Prognosis of pediatric patients transplanted for Ph+ chronic myeloid leukemia in the period from 1989 to 2006 in the Czech Republic

L. KROL[1], R. FORMANKOVA[1], P. KESLOVA[1], V. VAVRA[1], Z. ZEMANOVA[2], K. MICHALOVA[3], M. DOBROVOLNA[3], J. MORAVCOVA[3], P. KOBYLKA[3], E. IVASKOVA[4], J. NAVRATILOVA[5], J. STARY[1], P. SEDLACEK[1*]

[1]Department of Pediatric Hematology and Oncology, Teaching Hospital Motol, 2nd Medical School, Charles University in Prague, Czech Republic, e-mail: petr.sedlacek@lfmotol.cumi.cz, [2]Institute of Clinical Biochemistry and Laboratory Diagnostics, General Faculty Hospital, Ist Medical School, Charles University in Prague, [3]Institute of Hematology and Blood Transfusion, Prague, [4]Czech Stem Cell Donor Registry, Prague, [5]Czech National Marrow Donors Registry, Prague

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Chronic myeloid leukemia (CML) is a myeloproliferative disorder caused by clonal proliferation of primitive hematopoietic stem cell. The median age at diagnosis is 55 to 60 years with less than 10% of patients younger 20 years. Incidence of CML in children in the Czech Republic is 0.106 cases/100 thousands per year. Here we report outcome of 38 pediatric patients (median age 12.5 years; range 1.8 – 17.3) with Ph-positive CML diagnosed between years 1989 to 2006. Primarily chronic phase of the disease was diagnosed in 32 (84%) patients. 32 (84.2%) patients underwent hematopoietic stem cell transplantation (HSCT) with the median age at transplantation of 14.9 years (range 6.9 – 20.5 years). Out of transplanted patients 16 (50%) obtained graft from unrelated donor, 13 (41%) from matched sibling donor, 2 from haploidentical family donor and autologous transplantation has been performed in one case. 6 patients were not transplanted, 4 of them died (median 1.2 years from diagnosis), 2 are alive 0.6 and 17.8 years from the diagnosis. Overall survival (OS) in 25 patients after HSCT at our department during the whole period is 66.7% with 15/16 being in stable continuous molecular-genetic remission (94%). During the period of time results of transplantations have been significantly improved (p=0.0071). OS after HSCT until year 1997 is 25% while from year 1998 until now is 87.5%. All centers OS of patients after HSCT is 71%. Results of HSCT in children with CML obtained from the year 1998 at our center are fully comparable with results achieved in large and experienced centers. HSCT remains the only proven and effective method for the treatment of CML. Clinical studies assessing the role of tyrosine kinase inhibitors in children instead of early HSCT should be planned carefully in order to avoid sub-optimal outcomes.

Key words: Ph+ chronic myeloid leukemia, children, allogeneic hematopoietic stem cell transplantation, incidence, prognosis

CML is a myeloproliferative disorder caused by clonal proliferation of primitive hematopoietic stem cell. CML is more frequent in older people while in childhood it is extremely rare disease. The median age at diagnosis is 55 to 60 years. We have defined the incidence of CML in children up to 18 years in the Czech Republic for the whole study period and the average incidence is 0.106 cases/100 thousands per year. There are about 2-3 children newly diagnosed every year representing around 3% of all newly diagnosed childhood leukemia.

CML in childhood is typically characterized by biphasic or triphasic course. CML is mostly diagnosed in the first chronic phase (CP1). Up to 40% of patients are asymptomatic and the diagnosis is discovered on the basis of routine blood count examination. Classical symptoms include fatigue, weight loss, night sweats, splenomegaly, early satiety or anorexia. The median survival in CP1 before tyrosine kinase inhibitors (TKI) became available was approximately 35 to 65 months. After this period of time most of the patients progressed to the blastic phase – blast crisis (BC). There is an intermediate phase, known as the accelerated phase (AP), which foregoes BC in approximately 60% of patients. BC is characterized by presence of more than 30% of blasts in the peripheral blood or bone marrow. The blastic transformation

* Corresponding author

Table 1. Initial hematologic values (x10^9/L)

	Minimum	Maximum	Median
WBC	10.0	712.0	145.0
Platelets	79.0	3022.0	596.0

Table 2. Characteristics of conditioning regimens used prior HSCT (n=25 patients)

Unrelated donor		
fTBI 14.4 Gy	cyclophoshamide 120 mg/kg rATG 40 mg/kg	12
	without rATG	2
Matched sibling donor		
busulfan 16 mg/kg	cyclophosphamide 120 mg/kg	8
Other family donor		2
busulfan 16 mg/kg	cyclophosphamide 120 mg/kg rATG 40 mg/kg thiotepa 15 mg/kg	
Autologous HSCT		1
busulfan 16 mg/kg	cyclophosphamide 200 mg/kg	

can result from myeloid (50%) or lymphoid (25%) lineage and the rest 25% from undifferentiated precursor cells. It is relevant for the disease prognosis. Patients with lymphoid BC have a better complete remission rate and longer median survival than patients with myeloid or undifferentiated precursor lineages [1]. The median survival in BC before TKI were implemented was 3 to 12 months but even recently remains dismal.

AP median survival is 24 to 36 months. No exact definition of this stage is widely accepted. In most cases the IBMTR (International Bone Marrow Transplantation Registry) classification is used. This classification includes criteria like percentage of blasts in transformation and basophiles, WBC, trombocytes, presence of anemia, splenomegaly, positive cytogenetics and the other characteristics (e.g. chloromas, myelofibrosis)[2]. There are another two classifications – MDACC (MD Anderson Cancer Center)[3] and WHO (World Health Organisation)[4] classification. One criterion which is constant in all classifications is cytogenetic clonal evolution (CE), which occurs in 40% of patients. The most common chromosomal abnormalities associated with CE are trisomy 8, Philadelphia (Ph) chromosome, isochromosome 17 and some other abnormalities of chromosome 17. Abnormalities in all other chromosomes have been reported. Different abnormalities may have a different prognostic significance. Patient who have CE as the only criterion for AP have a better outcome than patients with other AP criteria [2].

Allogeneic HSCT still remains the only known curative option for patients with CML. There are two different approaches to preparative regimens in clinical practice, myeloablative and non-myeloablative. HSCTs with myeloablative conditioning are being used for more than 20 years now and their anti-leukemic effect is well known. This kind of regimen is associated according to different studies with 10-50% transplant related mortality (TRM) in the first two years after transplantation. This disadvantage leads to development of non-myeloablative regiment (RIC – reduced intensity conditioning). They are associated with lower level of TRM but with higher risk of both acute and chronic graft versus host disease (GvHD) and they have also a higher risk of relapse. There is the reason why the results of OS haven't changed significantly. Probability of 5-year survival is 60 to 80%[5-7]. The centre experience, qualified observation of minimal residual disease (MRD) after the transplantation and possibility of adoptive immunotherapy with the aim to averse the risk of relapse can markedly affect the results[8].

Patients and Methods

Patients. Between 1989 and 2006, 38 children (median age 12.5 years; range 1.8 – 17.3) with Ph-positive CML were diagnosed in the Czech Republic (17 girls and 21 boys). At presentation 32 (84%) patients were diagnosed in CP, 5 (13%) in AC and one patient was diagnosed as primary lymphoblastic blast crisis. Initial hematologic values at diagnosis are displayed in Table 1.

Cytogenetic examination for the confirmation of Ph chromosome positivity was performed in all 38 patients with positive results in all of them. In some patients there were the other chromosomal aberrations present: trisomy 8, variant complex translocation t(3;9;22) or t(9;17;22). After the method became available BCR-ABL has been proved by polymerase chain reaction (PCR). We examined 29 patients with positivity in all cases.

25 patients (median age at transplantation was 14.2 years; range 6.9 – 19.0) underwent allogeneic (n=24) or autologous (n=1) HSCT at our institution (median time from diagnosis to transplantation was 0.98 years, range 0.3 – 5.8).

The conditioning regimens and graft versus host disease prophylaxis varied according to the type of donor, details are shown in Table 2.

Methods. Engraftment is defined as the first out of 3 consecutive days when ANC $\geq$ 500 x10^{12}/L and platelets $\geq$ 20 x10^{12}/L with no platelet transfusion in previous 7 days. Variable number of tandem repeats (VNTR), short tandem repeats (STR), semi-quantitative or quantitative evaluation of BCR-ABL by fluorescent in situ hybridisation (FISH) and real-time PCR (RT-PCR) are used for monitoring of residual leukemia. The post-transplant monitoring scheme includes VNTR chimerism, monitored from day +14 until day +100 every week and then during every outpatient visit. BCR-ABL is evaluated in peripheral blood samples on days +30, +60 and +100, than less frequently depending on results. If positivity is detected, it is repeated each 4 – 6 weeks, otherwise on day +180 and then once a year.

Survival probabilities were calculated by the method of Kaplan-Meier. The log-rank test was used to compare survival curves. All quoted P-values are two sided and confidence intervals (CI) refer to 95% boundaries. All statistical analysis has been made on Stat-View software (SAS Institute, Cary, NC, USA).

Results

All patients were primarily treated with hydroxyurea (Litalir®, BMS); approximately 30% of patients were later on switched to interferon alpha (IFN-α, Roferon A®, Roche). Recently diagnosed patients were treated with imatinib mesylate (Glivec®, Novartis). Busulfan, cytarabine or mercaptopurine before HSCT were used sporadically in patients with signs of acceleration.

32/38 (84.21%) patients underwent HSCT. 16 (50%) obtained graft from unrelated donor (UD), 13 (41%) from matched sibling donor (MSD), 2 from haploidentical family donor and autologous transplantation has been performed in one case. 6 patients were not transplanted, 4 of them died (median 1.19 years from diagnosis, range 0.66 – 1.57 years), 2 are alive 0.6 and 17.8 years from the diagnosis, both treated with imatinib.

25 patients were transplanted at our department, 6 patients in the other transplant centers for adults in Czech Republic. 1 patient was transplanted in the Fred Hutchinson Cancer Research Center, Seattle, WA, USA. 14 (56%) patients transplanted at our department obtained graft from UD, 8 from MSD and 2 from haploidentical family donor. One patient underwent HSCT using Ph negative graft modified by long term culture. Further details could be found in Table 3. Median time between diagnosis and transplantation is 0.98 year (range 0.3 – 5.8 years) and it is significantly shorter in last few years. The median age of the patient at transplantation is 14.9 years (range 6.9 – 20.5 years).

Engraftment occurred in all our patients who underwent the HSCT. The only patient with autologous HSCT experienced primary graft failure. The median time for engraftment of granulocytes is 20 days (range 10 – 36 days). All patients but 2 (8.3%) reached complete chimerism verified by VNTR in the median time of 21.5 days (range 11 – 73 days). MRD was detected in 5 patients after day +100 (second run of RT-PCR was positive).

Acute graft versus host disease (aGvHD) developed in 14 (56%) of our patients, chronic limited GvHD in 3 (12%) and extensive in 1 (4%) patient respectively. See Table 4 for further informations.

Following HSCT molecular-genetic relapse was diagnosed in 3 (12.5%) of our 24 transplanted patients (in one patient three times). All these patients were successfully treated with donor lymphocyte infusions (DLI) from UD (1x) or MSD (2x). Patient who developed relapse three times (following MSD SCT) was repeatedly treated with IFN-α or imatinib [8].

Table 3. Patients, donors and transplantations characteristics in TH Motol (n=25 patients)

	Number	Percentage (%)
Sex		
boys	14	56.0
girls	11	44.0
HSCT		
CP	21	84.0
AP	3	12.0
BC	1	4.0
Source of hematopoietic stem cells		
bone marrow	20	80.0
peripheral blood	5	20.0
Conditioning regimen		
myeloablative	25	100.0
non-myeloablative	0	0.0
Graft donor		
matched sibling donor	8	32.0
other family donor	2	8.0
unrelated donor	14	56.0
Autologous HSCT	1	4.0
HLA matches in unrelated donors		
10/10	5	35.7
9/10	4	28.6
8/10	1	7.1
7/10	4	28.6

Table 4. Graft versus host disease and relapses in patients who underwent HSCT in TH Motol (n=25 patients)

	Number	Percentage (%)
Acute graft versus host disease		
grade 0 and I	16	64.0
grade II	6	24.0
grade III and IV	3	12.0
Chronic graft versus host disease		
absent	17	68.0
limited	3	12.0
extensive	5	20.0
Relapse		
hematologic	2	8.0
molecular – genetic	3	12.0

Two patients developed hematologic relapse 4.5 and 18 months after HSCT. One died of rapid disease progression, one achieved further remission after imatinib and DLI, but died in a consequence of secondary GvHD due to invasive fungal infection (*Aspergillus niger*).

The median follow-up of the whole group of living patients is 7.1 years (range 0.6 – 17.8 years). Overall survival (OS) in patients after HSCT at our department during the whole period is 66.7% (16/24), see Figure 1. 15 out of 16 patients (94%) are alive in continuous and stable molecular-genetic remission. During the period of time results of transplantations have been significantly improved (p=0.0071). OS after HSCT until year 1997 is 25% while OS achieved in

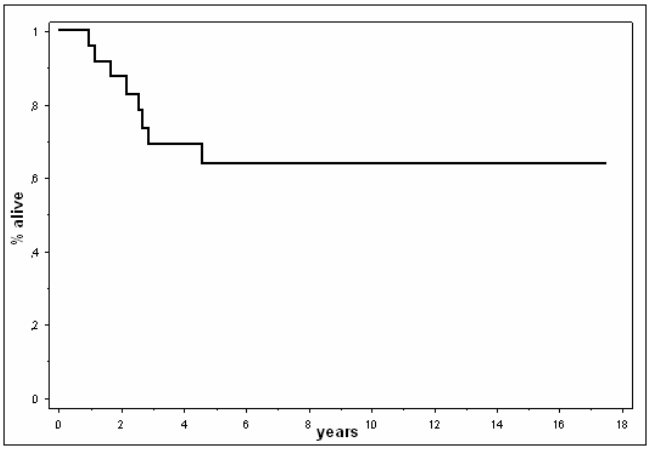

Figure 1. OS after HSCT at TH Motol during whole period of consideration

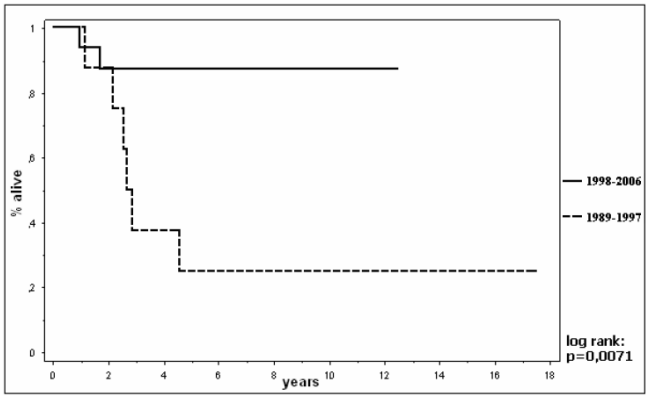

Figure 2. OS after HSCT at TH Motol (depends on the year of transplantation)

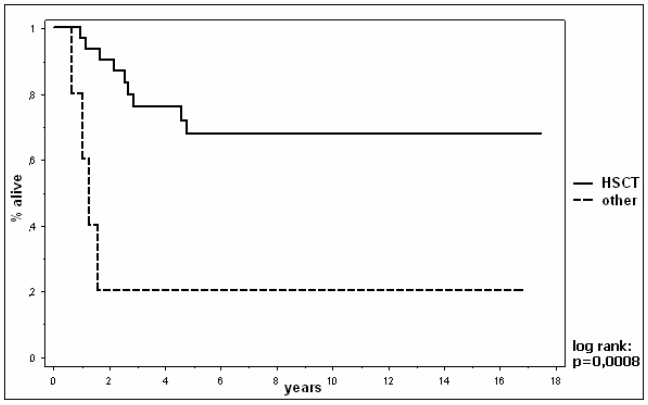

Figure 3. OS in 37 patients treated in the Czech Republic (1989-2006)

Table 5. Causes of death

	Number	Percentage (%)
Period 1989 – 1997 (n = 10)		
GvHD	3	30.0
Gneg sepsis	1	10.0
progression	4	40.0
ARDS[1]	1	10.0
unknown	1	10.0
Period 1998 – 2006 (n = 4)		
Gneg sepsis	1	25.0
CMV pneumonia	1	25.0
IFI[2]	2	50.0

[1] ARDS = Acute Respiratory Distress Syndrome; [2] IFI = Invasive Fungal Infection

patients transplanted from the year 1998 until now is 87.5%, see Figure 2. All centers OS of patients after HSCT is 71% compare to 33.3% in patients without transplantation (p=0.0008), see Figure 3.

Altogether 14 (36.8%) patients died, 4 for disease progression without previous HSCT and 1 which was transplanted in the USA (extensive chronic GvHD). The other 9 died following HSCT at our department (8 alloSCT, 1 autologous). Causes of death are listed in Table 5.

Discussion

The treatment of CML underwent a big development in the period of consideration. Over this period of time we have mostly used hydroxyurea and interferon alpha (IFN-α). We have tried to drive every patient to allogeneic HSCT with matched related or unrelated donor (in case of chronic phase) or alternative HLA mismatched donor for patients with signs of disease acceleration. There are two therapeutical options available now – life long therapy with tyrosine kinase inhibitors or allogeneic HSCT.

Imatinib is in clinical practice from 1998 and its introduction changed current management of CML therapy especially in adults. Imatinib was shown to be the most effective non-transplant strategy for frontline therapy in CML. The current analysis indicates that the introduction of imatinib resulted in a substantial decrease in the use of allogeneic HSCT for CML [9]. Although the results are excellent (a hematologic response is achieved in 80% of patients in accelerated phase and 25% achieved a major cytogenetic response[10, 11]), there are still some problems. Imatinib is in use for a short period of time only and it is difficult to determine long-time overall survival (OS) of patients. Furthermore it is known that imatinib is not able to eradicate the leukemic clone. Approximately 5% of patients have primary hematologic resistance and these patients can't achieve primary hematologic remission. In 30% of patients in CP1 primary cytogenetic resistance is documented and they therefore can't achieve primary cytogenetic remission. Many patients in AP do not respond to imatinib and others who respond may eventually later on develop re-

sistant disease. In addition 30% to 60% of patients in AP progress to BC or loose the hematologic response within 12 months of therapy. In the BC only 10% to 15% of patients have a durable response [12].

The mutation in BCR-ABL kinase domain is responsible for the resistance. This knowledge leads to development of the second generation of TKI such as dasatinib or nilotinib which are able to inhibit the function of the most BCR-ABL kinase mutant forms. Still there remains a group of patients with T315I-BCR-ABL imatinib-resistant mutation who are also primary resistant to dasatinib and nilotinib or the response is not durable. For this resistant mutation therefor it will be necessary to develop new targeted therapy. There are some new agents (ON012380, LBH589B and MK-0457) which seems to be able to inhibit this kind of mutant kinase [13].

The role of non-myeloablative regimens (RIC- reduced intensity conditioning) is one of the most discussed questions today. Their introduction invoked very optimistic expectations because their usage was associated with reduction of TRM (transplantation related mortality). TRM in conventional regimens is 10 – 50%. But finally the 5-years OS is just 60 – 80%, what is comparable with myeloablative regimens. The reason is that the RIC is associated with higher level of relapses namely hematologic. GvHD is more common, especially chronic and extensive.

GvHD is the most frequent cause of death of patients after HSCT. GvHD is mediated by donor T-cells. These lymphocytes are also responsible for graft versus leukemia effect (GvL). This finding led to development of donor lymphocytes infusion (DLI) approach which can induce complete remission of CML in emerging or fully manifested relapse after HSCT.

Effect of DLI depends on three essential factors: a type of relapse (hematologic, cytogenetic and molecular-genetic), a phase of the disease after relapse and donor CD3+ T-cells dosage [14, 15]. The beneficial effects of DLIs are often associated with GvHD or myelosuppression. There is the reason for an administration approach known as escalating doses regimen (EDR) proposed by Dazzi et al. [16] which is associated with a lower incidence of GvHD and higher efficiency in remission achievement.

These observations led to new transplantation protocols with CD3 T-cells depleted grafts in combination with DLI during the relapse. This new regimen had to be associated with decreased incidence of GvHD and lower TRM. OS of these patients remains in the range 72 – 89%[17].

In the case of relapse there are some other options of adoptive therapy, e.g. re-transplantation or use of TKI. Re-transplantation might be associated with excessive toxicity and therefore higher morbidity and mortality. TKI might be used separately or in combination with DLI[18, 19]. The results of imatinib for treatment of post-transplant relapse seemed promising at the beginning but some new studies show that the results are not supposed to be definitive [20].

Our department participates in the development of new European procedures for treatment of CML in children with the use of TKI and sequential allogeneic HSCT. Our goal in the era of TKI is to achieve the best control of chronic phase using TK inhibitors and to provide the patient with safe HSCT using the best matched donors.

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