# STUDY THE APPLICATION OF PUBLIC CORD BLOOD STEM CELL TRANSPLANTATION FOR LEUKEMIA TREATMENT (FROM 10 - 2016 TO 9 - 2018)

***Bach Quoc Khanh[1]; Tran Ngoc Que[1]; Nguyen Ba Khanh[1,2]; Vo Thi Thanh Binh[1]***
***Dang Thi Thu Hang[2]; Nguyen Vu Bao Anh[1,2]; Nguyen Tuan Tung[3]***
***Kieu Thi Van Oanh[3]; Vu Duy Hong[1]; Duong Quoc Chinh[1]***
***Hoang Thi Thanh Nga[1]; Nguyen Anh Tri[1]***

**SUMMARY**

*Objectives: To describe the quality of cord blood stem cell stored in Stem Cell Bank of National Institute of Hematology and Blood Transfusion, to assess the initial results of cord blood transplantation for acute leukemia treatment. Subjects and method: Interventional, retrospective and prospective study. Subjects included 3,466 public cord blood units which were successfully stored, 05 patients with acute leukemia, transplanted by cord blood stem cell, in National Institute of Hematology and Blood Transfusion, from October of 2016 to September of 2018. Results: After 02 years of following up, among 3,466 stored public cord blood units, the mean total nucleated cell was 130.4 ± 41.9 x $10^7$, the mean CD34 cell was 45.9 ± 35.5 x $10^5$, the post-thawed viability of stem cells was 84.7 ± 3.4%; there were 30 acute leukemia patients who were indicated for stem cell searching in public cord blood bank and the rate of successful search was 97.7%; 05 patients were transplanted using the matched stem cell units; among that, 02 patients achieved stable engraftment; the most common complications were mucosal ulceration (100%), bacteremia (100%) and CMV reactivation (100%); graft versus host disease happened in 01 case; 03 mortality cases were all due to severe sepsis. Conclusion: Allogeneic cord blood transplantation is a promising and effective method for the treatment of acute leukemia.*

** Keywords: Acute leukemia; Allogeneic; Stem cell transplantation; Cord blood; Stem cell.*

## INTRODUCTION

Acute leukemia is one of the most malignant hematological diseases with very high mortality if treated by standard chemotherapy. Today, thanks to allogeneic stem cell transplantation, many acute leukemia patients have been cured and have long and stable lives. The most common source of stem cell for transplantation is from related donors which is only available for about 30% of patients. One of the effective alternative source for transplantation patients without related donors is public cord blood.

*1. National Institute of Hematology and Blood Transfusion*
*2. Hanoi Medical University*
*3. Bachmai Hospital*
***Corresponding author: Tran Ngoc Que (drque72@gmaol.com)***
***Date received: 20/10/2018***
***Date accepted: 02/12/2018***

Cord blood stem cell transplantation has been applied in many developed countries for the last 30 years with many achievements [1]. Cord blood stem cell has also been established at National Institute of Hematology and Blood Transfusion (NIHBT) since 2014. At this time, about 4,000 cord blood units have been stored for transplantation purpose, in addition to the routine source from related donors [2]. Beside stem cell creation, it is very important to apply and assess the results of stem cell transplantation for the treatment of acute leukemia. This may help to prove the cord blood quality and to encourage and to improve this kind of stem cell source.

So, we conducted this study with the objectives:

- *To describe the quality of cord blood stem cell sample/units stored in Stem Cell Bank of NIHBT.*

- *To assess the initial results of cord blood transplantation for acute leukemia treatment.*

## SUBJECTS AND METHODS

**1. Subjects.**

- 3,466 cord blood units which were collected, processed and cryopreserved in Stem Cell Bank of NIHBT.

- 30 acute leukemia patients were indicated for stem cell searching, in which 05 acute myeloid leukemia patients were allogeneic transplanted using cord blood, in Stem cell Transplantation Department of NIHBT.

* *Study time:* From October of 2016 to September of 2018.

**2. Methods.**

- Study design: Interventional, retrospective and prospective study.

- Convenient sampling.

- Criteria for cord blood collection, processing and storage: Healthy mothers and infants without chronic or hereditary diseases; the collected cord blood volume was ≥ 80 mL, no abnormal colour, no blood clot, no infection; the total nucleated cell was ≥ 1,000 x 106, MCV ≥ 95 fl; the stored units were negative with bacteria, fungus, common virus and abnormal hemoglobin components.

- Patient eligibility criteria: Patients with acute leukemia (both myeloid and lymphoblastic types), never transplanted before, achieved complete remission at time of transplantation, stable health status without any acute diseases or disorders (renal, liver or heart failure, severe infection…), unable to find any HLA (human leukocyte antigen)-matched related donors in their families.

- Cord blood selection criteria: From the public cord blood bank of NIHBT, HLA matched at least 4/6 locus HLA-A, -B and -DR at high resolution, the minimal dose of total nuclear cells was 2 x 107 cells/kg, the minimal dose of CD34+ cells was 0.8 x 105 cells/kg, no risk of impact by anti-HLA antibodies in patient's serum.

- Exclusion criteria: Patients with chronic leukemia, other hematological diseases, cord blood units unmet the selection criteria.

- Materials and equipment: Patient blood samples, cord blood sample, FC500 flow cytometer, automated cell count system DXH500, Luminex system and kits for HLA typing and anti-HLA detection, quantitative

PCR system for chimerism monitoring, FISH testing for mutation and chimerism monitoring, blood group and serology testing, microbiology testing for bacteria, CMV… system for colony forming assays, flow cytometer system for post-thawed cell viability assessment.

- Conditioning regimen: Busulfan (120 mg/m$^2$ day-8 to day-5); fludarabine (40 mg/m$^2$ day-8 to day-3); etoposide (20 mg/kg day-4 to day-2); stem cell transplantation at day 0.

- Graft vs. host disease prophylaxis: cyclosporine A + methotrexate.

- Engraftment criteria:

+ Blood cell recovery determined by: Absolute neutrophil ≥ 0.5 G/L in 3 consecutive days; platelet count ≥ 20 G/L in 3 consecutive days (without transfusion).

+ Chimerism assessed by quantitative PCR test: < 5% of donors defined as graft failure or rejection; 5 - 95% defined as mixed chimerism; > 95% defined as complete chimerism.

+ Graft failure: Diagnosed if engraftment criteria of neutrophils can not be met until day 35 [3].

+ Study indexes: Patients characteristics (age, gender, diagnosis); cord blood characteristics (HLA matched level, cell doses, blood group compatibility, sex); engraftment, complication, survival results;

+ Criteria for mucositis grade [4]: Grade 0: no oral mucositis; grade 1: erythema and soreness; grade 2: ulcers, able to eat solids; grade 3: ulcers, requires liquid diet (due to mucositis); grade 4: ulcers, alimentation not possible.

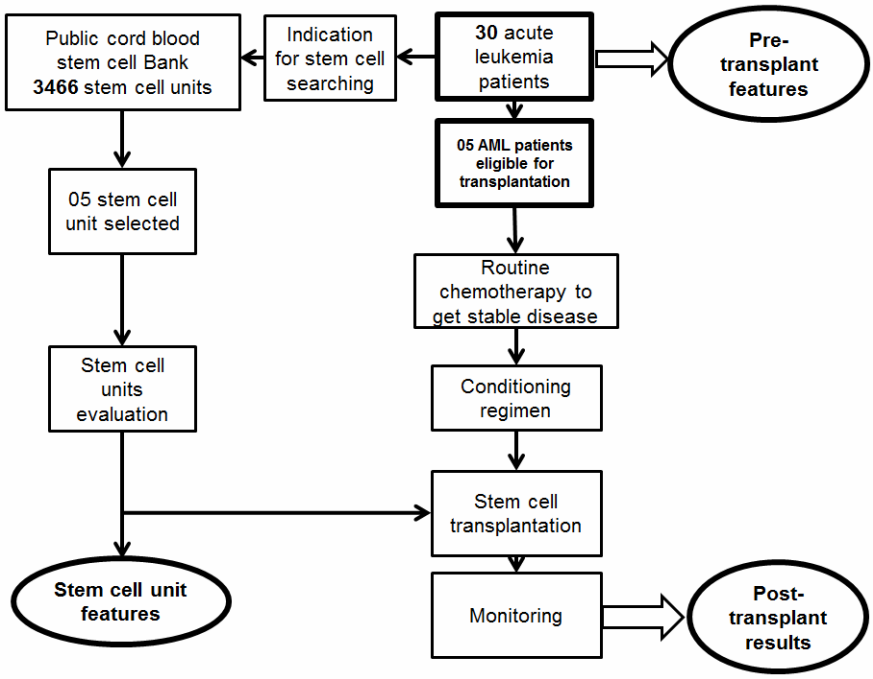


*Figure 1:* Diagram of study process.

* *Statistical analysis:* Proportions, medians were calculated using SPSS 16.0.

## RESULTS

### 1. Subject characteristics.

*Table 1:* General features of processed and preserved cord blood units in study.

| Indexes | X ± SD | Min | Max |
|---|---|---|---|
| Mean total nucleated cells ($10^7$ cells) (n = 3,466) | 130.4 ± 41.9 | 100.1 | 497.7 |
| Mean CD34+ cells ($10^5$ cells) (n = 3,466) | 45.9 ± 35.5 | 3.9 | 365.0 |
| Mean viability (%) | 84.7 ± 3.4 | 81 | 99.8 |

*Table 2:* Stem cell searching results for acute leukemia patients.

| Indexes \ Results | Number of patients | |
|---|---|---|
| | n | % |
| HLA-matched at least 6/6 | 7 | 15.5 |
| HLA-matched at least 5/6 | 28 | 62.2 |
| HLA-matched at least 4/6 | 44 | 97.7 |
| No HLA-matched unit found | 1 | 2.3 |
| Total | 30 | 100% |

The rate of successful searching for an 4/6 HLA locus matched stem cell unit was 97.7%, the rate for 6/6 matched unit was 15.5%.

*Table 3:* Pre-transplant features of patients.

| Patient | Age | Gender | Diagnosis | Disease stage | Weight (kg) |
|---|---|---|---|---|---|
| 1 | 27 | Female | AML | CR 2 | 56 |
| 2 | 32 | Female | AML | CR 1 | 52 |
| 3 | 21 | Female | AML | CR 2 | 56.5 |
| 4 | 33 | Female | AML | CR 1 | 46 |
| 5 | 24 | Female | AML | CR 1 | 45 |

*(AML: Acute myeloid leukemia; CR: Complete remission)*

Among 05 transplanted cases, 02 patients achieved second complete remission, all patients were adult with weight ranging from 45 - 56.5 kg.

*Table 4:* Feature of transplanted cord blood units.

| Patient | HLA matched level | Gender mismatch | ABO group mismatch | CD34 cell dosage ($10^5$/kg) | TNC dosage ($10^7$/kg) |
|---|---|---|---|---|---|
| 1 | 4/6 | No | No | 2.04 | 5.33 |
| 2 | 5/6 | Yes | Yes | 7.85 | 3.93 |
| 3 | 5/6 | Yes | No | 1.06 | 2.22 |
| 4 | 6/6 | No | No | 1.05 | 3.90 |
| 5 | 5/6 | No | Yes | 2.28 | 4.92 |

*(TNC: Total nucleated cell)*

All transplanted cord blood units were at least 4/6 HLA matched with the patients, the CD34 dosage ranged from 1.05 - 7.85 x $10^5$/kg, TNC dosage ranged from 2.22 - 5.33 $10^7$/kg, there were 03 cases with ABO blood group mismatch between patients and cord blood units.

*Table 5:* Cord blood stem cell transplantation results for acute leukemia patients.

| Patients | Total follow-up time | Neutrophil recovery time | Platelet recovery time | Mortality | Relapse |
|---|---|---|---|---|---|
| 1 | 17 days | Not yet | Not yet | Yes | - |
| 2 | 360 days | 10 days | 33 days | No | No |
| 3 | 58 days | Graft failure | Graft failure | Yes | - |
| 4 | 120 days | 16 days | 56 days | No | No |
| 5 | 30 days | Not yet | Not yet | Yes | - |

There were 02 patients with stable engraftment, 03 patients died in 17 - 58 days post-transplant, no patient relapsed.

*Table 6:* Features of complication after cord blood stem cell transplantation.

| Patients | Chimerism | Oral mucositis | CMV reactivation | GvHD | Bacteremia | Mortality cause |
|---|---|---|---|---|---|---|
| 1 | - | Grade III | Yes | No | Yes | Bacteremia |
| 2 | 100% | Grade III | Yes | Skin, grade I | Yes | - |
| 3 | 0% | Grade III | Yes | No | Yes | Bacteremia |
| 4 | 100% | Grade III | Yes | No | Yes | - |
| 5 | 0% | Grade III | Yes | No | Yes | Bacteremia |

100% of the patients had oral mucositis grade III, CMV reactivation and bacteremia. There were 03 patients who died because of bacteremia, 01 patient had mild GvHD.

## DISCUSSION

Cord blood units which were stored in Stem Cell Bank of NIHBT had good quality, the mean TNC was 130.4 x $10^7$ cells/unit (*table 1*). According to international guidelines, the minimum TNC dose for transplantation is 2.0 x $10^7$/kg of patient weight, so the cord blood units of NIHBT can be applied for patients with mean weight of about 65 kg [5]. The mean CD34 cells contained in each unit was 45.9 x $10^5$ cell/unit (*table 1*). With the minimum CD34 dose required as 0.8 x $10^5$ cells/kg, they can be applied for patients who weigh 57 kg [6]. The total number of cord blood stem cell units stored in Stem Cell Bank were 3,466 units.

This could provide the successful searching rate at 97.7% for 30 acute leukemia patients who were indicated for transplantation (*table 2*). This is a very important advantage of stem cell from cord blood because the HLA matching level only requires at least 4/6 locus HLA-A, -B and -DR, so the searching rate can be higher and the number of stored units for searching can be lower compared to other sources of stem cell [5].

Among 30 cases of stem cell searching, there were 05 patients who were transplanted using the matched cord blood units. All 05 cases were adult acute myeloid leukemia patients who achieved complete remission before transplantation (*table 3*). Commonly, one disadvantage of cord blood stem cell is the low volume and cell dose so the usual application is for pediatric patients [7]. However, transplanted patients in NIHBT were not only limited to pediatric cases with low weight but also applied to adults with weight about 45 - 56.5 kg (*table 2*). The cell doses of those units were 2.22 - 5.33 x $10^7$ TNC/kg and 1.05 - 7.85 x $10^5$ CD34 cell/kg that were all qualified for international standard and even better (*table 4*) [5, 6]. This was because all cord blood units stored in public bank of NIHBT were selected by high level of cell count before processing and cryopreservation [2].

About transplantation results, 02 out of 05 patients achieved engraftment while the other 03 patients could not achieve engraftment and died because of complication (*table 5*). For 02 patients with engraftment, the recovery time of neutrophils were 10 and 16 days, of platelets were 33 and 56 days, which were similar to results from reports about cord blood transplantation and longer when compared to that from adult stem cell sources [8, 9]. The main reason is that the cell doses of cord blood are significantly lower than that of mobilized peripheral blood or bone marrow fluid. Moreover, the delayed engraftment can be affected partially by CMV reactivation which happened in 100% of cases (*table 6*). This virus can cause marrow suppression, cellular growth limitation and even severe damages to transplantation patients [10]. The cord blood stem cell also has another disadvantage which is higher graft rejection rate than other stem cell sources [11]. Actually, there were 03/05 patients in our study did not get engraftment and died because of severe bacteremia (*table 5*).

Common complications during cord blood stem cell transplantation were oral mucositis (100%), bacteremia (100%) and CMV reactivation (100%) (*table 6*). Reports of Burik (2007), Victor (2011) also recognized the high rate of those complication in cord blood transplantation patients [12]. The causes may be long cell recovery time, especially neutrophils, as well as naïve immune function of cord blood cells that lead to weaker infection resistance when compared to other sources of stem cells. This might be the reason of 03 mortality cases (*table 5*). In contrast, severe GvHD in this type of transplantation is very low. Only 01 patient had mild GvHD and 4/5 patients showed no symptom of GvHD (*table 6*). This was similar to other reports, which confirmed that low rate of severe GvHD is an important advantage of cord blood stem cell transplantation [1].

At this time, both patients with engraftment are stable at 120 and 360 days of follow-up (*table 3*). The cord blood transplant results of our study were not different from other published reports. Patients in study by Laughlin et al (2004) had 2 years-overall survival of 26% [11]. In study of Matsumura et al (2012), the 2 years overall survival was 36%. Jaime et al (2013) reported the overall survival after 3 years of cord blood transplantation as 44%. This confirms that the quality of stem cell as well as cord blood transplantation in Vietnam is very promising, which may help patients gain more chances to be treated by stem cell technology if they can not find related stem cell donors.

## CONCLUSION

The initial results of application of cord blood stem cell transplantation for acute leukemia in National Institute of Hematology and Blood Transfusion had given some conclusions:

- For 3,466 cord blood units stored at NIHBT, the mean total nucleated cell was 130.4 x $10^7$ cells/unit, the mean CD34 cell was 45.9 x $10^5$ cells/unit, which can be applied for adult patients.

- The successful rate of finding a 4/6 HLA locus matched stem cell unit for 30 acute leukemia patients was 97.7%.

- Among 05 transplanted patients, 02 cases had got stable and long engraftment, 03 cases without engraftment died due to severe bacteremia.

- The most common complication during transplantation process included mucositis, CMV reactivation, bacteremia. Graft vs. host disease only occurred in 01 case.

## RECOMENDATION

Cord blood stem cell transplantation should be carried on with larger samples to evaluate more accurately the effectiveness of this source in acute leukemia treatment.

## REFERENCES

*1. Trần Ngọc Quế, Nguyễn Bá Khanh, Lê Xuân Thịnh và CS*. Thành công bước đầu trong xây dựng ngân hàng tế bào gốc máu dây rốn cộng đồng ở Việt Nam. Y học Việt Nam. 2015, 429, tr.338-344.

*2. E.Gluckman*. History of cord blood transplantation. Bone Marrow Transplantation. 2009, 44, pp.621-626.

*3. Department of Clinical Haematology Oxford BMT Programme*. The diagnosis and management of primary and secondary graft gailure after haemopoietic stem cell transplant. 2017, 1, 2.

*4. D.E. Peterson, R.J. Bensadoun, F. Roila*. Management of oral and gastrointestinal mucositis: ESMO Clinical Practice Guidelines. Annals of Oncology. 2011, 22, pp.78-84.

*5. United Kingdom Paediatric Bone Marrow Transplant Group, British Society for Histocompatibility and Immunogenetics, The British Transplantation Society, et al.* Guidelines for selection and HLA matching of related, adult unrelated donors and umbilical cord units for haematopoietic progenitor cell transplantation. 2013.

*6. Duncan Purtill, Katherine Smith, Sean Devlin et al.* Dominant unit CD341 cell dose predicts engraftment after double-unit cord blood transplantation and is influenced by bank practice. Blood. 2014, 124, pp 905-912.

*7. Karen K. Ballen, Eliane Gluckman, Hal E. Broxmeyer.* Umbilical cord blood transplantation: the first 25 years and beyond. Blood. 2013, 122 (4), pp.491-498.

*8. Changcheng Zheng, Baolin Tang, Wen Yao et al.* Comparison of unrelated cord blood transplantation and HLA-matched sibling hematopoietic stem cell transplantation for patients with chronic myeloid leukemia in advanced stage. Biol Blood Marrow Transplant. 2013, 19, pp.1708-1712.

*9. William Tse, Mary J. Laughlin.* Umbilical cord blood transplantation: A new alternative option. Hematology Am Soc Hematol Educ Program. 2005, pp.377-383.

*10. Rafael de la Cámara*. CMV in hematopoietic stem cell transplantation. Mediterr J Hematol Infect Dis. 2016, p.8.

*11. M.D. Mary J. Laughlin, M.B. Mary Eapen B.S, M.D. Pablo Rubinstein et al.* Outcomes after transplantation of cord blood or bone marrow from unrelated donors in adults with leukemia. N Eng J Med. 2004, 351 (22), pp.2265-2275.

*12. van Burik J.A, Brunstein C.G* . Infectious complications following unrelated cord blood transplantation. Vox Sang. 2007, 92 (4), pp.289-296.