INTRODUCTION

Stem cell transplantation (SCT) is nowadays a routine therapy for many malignant and non-malignant hematological disorders (1–5). Two main antitumor mechanisms have been recognized in SCT. First is based on aggressive myeloablative chemotherapy with or without total body irradiation (6–7) while the second one is based on immune antitumor mechanism mediated by donor immunocompetent T-cells (the graft-versus tumor reaction (GvT) and/or graft-versus leukemia reaction (GvL) (8–10). However, still the main problem of SCT is high treatment-related toxicity (TRT) and/or treatment-related mortality (TRM) accompanied by the mortality rate related to transplant between 25–40% (11, 12). Recently more efficient supportive care, better control of infections and main complication graft-versus-host disease (GvHD) significantly reduced the mortality rate due to transplant (13). In this respect reduced intensity conditioning (RIC) substantially decreased early toxicity and mortality complications of standard myeloablative conditioning (14–16). Therefore RIC as immunosuppressive conditioning allow the engraftment of donor cells which than can mediate GvL. Consequently in many centers much older patients are undergoing transplantation. Instead of basic malignant disease, the indication for SCT mainly depends on patient’s performance and co-morbidity score (so-called biological age) (17–18). SCT from matched unrelated donor (MUD) become eligible for many patients (19–20). With new high resolution technique for HLA-typing, combined with constantly increasing number of the volunteer donors in the world-wide Registries a chance to find e compatible donor is generally more realistic. Thus results of transplantation with an unrelated donor compatible in 10 out of 10 antigens practically correspond to those with related identical donor (21). Furthermore stem cells from cord blood (22) and new data from allografting with haploidentical donor cells are also very encouraging (23). All these new developments suggests that in the future there will be no problem to find appropriate donor for high risk patients.

THE HISTORY OF SCT IN UNIVERSITY HOSPITAL CENTER, ZAGREB

Main SCT events

At University Hospital Center Zagreb first allogeneic SCT had been performed on February, 8th, 1983. Five years later, in 1988 autologous
SCT program have been started, mostly in myeloma, lymphoma and acute myeloid leukemia but until 2005 transplantation program has been performed only sporadically. From 2005 onward we have been using transplant with stem cells obtained from cord blood of unrelated donor. For the first time in 2006 an adult patients received two unrelated cord blood. In 2012 transplant with stem cells from haploidentical donor was given in adult patient with aggressive lymphoma.

During the 30 years period 659 patients underwent allogeneic transplantation in our institution. Figure 1 summarizes the main transplant events whereas indications for transplants are given in Figure 2. The number of transplants performed per three year period is given in Figure 3.

From 2011 we have been treated more than 50 patients per year which is twice more than before 2009. This is mostly because from 2010 we are routinely performed SCT from unrelated donor and transplant with reduced intensity conditioning in older patients.

**Early phase of SCT in Zagreb**

During the 1970-ies SCT had been recognized as new and effective therapy mainly for leukemia. At the beginning SCT had been performed at the end stage of hematological malignancies (24). First results clearly proved that SCT treatment was feasible and effective (25–26) but still at that time being only experimental treatment. The main complication GvHD and the beneficial anti-immune tumor effect were recognized clinically, but still without scientific explanation. Nevertheless SCT in many countries had become an increasing treatment options for many hematological disorders.

At that time, in Croatia, a group of immunologists at Institute Rugjer Boskovic studied experimental bone marrow transplantation in mice and they were the first in the world who demonstrated that transplanted lymphoid cells can mediate both anti-leukemia effect (27) and the antileukemia effect of graft-versus-host reaction (28). They organized in 1979 a workshop on the necessities and possibilities for clinical SCT in Croatia (29–30) and proposed that clinical SCT project should be organized.
SCT in Zagreb, Croatia

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and located at University Hospital Center Zagreb. Before clinical SCT was started, Interdisciplinary transplant team (TT) was formed including experts from different clinical and laboratory fields, which proposed the Protocol of therapy for SCT and produced manual with defined indications and priorities for SCT.

First clinical allogeneic transplantation had been performed on 8th of February 1983, in patients with severe aplastic anemia. The source of stem cells was marrow from HLA-identical sibling donor (31).

"Is the transplant rational and priority in the country with relatively low income?" This question was raised at that time by many physicians. Many of them had been against the high tech medicine, primarily because of financial problems. They were asking us: "What is more important transplantation or vaccination? There is no money for the basic medical needs such as vaccination, and you are doing SCT?!" they said. The only possible answer was: "Of course, vaccination is more important, but we need vaccination and transplantation". We tried to elaborate this problem in Sounding Board article: Transplanted technology: Third world options and first answer was: "Of course, vaccination is more important, but we need vaccination and transplantation". We tried to elaborate this problem in Sounding Board article: Transplanted technology: Third world options and first world science (37).

Let me cited some of the relevant part of this article to explain the needs for transplant at that time:

"In the end, only two options were feasible: continue sending patients abroad or do bone marrow transplantation at home. The latter was in all ways preferable... Economically, a domestic transplantation program was far cheaper, since salaries and hospital costs are low and paid in dinars.... Overall five transplantations could be done at home for the price of single one abroad.

In addition Transplant project really was speed-up "engine" for the development of clinical medicine in Zagreb. Namely the prerequisite for SCT was organization on much higher level of quality of many clinical wards and clinical laboratories such as immunology and HLA-typing, transfusion medicine, pathology, microbiology especially diagnostics of viral and fungal infections, biochemistry and last but not least molecular biology which are not only important for routine work of SCT program.

**SCT AT UNIVERSITY HOSPITAL CENTER ZAGREB – STANDARD THERAPY FOR MANY HEMATOLOGICAL MALIGNANT TUMORS**

After first clinical experience with severe aplastic anemia, the program has been expanded, and SCT was offered to patients with acute leukemia and chronic myeloproliferative neoplasms. For CML before the era of tyrosine kinase inhibitors, SCT was the treatment of choice. During that period our first professional and scientific interest was to reach the therapeutic outcome similar to the most European Blood and Marrow Transplant (EBMT) Centers. So we decided to optimize diagnostic and treatment approach of SCT by standardizing diagnosis and therapy for skin GvHD (38–39), proposed criteria for selection of donor for allografting (40), by introducing new methods for monitoring the chimerism after transplantation by measuring isoenzymatic polymorphism of erythrocyte phosphoglucomutase-1 (41–42), by successful elimination of antibodies by plasma exchange in ABO incompatible SCT (43) and the monitoring of immunologic recovery after transplant (44). In addition, original modification of total body irradiation (TBI) created by our physicist (45) have been still using for the standard myeloablative conditioning (50).

In the mid-1990-ies we have enough clinical data to publish our early results of survival and disease-free survival for AML and ALL patients. For AML patients allografted in 1st complete remission leukemia-free survival at 5 year period was 68%. The relapse rate for this group of patients was pretty low, 12% at 5 years (41). For ALL patients in 1st complete remission leukemia free survival and probability of leukemia relapse at 3 years was 52% and 20% respectively (42). Our prospective clinical study on GvDH prophylaxis clearly showed that cyclophosphamide in combination with short methotrexate is most effective prophylactic regimen (43).

At the end of the last century SCT program in our Center has become routine and standard therapy for malignant as well as for non-malignant hematological disorders. The problem of transplant at that time was in general the availability of the donors. In order to expand the program we started with unrelated marrow transplant program in the early 90-ies. In our HLA-typing Center, serology due to its inability to identify all specific products of the HLA alleles has been widely replaced with DNA-based typing methods. It has been shown that in order to improve the clinical outcome of SCT from an unrelated donor, it is essential to identify and match patient and donor’s HLA genes at the allele level (44, 45). The MUD program during the last two year have been expanded and now more than 50% of patients receive stem cell from unrelated donor (Figure. 4.). Organization of Croatian Registry known under name "Ana Rukavina" with more than 30,000 volunteer donors is the second very important event that significantly influenced MUD program in Croatia and our Center (45, 46).

As stated before stem cells from cord blood as source for transplantation have been used sporadically. At the beginning transplant with cord blood was reserved for patients with non-hematological disease i.e. aplasia and Fanconi anemia. In 1991 we have been able to perform transplant in a very young child with Philadelphia positive CML with stem cells obtained from cord blood of her sister. That was the first report of cord blood transplantation for patients with Philadelphia positive CML (47, 48). SCT has the potentiality to cure primary immune deficiency syndromes, predominantly severe combined immune deficiency (SCID), which can be corrected by partial reconstitution of normal immune cells. In other words full donor chimerism of the affected cell subset may not be required.
SCT have been also applied for lymphoma treatment, not as the treatment of choice, but mostly for patients in advantage stage of disease (49). In the recent years SCT with RIC conditioning as a second line therapy significantly improved the outcome for patients with SLL/CLL (50).

**SCT and International collaboration**

By using SCT as a standard therapy we have been able to join several international cooperative groups, and to participate in many clinical studies. More than 20 years, Zagreb center is an active member of Leukemia and Lymphoma Group of EORTC. EORTC leukemia group was the first one which in prospective clinical trial analyzed the outcome of SCT as a postremission therapy for patients with AML (51). Patients with HLA-identical sibling achieving complete remission after induction therapy were assigned to undergo allogeneic bone marrow transplantation; the others were randomly assigned to undergo autologous bone marrow transplantation or a second course of intensive chemotherapy.

In AML patients who achieved first complete remission allogeneic as well as autologous SCT results in better disease free survival than intensive consolidation chemotherapy (52). In the subsequent studies, according to intention to treat (53). AML patients younger than 46 years with an HLA-identical sibling donor were assigned to undergo allogeneic (allo) stem cell transplantation (SCT), and patients without such a donor were planned for autologous (auto) SCT.

The DFS rates in patients with and without a sibling donor were similar in patients with good/intermediate risk. Contrary to that in patients with bad/very bad risk cytogenetics DFS for those with donor and without donor were 43.4% and 18.4%, respectively. In younger patients (15–35 years), the difference was more pronounced. The strategy to perform early allo-SCT led to better overall results than auto-SCT, especially for younger patients or those with bad/very bad risk cytogenetics. This data were also proved in AML-12 study (54).

The similar clinical trials have been investigated in high-risk myelodysplastic syndrome (MDS) and secondary AML. The first large EORTC leukemia group prospective study showed the feasibility of both alloSCT and ASCT. This treatment approach leads to a relatively high remission rate, and the majority of patients in remission received the SCT in first complete remission (69). The subsequent studies investigate whether this approach is better than treatment with chemotherapy only. The data was pooled with the other European studies with the intention to investigate the outcome of patients with and without an HLA-identical sibling donor. After a common remission-induction and consolidation course, patients with an HLA-identical sibling donor were scheduled for allogeneic transplantation and patients lacking a donor for autologous transplantation. This analysis showed that patients with high-risk MDS and secondary AML may benefit from both allogeneic and autologous transplantation. We were unable to demonstrate a survival advantage for patients with a donor compared to patients without a donor (56).

Evaluation of older patients, i.e. patients an older 60 years with newly diagnosed MDS/AML and impairments in activities of daily living, Karnofsky Index of <80%, quality of life/fatigue=50 are likely to have poor outcomes, and they are not the candidate for SCT (57). In two EORTC studies ALL-3 and ALL-4, for acute lymphoblastic leukemia SCT was proved as the best antileukemia treatment. Because of high TRM there was no advantage in survival for SCT group compared to chemotherapy (58, 59).

As the member of EBMT we have been participating in many retrospective studies. For many years we have been chairing EBMT group for outreach program. This gave us the opportunity to collect the data from Central and Eastern European countries and to compare the outcome with EBMT data from well developed countries. The published data of transplant in these countries clearly showed that SCT is very effective therapy for patients with acute leukemia, especially for patients transplanted in first CR (60, 61). But transplant rates were substantially lower in eastern European countries for autologous, allogeneic, and unrelated HSCT than in Western Europe. There was a clear correlation between economic factors, measured as gross national income per capita, and transplant rates for low-income countries. There was also a clear correlation between team density (number of teams per 10 million population) and transplant rates. These data document that economic factors can only partly explain the differences in transplant rates between eastern and western European countries (76). Another important factor seems to be the access to the therapeutic procedure(s). These data provide a basis for adequate health care planning.

Another important analysis was done for the Eastern Mediterranean (EM) region (63). In comparison to Europe and North America, differences in patterns of diseases and pre-SCT general status, particularly for patients with BM failure, are described. Other differences including high sero-positivity for CMV, hepatitis B and C infection, and specific observations about GVHD and its relation to genetically homogeneous communities are also evident. A total of 7617 SCTs have been performed by these programs including 5701 allogeneic SCTs. The observed area has low-SCT team density (1.56 teams per 10 000 sq km area vs <1–6 teams in Europe) while gross national income per capita, and transplant rates for low-income countries. There was also a clear correlation between team density (number of teams per 10 million population) and transplant rates. These data document that economic factors can only partly explain the differences in transplant rates between eastern and western European countries (76). Another important factor seems to be the access to the therapeutic procedure(s). These data provide a basis for adequate health care planning.

The only way to substantially improve leukemia therapy is by cooperative research. To optimize research, the European LeukemiaNet (64) integrates 105 national leukemia trial groups and networks, 105 interdisciplinary partner groups and about 1,000 leukemia specialists from 175 institutions. Their ultimate goal is to take care for
The evolving role of stem cell transplantation in lymphoma, Bone Marrow Transplant 15: 460–473.

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FUTURE AND CONCLUSIONS

During the 30 years period SCT has been established as a standard and routine therapy at University Hospital Center Zagreb for many malignant and non-malignant hematological disorders. Transplant has significant influence on the quality level and the development of many clinical diagnostic and treatment facilities. In the future by in vitro manipulation and selection of stem cells one might expect further development of SCT in terms of its safety and efficacy. In our hospital we are able to isolate and cultivate bone marrow derived mesenchymal stem cells (MSCs), multipotent nonhematopoietic cells or bone marrow stromal cells (65).

Clinical interest for MSCs was initiated by the observation that MSCs are immuno-privileged cells that display immuno-modulatory properties in vitro. Ex vivo expanded MSCs have therefore become a new type of cellular therapy in development with a wide range of potential clinical applications. Infused MSCs suppress graft versus host disease, support engraftment of transplanted allogeneic hematopoietic stem cells and stimulate growth in patients with osteogenesis imperfecta. Although underlying immuno-modulatory mechanisms of action are not completely understood, potential benefit of MSC therapy justifies its clinical use in a broad range of disorders. They have capacity for self-renewal in a number of non-hematopoetic tissues. Moreover MSCs display a significant capacity for decreasing inflammation, modulating immune responses and protecting tissue from injury, including stroke and anoxic damage, stemmed from the assumption that stem cells differentiate and replace dead cells representing the basis for a new discipline, regenerative medicine. This new multidisciplinary field intended to repair, replace and regenerate cells tissue as well as organs for restoring or establishing normal function. The basic idea behind regenerative medicine is to cure human ailments by replacing failed body organs and damaged tissues with laboratory-grown cells and organs. Regenerative medicine employs small molecule drugs, medical devices, cell-based therapies and biologics. In a broader sense, the term includes advanced cell-based therapies, tissue engineering, stem cells and developmental biology, cellular therapeutics, biomaterials and gene therapy.

REFERENCES

1. GRATWOHL A, BALDOMERO H, FRAUENDORFER K, URBAN-ISPIZUA A; Joint Accreditation Committee, International Society for Cellular Therapy; European Group for Blood and Marrow Transplantation 2006 EBM7 activity survey 2004 and changes in disease indication over the past 15 years. Bone Marrow Transplant 37: 1069–85

2. BACKGALUPO A 2008 Treatment strategies for patients with severe aplastic anemia. Bone Marrow Transplant 42 (Suppl 1): S42–S44


4. JANTUNEN E, SUREDA A 2012 The evolving role of stem cell transplants in lymphomas, Blood 117: 660–73


Allogeneic compared with autologous stem cell transplantation in acute myeloid leukemia (AML) treated with intensive chemotherapy followed by donor buffy-coat infusion and chemotherapy for leukemia in relapse after marrow transplantation. Haematologica 89: 338–40

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