic approach in patients after allogeneic bone marrow (BM) or peripheral blood stem cell (PBSC) transplantation [3]. At present, VNTR/STR analysis is the approach most likely to give reproducible informative data after allogeneic transplantation [3]. A large number of VNTRs have been studied in various populations [9,10,17]. The discriminative power of a given VNTR depends on the number of alleles and their distribution in the population [30].

The aim of this work is to study certain number of VNTRs that were previously reported to be of value in chimerism detection, the relative distribution of their various alleles in Egyptian donor/recipient pairs, their discriminative power and potential value in the detection of chimerism in transplanted patients in both conventional ablative and nonmyeloablative transplants.

## PATIENTS AND METHODS

A total of 986 peripheral blood samples representing 105 patients and 148 donors presented to BMT Unit, National Cancer Institute, Cairo University and to Nasser institute between June 1999 and April 2002 were tested for seven different VNTR loci before allogeneic BMT. Eighty-four patients were followed post-transplantation including patients with severe aplastic anaemia (SAA), n=8; Fanconi anaemia, n=4; thalassaemia, n=5; CML, n=21; AML, n=27; MDS, n=5; ALL,n= 9; biphenotypic, n=1; CLL, n=1; NHL, n=2 and multiple myeloma, n=1.

*The samples were divided into:*

1- One hundred and forty eight donors who were studied for the distribution of various alleles of the studied VNTR loci as representatives of the normal healthy population.

2- One hundred and five patient /donor pairs (HLA identical siblings) prepared for allogeneic transplantation were screened to evaluate the discriminative and informative power of different VNTR loci in the detection of chimerism. A locus was informative if a unique band for the recipient was detected.

3- Eighty four transplanted patients (34 post-myeloablative and 50 post non-myeloablative conditioning regimens) were followed up post allogeneic transplantation using the informative VNTR to detect the state of chimerism. CC was considered when only donor pattern was present, whereas MC was considered when both host and donor patterns were present. When only host cells were detected the patient showed autologous recovery (AR). Follow up was performed at engraftment and as indicated thereafter in patients receiving myeloablative conditioning regimen while in patients receiving nonmyeloablative conditioning it was performed regularly at days 28 and 56 then as indicated thereafter.

• The myeloablative conditioning regimen consisted of cyclosphosphamide 60mg /Kg /day at days -3 and -2, combined with 250 cGY fraction of total body irradiation (TBI) daily for 4 consecutive days starting from day -7, or a combination of busulphan (myeleran) 4mg/ Kg/day X 4 and cyclophosphamide 60mg/Kg at days -3 and -2 for patients with ALL, AML, MDS, CML and NHL. Severe aplastic anaemia patients received cyclophosphamide 50mg/Kg X 4 at day -5 to day -2 combined with antithymocyte globulin (ATG) 10mg/Kg/ day for 3 days starting from day -5. Thalassaemic patients

received busulphan 3.5mg/ Kg/ day for 4 days starting from day -4 combined with cyclophosphamide 60mg/ Kg X 2 days starting from day -3 in addition to ATG 11mg/kg/day for 10 days starting from day -5. The nonmyeloablative conditioning regimen consisted of fludarabine 30mg /$m^2$/day for 4 days from day -7 combined with melphalan 70mg/$m^2$/day for 2 days (day -3 and -2) or fludarabine 30mg/$m^2$/day for 3 days from day -4 combined with TBI 2000 cGY on day -1.

• The regimen for the prophylaxis of GVHD in case of myeloablative transplant consisted of cyclosporine 3mg/Kg from day -1 till day +14 combined with methotrexate 15 mg/$m^2$ on days +1, +3 and +6. In case of nonmyeloalative transplant, it consisted of cyclosporine 3 mg/Kg/day from day -1, in addition to mycophenolate mofetil (MMF) 2 gm/day from day +1, both were continued till day +28 followed by chimerism study. On day+28, MMF was stopped, cyclosporine was gradually withdrawn till day +56 in case of CC or MC while it was abruptly discontinued in case of autologous recovery (AR). Donor lymphocyte infusion (DLI ) was given to patients with MC or AR on day 56.

• Peripheral blood stem cell (PBSC) was used in all patients.

• The infused allograft contained a median of 9 X$10^6$ CD34 cells/ Kg (range 3.1-37.8 X $10^6$ /Kg). For DLI, the median dose was 2 X $10^6$/Kg (range 1- 2.5 X $10^6$).

*Methods:*

Ten to 20ml of heparinized peripheral blood were collected. DNA extraction was performed by the salting out technique [16]. PCR amplification of seven different VNTRs loci ( Apo-B, YNZ-22, 33.6, 33.1, 33.4, D1S80 and H-Ras) was performed, all oligonucleotide primers were synthesized commercially (Pharmacia), primer sequences and amplification cycles (Tables 1, 2) were obtained from previously published data [12,33,47]. All reactions were performed in a volume of 50ul containing 50mmol/L KCl, 10mmol/L Tris-HCl (pH 8.3), 1.5mmol/L MgCl2, 0.01%(wt/vol) gelatin, 12.5 pmol each primer (Pharmacia), 250ng template DNA, 0.2 mmol/L each dNTPs (Amersham Pharmacia) and 2.5 units Taq polymerase (Promega). Products were separated on 2% agarose gel containing ethidium bromide for 1 hour at 100 volts, visualized using ultra violet transilluminator and photographed. Alleles were characterized by their molecular weight determined relative to a 1Kb DNA ladder (Promega) run as a marker. The different alleles of a given locus were numbered in an ascending fashion according to the size of alleles detected in the current study.

## RESULTS

The number of cases tested and alleles detected according to each VNTR used are presented in table 3. The number of alleles detected at each locus as tested in a group of donors was lower compared to the maximum number reported in the literature. The frequency and distribution of alleles of the tested VNTR loci using information generated from DNA samples obtained from a group of donors are presented in fig. 1 (a,b). In our series the sizes of alleles ranged from 435 bp to 1006 bp for Apo-B, from 666 bp to 2375 bp for 33.1, from 364 bp to 993 bp for 33.6, from750 bp to 1500 bp for 33.4, from 238bp to 938bp for YNZ-22, from 488 bp to 768 bp for D1S80 and from 1000 bp to 2500 bp for H-ras (Figs. 2,3). The heterozygosity index ranged from 28% for YNZ-22 to 84% for Apo-B (Table 4).

The discriminative power in the detection of the state of chimerism in 105 patient and donor pairs attending allogeneic transplantation was calculated for the VNTR loci (Apo-B, YNZ-22, 33.6, 33.1, 33.4 and D1S80). It ranged from 46% to 58% (Table 5). For H-Ras, only 8 patient/ donor pairs were tested, it was informative in a single pair. As one allele was present in 60% of cases, this marker was excluded from chimerism detection thereafter.

Using this panel of loci, informative differences were present in 80/84 (95%) of matched donor- recipient pairs. Accordingly 4 patients could not be followed up post nonmyeloablative transplantation and will not be mentioned thereafter. Eighty patients were followed up post allogeneic transplantation using the informative VNTR locus. Apo-B was used in 25/84 (30%), YNZ-22 in 20/84 (24%), D1S80 in 19/84 (22%), 33.6 in 10/84 (12%), 33.1 in 5/84 (6%) and 33.4 in 5/84 (6%). Patients receiving myeloablative conditioning were evaluated at engraftment and as indicated thereafter; patients receiving nonmyeloablative conditioning regimen were regu-

larly tested at 28 and 56 days then as indicated (Table 6 a,b) (Figs. 4,5).

In patients receiving myeloablative conditioning, complete chimerism was detected in 31/34 (91%) of patients, while MC was detected in 2/34 (6%); one thalassaemic patient maintained a haemoglobin level between 9-11g% and the other was suffering from severe aplastic anaemia and relapsed 4 months after transplantation. One/34 showed failure of engraftment; he was suffering from SAA and died thereafter. In post nonmyeloablative conditioning, complete chimerism was detected in 29/46 (63%) of patients of which 2 (AML, n=1; MDS, n=1) reverted to MC at day 56, the AML case showed persistent MC post-DLI and relapsed 4 months after the transplant, while the MDS case rejected the graft 5 months post transplantation. Mixed chimerism at day 28 was detected in 9/46 (20%) (CML, n:7; AML, n:2); of which 8 were followed up at day 56. Four/8 (CML, n=2, AML, n=2) were converted to CC after rapid tapering of immunosuppresion. One/4 (AML) is still in haematological remission at 11 months pot-transplantation, 1 CML case lost donor cells and reverted to host phenotype (AR) after DLI but is still in haematological remission at 14 months after transplantation while the remaining 2 (AML n=1, CML n=1) died from infection. The other 4/8 (all were CML) showed persistent MC at day 56 after rapid tapering of immunosuppresion, one of them was converted to CC at 3 months, the other 3 had persistent MC after DLI and relapsed within 3-5 months after transplantation. Eight /46 (17%), who were CML cases, showed only host phenotype (AR) at day 28. Seven were followed up after DLI; 2 achieved MC with one changing into CC within 6 months after transplantation and the other reverting to host phenotype and relapsed within 11 months after transplantation. Four of the remaining 5 cases subsequently relapsed within a period ranging from 4-12 months of transplantation while 1 is still in haematological remission after 10 months of transplantation.

Table (1): VNTR PCR primers.

| Gene | Sequence | |
|---|---|---|
| Apo-B | 5' CCTTCTCACTTGGCAAATAC 3' | (Ref. 12) |
| | 5' ATGGAAACGGAGAAATTATG 3' | |
| 33.1 | 5' CGTGTCACCCAC_AAGCTTCT 3' | (Ref. 47) |
| | 5' TGCTTTCTCCACGGATGGGA 3' | |
| 33.4 | 5' ATGGGGGACCGGGCCAGACC 3' | (Ref.47) |
| | 5' CCAGGAGGCCACCAGAACCT 3' | |
| 33.6 | 5' TGTGAGTAGAGGAGACCTCAC 3' | (Ref. 47) |
| | 5' AAAGACCACAGAGTGAGGAGC 3' | |
| H-Ras | 5' TTGGGGGAGAGCTAGCAGGG 3' | (Ref.47) |
| | 5' CCTCCTGCACAGGGTCACCT 3' | |
| YNZ-22 | 5' GGTCGAAGAGTGAAGTGCACAG 3' | (Ref.47) |
| | 5' GCCCCATGTATCTTGTGCAGTG 3' | |
| D1S80 | 5' GAAACTGGCCTCCAAACACTCCCCGCCG 3' | (Ref.13) |
| | 5' GTCTTGTTGGAGATGCACGTGCCCCTTGC 3' | |

Table (2): PCR conditions.

| VNTR Locus | PCR | | | |
|---|---|---|---|---|
| | Denaturation | Annealing | Extension | Cycles |
| Apo-B | 94ºC, 1min. | 58ºC, 6min. | | 26 |
| 33.1,33.4, 33.6 and H-ras | 95ºC, 1min. | 64ºC, 2min. | 72ºC, 6min. | 20 |
| YNZ-22 | 95ºC, 1min. | 55ºC, 1min. | 72ºC, 2min. | 30 |
| D1S80 | 94ºC, 1min. | 66ºC, 1min. | 70ºC, 5min. | 28 |

An initial 7 min. hot-start denaturation step was carried out at 100ºC for Apo-B, 33.1, 33.4, 33.6 and H-ras before addition of Taq polymerase. After the last cycle PCR was ended by extra elongation step at 72ºC for 10 min. for 33.1, 33.4, 33.6 and H-ras, PCR reactions were stoppedat 4ºC.

Table (3): Number of cases examined and alleles detected according to each VNTR used in tested patient/donor.

| VNTR Locus | Number Tested | Number of alleles | |
|---|---|---|---|
| | | Detected | Reported |
| Apo-B | 182 (38+72 couples) | 12 | 14 (Ref.23) |
| 33.1 | 104 (40+32 couples) | 8 | 10 (Ref.47) |
| 33.6 | 120 (34+43 couples) | 10 | 13 (Ref.47) |
| 33.4 | 73 (25+24 couple) | 7 | 9 (Ref.47) |
| YNZ-22 | 161 (33+64 couples) | 9 | 14 (Ref.47) |
| D1S80 | 92 (46 couples) | 9 | 16 (Ref.13) |
| H-Ras | 39 (23+8 couples) | 7 | 11 (Ref.47) |

Table (4): Heterozygosity index.

| Allele | Detected | Reported |
|---|---|---|
| Apo-B | 84% | 79% (Ref.47) |
| 33.1 | 73% | 66% (Ref.24,38) |
| 33.6 | 68% | 67% (Ref.24)<br>66% (Ref.38) |
| 33.4 | 71% | 70% (Ref.24) |
| YNZ-22 | 28% | 54%-89% (Ref.17) |
| D1S80 | 79% | 81% (Ref.13) |

Table (5): Discriminative power of different VNTR loci in detection of chimerism following allogeneic SCT.

| Locus | No.of pairs | Non discriminative | Discriminative | | |
|---|---|---|---|---|---|
| | | | Haplo-identical | Non-identical | % |
| Apo-B | 72 | 31 | 37 | 4 | 57 |
| 33.1 | 32 | 16 | 12 | 4 | 50 |
| 33.6 | 43 | 21 | 19 | 3 | 51 |
| 33.4 | 24 | 13 | 10 | 1 | 46 |
| YNZ-22 | 64 | 27 | 19 | 18 | 58 |
| D1S80 | 46 | 22 | 20 | 4 | 52 |

Haploidentical: Sharing one allele.
Non-identical : No shared alleles.

Table (6-a): Status of chimerism in allogeneic PBSC transplanted patients receiving myeloablative conditioning regimen.

| UPN | Diagnosis | VNTR | State of chimerism | |
|---|---|---|---|---|
| | | | At Engratment | Others |
| 2 | SAA | Apo-B | AR | |
| 9 | SAA | YNZ-22 | CC | D+104: CC |
| 13 | SAA | Apo-B | CC | |
| 14 | SAA | Apo-B | CC | |
| 17 | SAA | 33.6 | CC | |
| 20 | SAA | YNZ-22 | CC | |
| 27 | SAA | Apo-B | CC | |
| 32 | SAA | Apo-B | | D+ 40: MC |
| 1 | Fanconi | Apo-B | CC | |
| 22 | B Thalassemia | 33.4 | CC | |
| 26 | B Thalassemia | Apo-B | MC | D+90:MC |
| 31 | B Thalassemia | D1S80 | CC | |
| 33 | B Thalassemia | D1S80 | CC | |
| 34 | B Thalassemia | D1S80 | CC | |
| 3 | CML | YNZ-22 | CC | 4m: CC<br>6m: CC |
| 11 | CML | 33.1 | CC | |
| 19 | CML | D1S80 | CC | |
| 4 | AML | 33.6 | CC | D+66: CC<br>D+140: CC |
| 5 | AML | 33.6 | CC | D+50: CC<br>D+78: CC |
| 6 | AML | YNZ-22 | CC | D+50: CC |
| 7 | AML | Apo-B | CC | D+45: CC<br>6 m: CC |
| 8 | AML | Apo-B | CC | D+50: CC<br>D+80: CC |
| 10 | AML | Apo-B | CC | |
| 12 | AML | Apo-B | CC | |
| 18 | AML | 33.1 | CC | |
| 24 | AML | Apo-B | CC | |
| 25 | AML | Apo-B | CC | |
| 28 | AML (M4) | Apo-B | CC | |
| 29 | AML | D1S80 | CC | D+108:CC |
| 15 | ALL | 33.6 | CC | |
| 16 | ALL | 33.6 | CC | |
| 21 | ALL (CR2) | D1S80 | CC | |
| 23 | ALL | D1S80 | CC | |
| 30 | ALL | Apo-B | CC | |

UPN: Unique patient number, CC: Complete chimerism,
MC: Mixed chimerism, AR: Autologous recovery,
D: Day, M.:Month

Table (6-b): Status of chimerism in allogeneic PBSC transplanted patients receiving nonmyeloablative conditioning regimen.

| UPN | Diagnosis | VNTR used | State of chimerism | | |
|---|---|---|---|---|---|
| | | | Day 28 | Day 56 | Others |
| 23 | Fanconi anaemia | YNZ-22 | CC | CC | |
| 38 | Fanconi anaemia | YNZ-22 | CC | | |
| 41 | Fanconi anaemia | 33.1 | MC vs CC | MC vs CC | |
| 5 | Accelerated CML | 33.6 | MC | | |
| 10 | CML | Apo-B | MC | MC | |
| 11 | CML | YNZ-22 | MC | | DLI 2:AR |
| 13 | CML | YNZ-22 | MC | MC | DLI 1:AR<br>DLI 2:AR |
| 21 | CML | YNZ-22 | MC | CC | D+ 98:AR<br>DLI 2:AR |
| 22 | CML | Apo-B | AR | AR | DLI 1:AR<br>DLI 2:AR |
| 24 | CML | D1S80 | MC | CC | |
| 25 | CML | D1S80 | AR | AR | DLI 1:AR<br>DLI 2:AR |
| 26 | CML | D1S80 | AR | AR | DLI:AR |
| 27 | CML | D1S80 | AR | AR | DLI 1:MC<br>D+65 post-DLI 1: CC |
| 29 | CML | 33.4 | AR | MC | DLI 1:AR<br>DLI 2:AR |
| 30 | CML | 33.1 | CC | | |
| 31 | CML | D1S80 | CC | | |
| 33 | CML | D1S80 | AR | AR | DLI 1: AR<br>DLI 2: AR |
| 36 | CML | Apo-B | AR | | |
| 37 | CML | YNZ-22 | AR | AR | DLI 1:AR<br>DLI 2:AR |
| 49 | CML | Apo-B | CC | CC | |
| 50 | CML | 33.1 | MC | MC | Days<br>+70,+80:MC 3 months:CC |
| 3 | AML(M2) | YNZ-22 | CC | CC | |
| 6 | AML | D1S80 | CC | CC | |
| 12 | AML | 33.6 | CC | CC | |
| 14 | AML | 33.6 | CC | CC | D+ 102:AR |
| 15 | AML | 33.6 | CC | CC | |
| 16 | AML | 33.4 | CC | CC | |
| 18 | AML | 33.4 | CC | MC | DLI :MC |
| 19 | AML | Apo-B | CC | | |
| 20 | AML | YNZ-22 | CC | CC | |
| 32 | AML | YNZ-22 | MC | CC | 4m.:CC |
| 35 | AML | YNZ-22 | MC | CC | |
| 40 | AML | D1S80 | CC | CC | |
| 43 | AML | YNZ-22 | CC | | |
| 47 | AML | YNZ-22 | CC | | |
| 48 | AML | D1S80 | CC | CC | |
| 2 | MDS(RA) | YNZ-22 | CC | CC | |
| 17 | MDS | Apo-B | CC | | |
| 39 | MDS | D1S80 | CC | | D+ 90: MC |
| 42 | MDS | YNZ-22 | CC | | |
| 45 | MDS | Apo-B | CC | CC | |
| 4 | ALL | Apo-B | MC vs CC | AR | |
| 7 | ALL | Apo-B | MC vs AR | | Post-reinduction<br>MC vs AR |
| 8 | ALL | YNZ-22 | CC | CC | |
| 9 | ALL | Apo-B | MC vs CC | | Post-reinduction AR |
| 1 | Biphenotypic AL | 33.6 | CC | CC | D+115:MC<br>D+220:CC |
| 28 | CLL(stageII) | Apo-B | CC | CC | |
| 34 | NHL | D1S80 | CC | CC | D+90: MC |
| 44 | NHL | 33.1 | CC | | |
| 46 | Multiple Myeloma | Apo-B | CC | CC | |

UPN: Unique patient number, CC: Complete chimerism, MC: Mixed chimerism,
AR: Autologous recovery, DLI: Donor lymphocyte infusion, D: Day, M.:Month

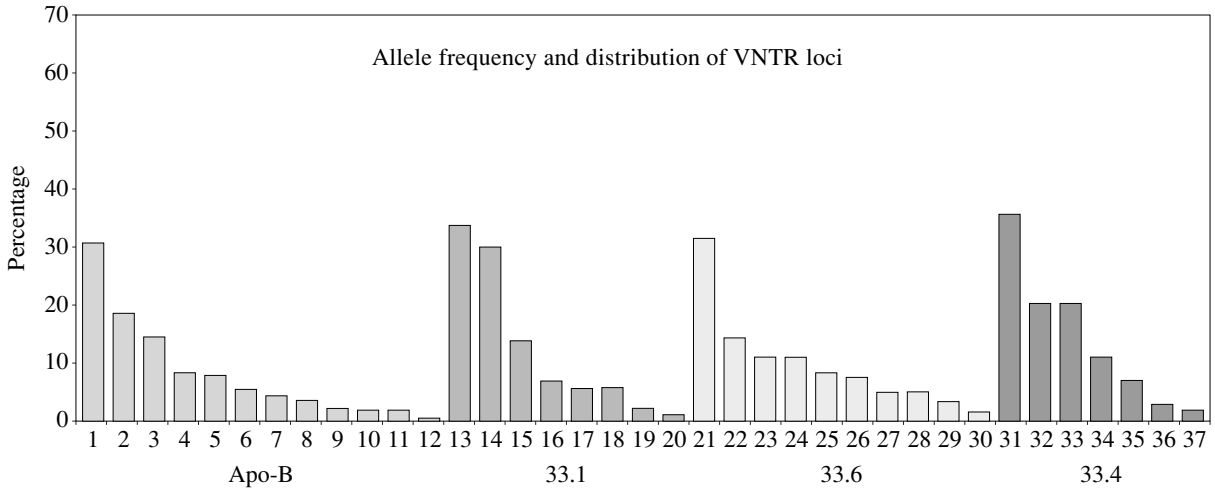


Fig. (1-A).

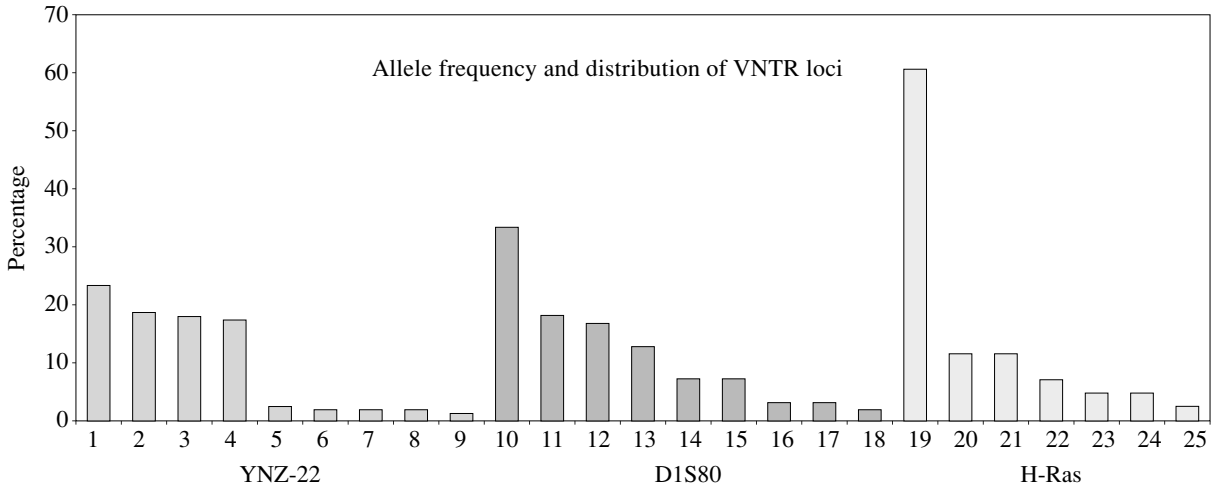


Fig. (1-B).

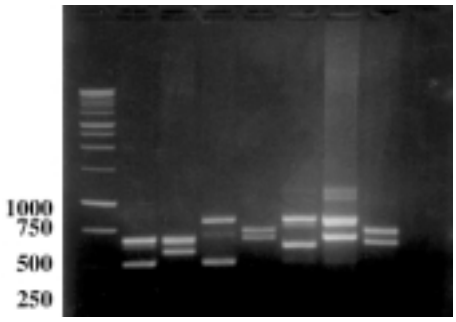


Fig. (2): Polymorphism of the VNTR locus Apo-B showing 6 different alleles.

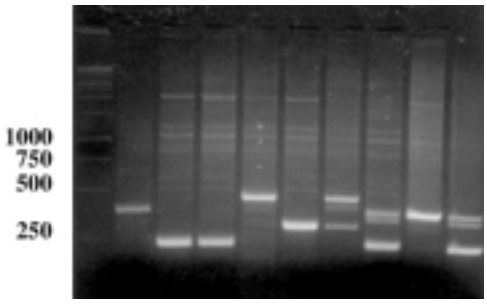


Fig. (3): Polymorphism of the VNTR locus YNZ-22 showing 4 different alleles.

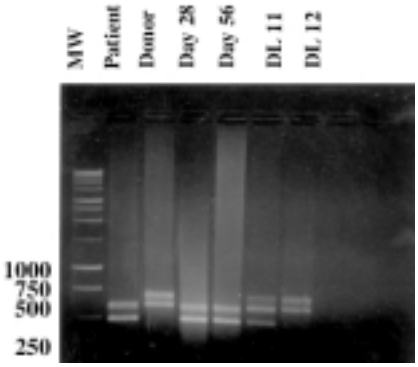


Fig. (4): Follow up of chimerism in patient UPN 27 post-nonmyeloablative transplant by amplification of the VNTR locus DIS80. Lane 1: MW marker (1kb); Lane 2: pre transplant pattern of the patient; Lane 3: his donor pattern; Lanes 4, 5: represent the patient at day +28 and +56 post transplantation showing only patient specific pattern indicating autologous recovery (AR); Lane 6: represents analysis of chimerism post DLI1 showing both patient and donor patterns indicating conversion to mixed chimerism (MC); Lane 7: represents analysis of chimerism post DLI2 showing only donor pattern indicating conversion to complete chimerism (CC). The patient is in haematological remission. UPN: unique patient number, MW: molecular weight, DLI; donor lymphocyte infusion.

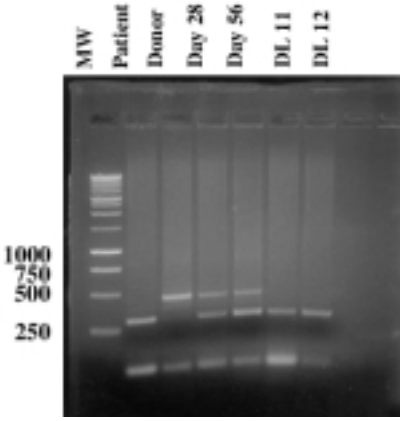


Fig. (5): Follow up of chimerism in patient UPN 13 post-nonmyeloablative transplant by amplification of the VNTR locus YNZ-22. Lane 1: MW marker (1kb); Lane 2: pre transplant pattern of the patient; Lane 3: his donor pattern; Lanes 4, 5: represent the patient at days +28 and +56 post transplantation showing both patient and donor patterns indicating mixed chimerism (MC); Lanes 6, 7 represent analysis of chimerism post DLI 1 and 2 respectively showing only patient pattern indicating autologous recovery (AR). The patient relapsed within 107 days after transplantation.

## DISCUSSION

Allogeneic stem cell transplantation is an effective, potentially curative treatment of advanced or high risk haematologic malignancies, as well as other malignant and non-malignant disorders [**4,39,45**].

To achieve successful engraftment in conventional transplants, myeloablative preparatory regimens have been used for the purpose of reducing the cell burden of the leukemia clones and suppressing or eliminating immunocompetent host cells that might inhibit engraftment of the incoming donor stem cells [**8**]. Chimerism detection is used to assess engraftment and to detect impending relapse. However, high dose chemo-radiotherapy with allogeneic transplantation is associated with significant morbidity and mortality due to the toxicity of the preparatory regimen, GVHD and the immunodeficient state that accompanies the procedure [**40**]. Non-myeloablative, relatively non-toxic and tolerable, conditioning regimens have been designed, not to eradicate malignancy but rather to provide sufficient immunosuppression to achieve engraftment and to allow induction of graft versus leukemia (GVL) as the primary treatment. This type of transplantation must be performed under the guidance of chimerism status [**14, 40, 41**].

The chimerism status after allogeneic transplant may be influenced by several factors such as the conditioning regimen, GVHD prophylaxis or treatment, the histocompatibility between the recipient and the donor, the occurrence of acute or chronic GVHD and the type of transplant [**20**].

The use of VNTR was a break through in the detection of chimerism status. The first described uses of VNTRs for chimerism analysis relied on Southern hybridization. Subsequently, PCR amplification of these loci was found to be a reliable, rapid and sensitive alternative method [**26**]. The number and frequency of detected alleles as determined by PCR reflected the level of polymorphism at the tested loci. An informative locus is one for which at least one recipient allele has a different number of repeats than the donor allele(s) [**51**]. The discriminative power of these loci depends on the number of alleles detected and their distribution in the population [**30**]. In this work, the lower number of alleles detected in our series compared to those reported in the literature [**13,17,23,47**] may be attributed to

missing larger alleles due to preferential amplification of small ones [17], or to an indirect result of the different PCR conditions or the subsequent detection steps utilized [50], however, these explanations are unlikely since the incidence of heterozygosity in our series is comparable to that reported in literature with the exception of YNZ-22 (Table 4). Alternatively the lower number of alleles detected in our work could be a real finding due to ethnic differences between different populations. Being the first VNTR study concerning the Egyptian population, we don't have other Egyptian data to compare with. As the choice of VNTR in the study of chimerism depends on its discriminative power (informativity), i.e. the number of polymorphic alleles and their relative frequency [30], the allele frequency distribution of the 7 VNTRs tested has been determined. Although the alleles observed in the different ethnic groups may be comparable, the relative frequency may vary significantly [9]. In our series, due to uneven distribution of its alleles with one allele (nº2) constituting 60% (Fig. 1), H-Ras was not informative and was not used for the detection of chimerism. In this work, heterozygosity index ranged from 28% for YNZ-22 up to 84% for Apo-B, the high incidence of homozygosity observed with YNZ-22 could be due to misclassification of a heterozygous individual as homozygous since small alleles are preferentially amplified when present in a heterozygous state with a large allele [17]. However, the undetected alleles, unlike its application in population studies, may not be of value in the study of chimerism as the aim is to find a discriminative locus whether or not we are missing an allele. The extent of polymorphism observed at these loci makes them useful markers for monitoring the state of chimerism following allogeneic transplantation; the discriminative power ranged from 46% to 58% in our series of 105 pairs of patients and donors screened for an informative locus using these loci.

In our series, 84 allogeneic transplants were followed for the state of chimerism post-transplantation (34 using myeloablative and 50 using nonmyeloablative regimens). The system adopted for screening for discriminative locus comprised evaluation of Apo-B followed by YNZ-22, D1S80 and 33.6 on account of better separated bands and favorable amplification of shorter alleles [17,51]. The alleles of 33.1 and 33.4 are of larger size and hence their amplification is not as efficient. Accordingly Apo-B was used in 30%, YNZ-22 in 24% and D1S80 in 22%; the three together accounting for 76% of our cases. Using this panel of loci, informative differences were present in 95% of matched donor-recipient pairs. The reported frequency of informative VNTR loci varies at different laboratories. Muniz et al. [30] reported a frequency of 37% for D1S80 in their recipient -donor pairs; whereas Stuppia et al. [43] reported a frequency of 44% for Apo-B and 25% for D1S80. However, the comparison may not be absolutely valid. We did not test all the loci for all the pairs, this was done only for 14 pairs. Subsequently, we developed a system of consequential testing according to the most frequently informative loci; once we obtained one the search was stopped. However the difference could still be partly true on account of ethnic variations and the number and frequency of alleles for each locus which will make their discriminative power different in various populations [30].

Complete engraftment or complete chimerism (CC) refers to the inability to detect recipient cells which depends on the sensitivity of the detection method [48]. In general the sensitivity using VNTR-PCR is between 1- 5% depending on the marker and the detected alleles [20,48,51]. In our series, as expected, the chimerism analysis performed in the early post- transplant period (on day+28 and +56) showed higher incidence of CC, with myeloablative conditioning (91%) than with nonmyeloalative (63%) as in the latter strategy the recipient haemopoeitic system is not completely ablated. In this work, 2 patients (AML M4, n=1; MDS-RAEB, n=1) were converted post-nonmyeloablative conditioning on day 56 from CC to MC. The AML case showed persistent MC post-DLI and relapsed 4 months after the transplant, while the MDS case rejected the graft 5 months post-transplantation. One possible explanation for this finding might be the level of sensitivity so that a low level of MC could not be detected at day 28 which became overt at day 56. Apparently, a residual malignant clone that would have been revealed by minimal residual disease (MRD) detection could not be overcome by the nonmyeloablative approach.

When both host and donor cells are present, mixed chimerism (MC) is considered. Mixed chimerism describes a dynamic balance between host and donor haematopoietic cells and it may

be a stable or transient phase [32,42]. In patients undergoing non-myeloablative transplantation the occurrence of transient MC was expected to be more frequent compared to patients after myeloablative conditioning. In our series the incidence of MC was 6% post myeloablative and 20% post nonmyeloablative regimens. The latter figure is comparable to an incidence of 24% reported by Wasch et al. 2000 [52]. The dynamics of MC correlates also with the underlying disease. In the current study, 2 patients with anaemia ( aplastic anaemia, n=1; thalassaemia, n=1) had persistent MC. It was an important tool to differentiate between graft failure and the other causes of pancytopenia in severe aplastic anaemia [48] which was the case in our patient (UPN 32) that ended to graft rejection 4 months after transplantation. MC could be detected in patients with anaemia over a long period (220 days to 4 years) without graft failure. MC is a common finding following SCT for thalassaemia, often persists and is compatible with long term cure [2,7]. Failure to change to complete chimerism in these cases is not alarming provided that the patient is keeping a transfusion independent reasonable haemoglonin level. However, residual host cells > 30% have a significant lower probability of disease free survival (DFS) and a higher probability of clinical rejection [3]. In patients with malignancies, MC is most often transient and conversion to CC, autologous reconstitution or relapse occurs either spontaneously or following immune manipulation [42]. In this work, the incidence of MC correlated with the underlying disease with a higher incidence in CML (35%) than in other diseases. A similar incidence of 35% in CML was reported by Gonzalez et al. [22]. In both series, this was probably due to less intensive conditioning. The standard method for follow up of allogeneic BMT in CML is the detection of BCR/ABL chimeric gene by RT/PCR [34,37]. This method is much more sensitive than detection of chimerism by VNTRs. However, a sizable portion of patients in long -remission after allogeneic transplantation test BCR-ABL positive [21]. Its detection during the first 6 months has no clinical significance and does not invite any clinical intervention [29]. On the other hand, MC by VNTR in CML may predict haematologic relapse several months earlier in patients who are persistently BCR-ABL positive post transplantation [36], calling for immunotherapy by donor lymphocyte infusion (DLI).The effectiveness of adoptive immunotherapy by donor lymphocyte infusion (DLI) to produce clinical remission in patients with MC is well documented especially in CML [6,35,44]. A similar GVL effect can be induced by rapid tapering of immunosuppression [1]. However, the efficiency of these interventions has to be monitored by chimerism analysis. In our series, transient MC converted to CC after rapid tapering of immunosuppression was observed in 5/8 (62%) of patients presented by MC post nonmyeloablative regimen (CML, n=3; AML, n=2). On the other hand, DLI failed to convert the remaining 3 cases to CC who reverted to host phenotype (AR) and relapsed within 3-5 months after transplantation. Thus the analysis of haematologic chimerism is a useful tool to monitor the efficiency of DLI and other therapies in avoiding or treating relapse in patients with mixed chimerism.

The incidence and significance of MC following allogeneic transplantation has been the subject of much investigation and debate. Some studies have shown that MC is associated with a higher incidence of relapse [7,25] whereas others have found MC to possess little or no clinical significance [15,49]. This controversy may be attributed, at least partly, to differences in the sensitivity of the methods used to detect MC [31, 42]. MC detection using VNTR or STR has a sensitivity limit > 0.1%. However, detection of MRD using a disease marker is much more sensitive with a sensitivity up to 1 in $10^6$. Sensitivity may be increased using lineage- specific analysis of chimerism [28,55]. However, in the absence of specific tumor marker for MRD analysis, chimerism testing is the only tool to detect host cells [42].The recent studies using PCR-based serial analysis of VNTR showed that patients with increasing MC have the highest risk of relapse [5,27,55] i.e. follow up rather than a single determination will be more relevant to predict impending relapse. The percentage of recipient cells can be calculated from the relative intensity of recipient-specific PCR products compared with the sum of recipient and donor specific PCR products [50].

In this work, graft failure with autologous recovery was detected in one patient with aplastic anaemia who received myeloablative regimen. In the group of patients who received nonmyeloablative conditioning, a higher proportion presented with autologous recovery (8/46, 17%);

all the cases were CML. Seven patients received DLI and the efficiency of the GVL effect was monitored by chimerism analysis. Conversion to MC occurred transiently in 2 patients, one was subsequently converted to CC while the other reverted to host phenotype and relapsed within 11 months after transplantation. Although the remaining 5 patients had persistent host phenotype after DLI; the onset of haematological relapse varied from 4-12 months after transplantation in 4 patients while haematological remission is still observed in one patient at 10 months post transplantation. It is interesting to evaluate whether the absence of molecular engraftment within a certain time span post-transplant is predictive of graft failure.

In conclusion, sequential molecular analysis of chimerism using VNTRs after allo-SCT has been shown to be useful for predicting engraftment, graft failure, rejection or impending relapse. It can help in the differential diagnosis of pancytopenia after ASCT. It is a useful indicator for the use and monitoring of DLI or other immunomodulatory interventions.Though it is less sensitive than MRD detection, yet it may be the only available tool in a number of cases. Moreover, chimerism anaslysis provides a rational for assessing different conditioning regimens. From our results we can conclude that measurement of chimerism is not essential for detection of engraftment in myeloablative transplantation using conventional conditioning, however, it may be useful in long term follow up to detect heralding relapse. Nonmyeloablative regimen, on the other hand, must be performed under the guidance of chimerism analysis to secure original engraftment and help decision on interventions such as DLI. Sensitivity may be increased using lineage-specific analysis. Quantitative evaluation of the dynamics of MC is the best predictor of impending relapse.

## REFERENCES


1- Abraham R., Szer J., Bardy P. and Grigg A.: Early cyclosporine taper in high -risk sibling allogeneic bone marrow transplants. Bone Marrow Transplantation, 20: 773 - 777, 1997.

2- Amrolia P.J., Vulliamy T. Vassiliou G., Lausson S., Bryon J., Kaeda J., Dokal I., Johnson R., Veys P., Darbyshire P. and Roberts I.A.: Analysis of chimerism in thalassaemic children undergoing stem cell transplantation. British Journal of Haematology., 114 (1): 219-225, 2001.

3- Antin J.H., Cliids R., Filipovich A.H., Giralt S., Mackinnon S., Spitzer T. and Weisdorf D.: Establishment of complete and mixed donor chimerism after allogeneic lymphohematopoietic transplantation: recommendations from a workshop at 2001 tandem meetings. Biology of Blood and Marrow Transplantation., 7: 473-485, 2001.

4- Armitage J.: Bone marrow transplantation. New England Journal of Medicine, 330: 827-838, 1994.

5- Bader P., Beck J., Frey A., Schehgel P.G., Hebarth H., Handgretinger R., Einsele H., Niethammer D. and Klingebiel T.: Serial and quantitative analysis of mixed haematopoietic chimerism by PCR in patients with acute leukemias allows the prediction of relapse after allogeneic bone marrow transplantation. Bone marrow transplantation, 21 : 487 - 495, 1998.

6- Bader P., Beck J., Schehgel P.G., Handgreting R., Niethammer D. and Klingebiel T.: Additional immunotherapy on the basis of increasing mixed hematopoietic chimerism after allogeneic bone marrow transplantation in children wth acute leukemia. Is there an option to prevent relapse. Bone Marrow Transplantation, 20: 79-81,1997.

7- Bader P., Holle W., Klingebiel T., Handgretinger R. Nietharmmer P. and Beck J.: Quantitative assessment of mixed haematopoietic chimerism by PCR after allogeneic bone marrow transplantation. Anticancer Research, 16: 1759-1764, 1996.

8- Bader P., Stoll K., Huber S. and Geiselhart: Characterization of lienage-specific chimerism in patients with acute leukemia and MDS after allogeneic bone marrow transplantation before and after relapse. British Journal of Haematology, 108: 761-768, 2000.

9- Baird M., Balazs I., Giusti A., Miyalaki L., Nicholas L., Wexler K., et al.: Allele frequency distribution of two highly polymorphic DNA sequences in three ethnic groups and its application to the determination of paternity. American Journal of Human Genetics, 39: 489-501, 1986.

10- Balazs I., Baid M., Clyne M. and Meade E: Human population genetic studies of five hypervariable DNA loci. American Journal of Human Genetics, 44: 182 - 190, 1989.

11- Blume K.G., Beutler K.J., Bross K.J., Schmidt G.M., Spruce W.E. and Teplitz R.L.: Genetic markers in human bone marrow transplantation. American Journal of Human Genetics, 32: 414-419, 1980.

12- Boerwinkle E., Xiong W., Fourest E. and Chan L.: Rapid typing of tandemly repeated hypervariable loci by PCR: application to apolipoprotein B 3' hypervariable region. Proceedings of the National Academy of Science of USA, 86: 212-216, 1989.

13- Budowle B., Chakraborty R., Giusti A.M., Eisenberg A.J. and Allen R.C.: Analysis of the VNTR locus D1S80 by PCR followed by high resolution PAGE . American Journal of Human Genetics, 48: 137-144, 1991.

14- Champlin R., Khouri, Shimoni A., Gajewski J. and Kornbeau: Harnessing graft versus malignancy: non-

myeloalative preoperative regimens for allogeneic haematopoietic transplantation. An evolving strategy for adoptive immunotherapy. British Journal of Haematology., 111: 18-29, 2000.

15- Choi S.J., Lee K-H., Lee J.H., Kim S., Chung H.J., Lee J.S., Mitterbauer G., Henn T., Haas O., Fodinger M., Kier P., Forstinger C. and Quehenberger P.: Prognostic value of haemopoietic chimerism in patients with acute leukaemia after allogeneic bone marrow transplantation: a prospective study. Bone Marrow Transplantion. 26: 327-332, 2000.

16- Davis L.G., Dibner M.D. and Battey J.F.: DNA extraction, Southern blot technique, probe labeling and hybridization In: Basic methods in molecular bioloby. Davis LG, Dibner MD and Battey (eds): Eserirer, Science New York. p42, 1986.

17- Deka R., De Croo S., Yu L.M. and Ferrell R.E.: Variable number of tandem repeat (VNTR) polymorphism at locus D17S5 (YNZ22) in four ethnically defined human populations. Human Genetic, 90: 86-90, 1992.

18- Diez-Martin J.L., Lamas P. and Gosalvez J.: Conventional cytogenetics and FISH evaluation of chimerism after sex-mismatched bone marrow transplantation and donor leukocyte infusion. Haematologica, 83: 408-415, 1998.

19- Dubovsky J., Daxberger H., Fritsch G., Printz D., Peters C., Mathes S., Gadner H., Lion T. and Muller-Berat N.: Kinetics of chimerism during the early post-transplant period in pediatric patients with malignant and non-malignant hematologic disorders: implications for timely detection of engraftment, graft failure and rejection. Leukemia, 13: 2060-2069, 1999.

20- Elmaagacli A.H., Beelen D.W., Becks H.W., Mobascher A., Stockova J., Trzensky S., Opalka B. and Schaeffer U.W.: Molecular studies of chimerism and minimal residual disease after allogeneic peripheral progenitor cell or bone marrow transplantation. Bone Marrow Transplantation, 18: 397-403,1996.

21- Gaiger A., Lion T., Kalhs P.: Frequent detection of bcr-abl specific mRNA in patients with chronic myeloid leukemia (CML) following allogeneic and syngeneic bone marrow transplantation. Leukemia., 7: 1766-1772, 1993.

22- Gonzalez M.: Debate round table: Comments concerning chimerism studies. Leukemia, 15: 1986-1988, 2001.

23- Helio T.: Concept of VNTR alleles: Comparison of apolipoprotein B 3' hypervariable region genotyping results obtained by three methods. Biochem. Biophy. Res. Comm., 181: 846-851, 1991.

24- Jeffreys A., Wilson V., Thein S.L.: Hypervariable "minisatellite regions" in human DNA. Nature, 314: 67-73, 1985.

25- Machinnon S., Barnett L., heller G. and O'Reilly: Minimal residual disease is more common in patients who have mixed T cell chimerism after bone marrow transplantation for chronic myelogenous leukemia. Blood, 83: 3409-3416, 1994.

26- Martinelli G., Trabetti E., Farabegoli P., Testoni N., Bandini G., Motta M.R., Vittone A., Terragna C., Pignatti P.F. and Tura S.: Early detection of bone marrow engraftment by amplification of hypervariable DNA regions. Haematologica, 82: 156-160, 1997.

27- Martino R., Caballero M.D., Camals C., Simon J.A., Salona C., Urbano-Ispizua A., Bargay J., Rayon C., Leon A., Sarra J., Oddriozola J., Conde J.G., Sierra J. and San Miguel J.: ALLO PBSCT Committee of the Spanish Group for Haematopoietic Transplantation (GETH), group GEL-TAMO.: Allogeneic peripheral blood stem cell tranplantation with reduced intensity conditioning: results of a prospective multicenter study: British Journal of Haematology, 115 (3): 653-659, 2001.

28- Mattsson J., Uzunel M., Tammik L., Aschan J. and Ringden O.: Leukemia lineage -specific chimerism analysis is a sensitive predictor of relapse in patients with AML and MDS after allogeneic stem cell transplantation. Leukemia, 15: 1976-1985, 2001.

29- Miyamura K., Barrett A.J., Kodera Y. and Saito H.: Minimal residual disease after bone marrow transplantation for chronic myeloid leukemia and implications for graft versus leukemia effect: a review of recent results. Bone Marrow Transplantation, 14: 201-209, 1994.

30- Muniz E.S., Plassa F., Amselem S., Goossems M. and Vernant J.P.: Molecular analysis of polymorphic loci to study chimerism after allogeneic bone marrow transplantation. Transplantation, 57: 451-456, 1994.

31- Nesci S., Manna M., Andreani M., Fattorini P., Graziosi G. and Lucarelli G.: Mixed chimerism in thalassaemic after bone marrow transplantation. British Journal of Haematology, 10: 143-146, 1992.

32- Ortega M., Escudero T., Caballin M.R., Olive T., Ortega J.J. and Coll M.D.: Follow up of chimerism in children with haematologic diseases after allogeneic haematopoietic progenitor cell transplants. Bone Marrow Transplantation, 24: 81-87, 1999.

33- Petz L.D., Yam P., Wallace R.E., Stock G. and Forman S.J.: Mixed haematopoietic chimerism following allogeneic bone marrow transplantation for haematologic malignancies. Blood, 70: 1331-1337, 1987.

34- Radich J.P., Gehly G., Gooley T., Bryant E., Clift R.A., Collins S., Edmands S., Kirk J., Lee A. and Kessler P.: Polymerase chain reaction detection of bcr-abl fusion transcript after allogeneic bone marrow transplantation for chronic myeloid leukemia: results and implications in 346 patients. Blood, 85: 2632-2638, 1995.

35- Roel A., Marcel G.H., Karen C. and Jan G.: Monitoring of residual disease and guided leucocyte infusion after allogeneic bone marrow transplantation by chimerism analysis of short tandem repeats. British Journal of Haematology, 110: 647-653, 2000.

36- Roman J., Martin C., Torres, Garcia A., Andres P., Garcia M.J. and Baiget M.: Importance of mixed chimerism to predict relapse in persistently bcr-abl long survivors after allogeneic bone marrow transplantation for chronic myelogenous leukemia. Leukemia

Lymphoma, 28: 541-550, 1998.

37- Roth M.S., Antin J.H., Ash R., Terry V.H., Gotlieb M., Silver S.M. and Ginsburg D.: Prognostic significance of Philadelphia chromosome-positive cells detected by polymerase chain reaction after allogeneic bone marrow transplant for chronic myelogenous leukemia. Blood, 79: 276-282, 1992.

38- Roux E., Helg C., Chapius B., Jeannet M. and Roosnek E.: Evolution of mixed chimerism after allogeneic bone marow transplantation as determined by granulocytes and mononuclear cells by polymerase chain reaction. Blood, 79: 2775-2783, 1992.

39- Santos G.W.: Bone marrow transplantation in leukemia. Cancer, 54: 2732-2740, 1984.

40- Slavin S.: New strategies for bone marrow transplantation. Current Opinion of Immunology., 12: 542-551, 2000.

41- Shimoni S., Giralt S., Khouri and Champlin R.: Allogeneic hematopoietic transplantation for acute and chronic leukemia: non-myeloablative preparative regimens and induction of GVL effect. Current Oncology Rep., 2: 132-139, 2000.

42- Shimoni S. and Nagler A.: Non-myeloablative stem cell transplantation (NST): chimerism testing as guidance for immune -therapeutic manipulations. Leukemia, 15: 1967-1975, 2001.

43- Stuppia L., Calabrese G., Bartolomera P.D., Piela R., Franchi P.G., Morizio E. and Palka G.: Retrospective investigation after bone marrow transplantation by PCR amplification of hypervariable DNA regions. Cancer Genetic Cytogenetic, 85: 124-128, 1995.

44- Thiede C., Bornhauser M., Oelschlagel U. and Brandel C.: Sequential monitoring of chimerism and detection of minimal residual disease after allogeneic blood stem cell transplantation (BSCT) using multiplex PCR amplification of STR markers. Leukemia, 15: 293 - 302, 2001.

45- Thomas E.D., Storb R. Clift R. A., Fefer A., Johnson L., Nieman P.E., Lender K.G., Glucksberg H. and Bukner C.D.: Bone Marrow Transplantation. New England Journal of Medicine., 292: 832-843, 1975.

46- Tsongalis G.J., Wu A.H., SilverH. and Ricci A.Jr.: Applications of forensic identity testing in the clinical laboratory. American Journal of Clinical Pathology, 112: S 93-103, 1999.

47- Ugozzoli L., Yam P., Petz L.D., Ferrara G.B., Champlin R.E., Forman S.J. , Koyal D. and Wallace R.B.: Amplification by the polymerase chain reaction of the human genome for evaluation of chimerism after bone marrow transplantation. Blood, 77: 1607-1615, 1991.

48- Van Deerlin V.M.D. and Leonard D.G.B.: Bone marrow engraftment analysis after allogeneic bone marrow transplantation in Domnita Crisan (ed.). In clinics in laboratory medecine, 20, n1, p197, 2000.

49- Van Leeuwen J.E.M., Van Tol M.J.D., Joosten A.M., Wijnen J.T., Verweij PJ. Khan P.M. and Vossen J.M.: Persistence of host-type haematopoieisis after allogeneic bone marrow transplantation for leukemia is significantly related to the recipient's age and/or the conditioning regimen, but is not associated with an increased risk of relapse. Blood 83: 3059-3067, 1994.

50- Wang L.J., Chou-Ryan L., Huang W., Haut P.R. and Kletzel: Evaluation of mixed hematopoietic chimerism in pediatric patients with leukemia after allogeneic stem cell transplantation by quantitative PCR analysis of variable number of tandem repeat and testis determination gene. Bone Marrow Transplantation, 29: 51 -56, 2001.

51- Wasch R., Bertz H., Kunzmann R. and Finke J.: Incidence of mixed chimerism and clinical outcome in 101 patients after myeloablative conditioning regimens and allogeneic stem cell transplantation. British Journal of Haematology, 109: 743-750, 2000.

52- Wasch R., Rrisser S., Hahn J., Bertz H., Engelhart M., Kunzmann R., Veelken H., Holler E. and Finke J.: Rapid achievement of complete donor chimerism and low regimen-related toxicity after reduced conditioning with fludarabine, carmustine, melphalan and allogeneic transplantation. Bone Marrow Transplantation, 26: 243 -250, 2000.

53- Witherspoon R.P., Schanfield M.S., Storb R., et al.: Immunoglobulin production of donor origin after marrow transplantation for acute leukemia or aplastic anaemia. Transplantation, 26: 407, 1978.

54- Zaccaria A., Rosti G., Testoni N., Santucci M.A., Tassinari A., Celso B. and Tura S.: Cytogenetic events after bone marrow transplantation for Ph' chromosome positive myeloid leukemia. Leukemia Research, 15: 289-296, 1991.

55- Zetterquist H., Mattsson J., Uzunel M., Nasman -Bjork H., Svenberg P., Tammik L., Bayat C., Winiarski J. and Ringden O.: Mixed chimerism in the B cell lineage is a rapid and sensetive indicator of minimal residual disease in bone marrow transplant recipients with pre-B cell acute lymphoblastic leukaemia. Bone Marrow Transplantion. 25: 843-851, 2000.