Hematologic Aspects of Myeloablative Therapy and Bone Marrow Transplantation

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The transplantation of bone marrow cells or isolated hematopoietic stem cells from the bone marrow or peripheral blood is a widely utilized form of therapy for patients with incurable diseases of the hematopoietic and immune systems. Successful engraftment of the transplanted stem cells in an adequately prepared recipient normally leads to bone marrow reconstitution over a period of several weeks, accompanied by more gradual reconstitution of the immune system. Since the recipient is profoundly ill during the initial treatment period, laboratory data is critical for monitoring engraftment, detecting residual/recurrent disease, and identifying problems that may delay bone

marrow reconstitution or lead to other medical complications. Accurate blood cell counts are imperative, and most bone marrow transplantation patients undergo periodic monitoring with bone marrow aspirates and biopsies with cytogenetic, molecular, and multiparametric flow cytometric studies. The potential complications of bone marrow transplantation include engraftment failure and delayed engraftment, infection, residual bone marrow disease, acute and chronic graft versus host disease, myelofibrosis, therapy-related acute leukemia, post-transplant lymphoproliferative disorders, and toxic myelopathy. *J. Clin. Lab. Anal.* 19:47–79, 2005.

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INTRODUCTION

Bone marrow transplantation is the infusion of hematopoietic stem cells into a patient with the intent of curing a systemic malignancy, inherited metabolic disease, or potentially fatal disease of the hematopoietic or immune systems. The term “bone marrow transplantation” is strictly used when the bone marrow is the source of the stem cells, while “stem cell transplantation” is the proper term for the transfusion of stem cells isolated from the peripheral blood. In this article, bone marrow transplantation (BMTx) will be used as a general term for both types of transplantation. Bone marrow transplantation is a curative, not a palliative, therapy performed in patients whose chance for cure is very small or negligible with “standard” techniques of chemotherapy and radiotherapy. Presently, bone marrow transplantation has been performed in more than 12,000 patients in the United States, at a steadily increasing rate of >3,000 patients/year by more than 180 active teams (1–4).

The majority of patients undergoing bone marrow transplantation receive high-dose chemotherapy or high-dose chemotherapy with total body radiation prior to the stem cell infusion to eradicate the immune and hematopoietic systems as well as all malignant cells within the body. “Myeloablative therapy” is also used as a treatment for many types of malignancy in which bone marrow transplantation has not been shown to be beneficial. Regeneration of a new bone marrow (“bone marrow reconstitution”) occurs during recovery from

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myeloablative therapy, either from stem cell progenitors or, less often, from residual host progenitors. The patient is critically ill during this period of time, and totally dependent upon excellent clinical care and accurate laboratory analysis. The hematology laboratory is especially important in the care of these patients, and provides routine cell counts as well as more sophisticated assays, including bone marrow evaluation, reticulocyte analysis, assessment of graft quality and bone marrow reconstitution, and other assays. This article addresses the basic principles of bone marrow transplantation and myeloablative therapy, the morphologic interpretation of the bone marrow aspirate and biopsy during bone marrow reconstitution, and the utilization of routine and special hematologic assays in the care of these patients.

FUNDAMENTAL PRINCIPLES OF MYELOABLATIVE THERAPY AND BONE MARROW TRANSPLANTATION

Isolated case reports of unsuccessful bone marrow transplants were reported in the late 1930s and early 1940s. Following the use of the atomic bomb in World War II, research into the effects of irradiation on the body showed a protective effect of spleen shielding or bone marrow infusions in lethally irradiated mice, and later experiments revealed that reconstitution of the bone marrow in animals was due to the engraftment of donor cells (5). Because of these discoveries, bone marrow transplantation was successfully utilized in the late 1950s and 1960s by Thomas (5) and others to treat leukemia patients who failed conventional therapy and had an identical twin. However, bone marrow transplantation did not become a routine, cost-effective form of therapy until an understanding of the major histocompatibility complex (MHC) and the development of effective techniques of tissue matching, immunosuppressive therapy, and supportive care for patients without bone marrow function (5).

BMTx is often compared to solid organ transplantation, but it is unique in several ways. Since bone marrow is a liquid "organ," the problems of compatible organ size, biliary and ureteral obstruction, and other surgical problems are not encountered. Since bone marrow is rapidly replenished by normal individuals, cadaver organs are not required, and the living donor sustains no permanent organ insufficiency. Patients can even supply their own bone marrow for later transfusion (autologous BMTx). However, transplant recipients receiving a marrow donation from another individual (allogeneic BMTx) face the problem of graft rejection, as well as a problem seldom encountered in solid organ transplantation, that of rejection of the host by the graft (graft-vs.-host-disease [GVHD]). In addition, bone

marrow transplant recipients are profoundly immunosuppressed until bone marrow reconstitution occurs, and they are extremely susceptible to opportunistic infections and other problems during this period. Therefore, BMTx is a very expensive undertaking with a significant morbidity and mortality rate. Consequently, the indications for this procedure are limited and potential recipients undergo a thorough screening process.

Indications for Bone Marrow Transplantation

The present indications for bone marrow transplantation include: (6)

- Rescue for high-dose chemoradiotherapy given for treatment of malignancy
- Replace a diseased hematopoietic or immune system
- Deliver anticancer immunotherapy
- Effect therapy for defective genes

The rationale for bone marrow transplantation in patients with bone marrow failure, malignancies, and congenital hematopoietic and immunodeficiency states is to supply normal stem cells for marrow repopulation after obliteration of the diseased marrow. An immunotherapeutic effect (i.e., graft-vs.-disease) also arises during allogeneic bone marrow transplantation from the action of functional donor immune cells in immunosuppressed host. BMTx is effective in the inherited metabolic diseases because of repopulation of the reticuloendothelial cell system and osteoclasts with donor-derived cells.

Specific diseases for which bone marrow transplantation is currently performed are listed in Table 1.

Stem cells for bone marrow transplantation are obtained from the bone marrow, peripheral blood, or umbilical cord blood of a newborn infant (7–9).

Obtaining Bone Marrow for Transplantation

Bone marrow for transplantation is obtained ("harvested") by multiple aspirations of the iliac crest over 2–3 hours, with the donor under general or spinal anesthesia (Fig. 1). Approximately $10\text{--}40 \times 10^9$ nucleated cells ($2 \times 10^8/\text{kg}$ of recipient weight), up to a maximum of 20 mL/kg of donor weight, are obtained during this procedure. The marrow aspirate primarily consists of stromal cells and erythroid, myeloid, monocytic, megakaryocytic, and lymphoid cell lines in various stages of development. However, undifferentiated stem cells are essential for long-term hematopoiesis for the recipient, while the early committed progenitors provide the earliest peripheral cells after engraftment and T lymphocytes are necessary for engraftment in the allogeneic setting. Particulate material in the marrow is removed by filtration. For ABO blood group incompatibility, plasmapheresis may be

TABLE 1. Indications for Bone Marrow Transplantation

Type of disease	Specific diseases
Primary and secondary bone marrow failure	Aplastic anemia Amegakaryocytosis/congenital thrombocytopenia Fanconi anemia Paroxysmal nocturnal hemoglobinuria (PNH)
Hematopoietic malignancies	Acute leukemias Chronic leukemias Myelodysplastic syndromes Myeloproliferative disorders Non-Hodgkin lymphoma Plasma cell diseases Histiocytic disorders
Solid tumors	Breast cancer Renal cell carcinoma Neuroblastoma Ewing sarcoma Testicular carcinoma
Congenital hematopoietic and immunodeficiency states	Immunodeficiencies (severe combined immunodeficiency, etc.) Granulocyte deficiencies (chronic granulomatous disease, etc.) Hemoglobinopathies (beta-thalassemia major, sickle cell disease)
Inherited metabolic disorders	Mucopolysaccharidoses Hurler's syndrome Hunter's syndrome Niemann-Pick disease Mucopolipidosis II
Other inherited diseases	Osteopetrosis Glanzmann thrombasthenia Lesch-Nyhan syndrome Cartilage-hair hypoplasia

required to remove isohemagglutinins, while differential centrifugation can be utilized to remove incompatible erythrocytes. Special processing ("purging") may also be performed to reduce the marrow burden of tumor cells, T lymphocytes, or other specific components that may have a deleterious effect on the recipient. After processing, the marrow cells are immediately given to the recipient via intravenous infusion or are frozen and stored for months to years for later transfusion.

Stem cells obtained from the peripheral blood are increasingly being used for BMTx. Hematopoietic growth factors are first given to the donor to stimulate stem cell production and mobilization from the bone marrow (8,10). A leukapheresis machine is used to isolate the donor's buffy coat, which contains the stem cells (11,12). Umbilical cord blood is another large, convenient source of stem cells for bone marrow transplantation (7).

Autologous bone marrow transplantation is performed with the donor's own bone marrow or peripheral

blood stem cells (Fig. 2). This procedure is often used in patients with hematopoietic malignancies, since it avoids both the difficult process of finding a compatible donor and immunologic problems caused from the infusion of nonself cells. Autologous BMTx is performed by harvesting the marrow prior to therapy, isolating and cryopreservation of stem cells and early hematopoietic precursors, and reinfusing the cells following marrow ablation (13). It may also be necessary to purge malignant cells from the harvest using anti-CD34 specific monoclonal antibodies to isolate the stem cells ("positive selection") or antitumor monoclonal antibodies to remove the malignant cells ("negative selection"). Autologous bone marrow transplantation has the disadvantage of a relatively high rate of posttransplantation disease relapse, either from transplanting small numbers of viable tumor cells or bypassing the beneficial graft-vs.-disease effect of allogeneic bone marrow transplantation.

Allogeneic bone marrow transplantation is performed with stem cells from another individual (Fig. 2) (10,14,15). Careful histocompatibility matching is required for this form of bone marrow transplantation, since the transplant will otherwise be rejected. The "perfect match" is an identical twin ("syngeneic transplant"); however, approximately 25% of siblings are HLA-identical and serve as excellent donors. However, many patients must rely on a matched unrelated donor (MUD). Since millions of individuals around the world have been typed as potential bone marrow donors, there is theoretically a 70% chance of finding a human leukocyte antigen (HLA) identical individual. However, in practice, the chance is only about 10% because the donor may not be available or have health problems that preclude the donation. The serious underrepresentation of non-Caucasian ethnic groups in the donor pool is also a major problem for non-Caucasian patients in view of the ethnic distribution of HLA antigens.

Ex vivo T-cell depletion (TCD) of the allograft is often used to reduce the risk of potentially severe graft-vs.-host disease. However, the increased incidence of graft failure, graft rejection, and disease relapse in patients receiving TCD bone marrow transplants suggests that some T cells are necessary for successful engraftment and elimination of therapy-resistant tumor cells (16).

Proper conditioning of the recipient prior to bone marrow transplantation can increase the chance of engraftment and decrease the chance of relapse. The conditioning regimen used to prepare patients for bone marrow transplantation is determined by the specific disease being treated and by any previous therapy (17). Good vascular access is essential, as well as proper nutritional support. Allogeneic transplantation

Fig. 1. Bone marrow harvest. **A:** Bone marrow aspiration from the posterior pelvis with a 30 mL syringe. **B:** Freshly aspirated bone marrow is filtered to remove particulate matter.

can be performed without immunosuppression in children with severe combined immunodeficiency syndrome (SCID). However, preconditioning with immunosuppression and/or radiation to prevent rejection is required in aplastic anemia, while patients with lymphohematopoietic malignancies may require both immunosuppression and intensive chemoradiotherapy to eradicate all normal and malignant hematopoietic and immune elements.

Successful engraftment is heralded in 2–3 weeks by the appearance of the progeny of donor stem cells in the circulation of the recipient. Cytogenetic techniques, isoenzyme studies, restriction fragment length polymorphisms (RFLP), or other procedures that can differentiate between donor and host cells are required to provide definitive proof of engraftment. In some patients, both host and donor stem cells may coexist, resulting in a state of “mixed chimerism.”

Care of the Bone Marrow Transplant Patient

The bone marrow recipient is profoundly immunodeficient and pancytopenic during the posttransfusion period. Recent improvements in the early survival of bone marrow patients have primarily resulted from advances in supportive care during this period (18). Blood and platelet transfusions are required for several

weeks, together with a protective environment, hyperalimentation, and prophylactic antibiotics. To prevent HLA sensitization or inadvertent engraftment with resulting GVHD, washed cells and irradiated blood products must be given to all prospective recipients. Platelet transfusion is a major problem, since the majority of patients (>90%) will become alloimmunized from random platelet transfusions, and refractory to further transfusions within 4–6 weeks. However, bacterial (*E. coli*, *Pseudomonas*), viral (herpes simplex, varicella zoster, adenovirus), and fungal (*Candida*, *Aspergillus*, *Nocardia*, *Pneumocystis*) infections are common and constitute the most serious posttransplantation problem (19). The recipient may have an increased risk of infection for up to 2 or 3 years, since immune functions usually take this long to return to normal. The highest mortality (85%) occurs with the interstitial pneumonias, particularly cytomegalovirus (CMV) (20,21). Frequent blood, urine, throat, nasopharyngeal, and stool cultures aid in the early diagnosis of infection, as well as serial serologic assays (particularly for CMV, herpes simplex virus (HSV), and herpes zoster). Bronchial washings and transbronchial lung biopsies may be performed for the diagnosis of *Pneumocystis*, CMV, and *Aspergillus*. The recent availability of monoclonal antibodies and DNA probes for many infectious agents is being greatly welcomed in this field.

Fig. 2. Types of bone marrow transplantation. **A:** Autologous bone marrow transplantation. **B:** Allogeneic bone marrow transplantation. **C:** Nonmyeloablative bone marrow transplantation. See text for detailed description.

The development of graft-vs.-host disease may further delay immune reconstitution and increase the risk of infectious complications. Other complications of BMTx include venoocclusive disease of the liver, cholecystitis, gastrointestinal bleeding, small bowel obstruction, pneumatosis intestinalis, necrotizing enteritis, and late complications such as acute myocardial infarction, secondary malignancies, clinical hypothyroidism, cataracts, neurologic toxic effects, and infertility/impaired sexual maturity (22–25).

Nonmyeloablative Bone Marrow Transplantation

Allogeneic stem cell transplantation after nonmyeloablative conditioning (“minitransplantation”) is the latest innovation in the field (Fig. 2). In this form of transplantation, chemotherapy and/or total body irradiation is first administered to eliminate the recipient’s bone marrow and to suppress their immune system, followed by the infusion of allogeneic bone marrow or stem cells (26–28). The engraftment of donor cells and the development of a chimeric state provides an excellent environment for the “graft vs. malignancy effect” (29,30). An infusion of donor lymphocytes “donor lymphocyte infusion” (DLI), is usually given later to stimulate the graft vs. malignancy effect and to reverse a potentially unstable mixed chimerism state (31–37).

The low mortality of nonmyeloablative transplantation permits its utilization in older or medically infirm patients who cannot tolerate conventional conditioning regimens. Patients receiving nonmyeloablative conditioning also incur less severe hematologic toxicity, require few (if any) erythrocyte and platelet transfusions, develop less severe gastrointestinal toxicity, and require less intensive supportive care measures compared to conventional myeloablative allogeneic transplants. Nonmyeloablative transplantation is under clinical evaluation for the treatment of nonhematopoietic chemorefractory disease, congenital immunodeficiency disease, sickle cell hemoglobinopathy, autoimmune diseases, and other illnesses, as well as hematologic malignancies. Many centers are able to perform nonmyeloablative transplants on an outpatient basis, and this technique is being examined in the matched unrelated and cord blood stem cell transplant setting. However, graft-vs.-host disease, disease relapse, and CMV infection remain major clinical challenges for nonmyeloablative stem cell transplantation.

HEMATOPOIETIC RECONSTITUTION AND BONE MARROW PATHOLOGY

Direct evaluation of the bone marrow plays a crucial role in patients undergoing marrow transplantation.

In addition to documenting the efficacy of marrow engraftment, bone marrow aspiration and biopsy is necessary to investigate posttransplant problems, such as delayed recovery, infection, or suspected disease relapse. Special diagnostic procedures, such as microbiologic culture, immunophenotypic analysis, and cytogenetic evaluation may also be required in selected patients.

During evaluation of the posttransplant bone marrow specimen, the pathologist must review other crucial information and specimens. These include:

- Pretransplant pathology specimens
- Pretransplant laboratory data, including ancillary data (immunophenotypic analysis, cytochemical stains, cytogenetic analysis, etc.)
- Date and type of marrow transplantation
- Clinical laboratory data
- Posttransplantation clinical course
- Types and doses of cytokines and other drugs
- Reason for bone marrow evaluation

Marrow examination should be goal-oriented, directed at answering the clinician’s questions, and solving important clinical problems. Therefore, communication with the requesting physician is crucial for optimal patient care.

Pretransplantation Bone Marrow Evaluation

The recipient’s bone marrow should be carefully examined prior to transplantation to confirm the clinical diagnosis and to determine the presence of pathology that may affect the efficacy or course of transplantation or the type of pretransplant conditioning regimen. In patients with leukemia, the presence of remission or relapse should be confirmed, and immunophenotypic analysis or cytochemical staining should be performed if necessary. Patients with lymphoma or solid tumors should undergo restaging with technically adequate, bilateral bone marrow specimens prior to transplantation to establish the most effective chemoradiotherapeutic protocol. Leukemia, myelodysplastic syndromes, and other diseases should be excluded in patients with severe pancytopenia and/or a history of aplastic anemia, by a thorough examination of adequate bone marrow aspirate and biopsy specimens.

Special consideration must be given to potential bone marrow transplant patients with bone marrow fibrosis, mast cell infiltration, and lymphoplasmacytic infiltrates. Diseases most commonly associated with bone marrow fibrosis include the myelodysplastic syndromes, acute and chronic myelofibrosis, chronic myelogenous leukemia (CML), acute leukemias, post-radiation therapy, and/or chemotherapy effect. Unless

severe, bone marrow fibrosis does not usually interfere with engraftment, and usually disappears during pretransplant conditioning. Severe fibrosis, with destruction of the marrow stromal architecture, may be a contraindication to marrow transplantation. However, allogeneic bone marrow transplantation has been shown to be the only curative option in primary myelofibrosis. Increased mast cells in the pretransplant marrow have been reported to be associated with an increased risk of graft rejection or failure to engraft in patients with aplastic anemia (38). Lymphoplasmacytic infiltrates are a special problem in patients with lymphoproliferative diseases, and should be carefully evaluated for morphologic features that may indicate marrow metastasis. Immunostaining, flow cytometry, and molecular analysis can greatly assist in the interpretation of these lesions, especially in patients with lymphomas of B-cell lineage. Aplastic anemia patients with lymphoplasmacytic marrow infiltrates have been shown in some studies to respond to immunosuppressive therapy with antithymocyte globulin (ATG), which may offer an alternative approach to BMTx.

Recent studies in the United States and Europe suggest that the attainment of a complete remission (CR) prior to transplantation is associated with improved disease-free and overall survival, making marrow evaluation in multiple myeloma, lymphomas, and leukemias essential in the timing and prognosis of transplantation therapy.

Morphologic Effects of Myeloablative Therapy

The morphologic features of the bone marrow in a patient receiving myeloablative therapy are determined by the overlapping processes of cell death and hematopoietic reconstitution (39). Aggressive chemotherapy alone, or successful conditioning for bone marrow transplantation with total body irradiation and aggressive chemotherapy, results in the obliteration of nearly all of the hematopoietic and immune cells over a period of several days. At the end of this period, the bone marrow is profoundly hypocellular, with an intact stroma containing a homogenous, periodic acid Schiff (PAS)-positive proteinaceous transudate resembling fibrinoid necrosis. A few residual plasma cells and macrophages are usually present, and vascular congestion, areas of nonspecific hemorrhage, small noncaseating granulomas, stromal edema, eosinophilia, mild reticulin fibrosis, sinus ectasia, osteocyte necrosis, and other anomalies may be found (Fig. 3) (39–44). Conventional chemotherapy without stem cell transplantation results in a variable degree of bone marrow suppression determined by host factors and whether the therapy is curative or palliative (39). Nonmyeloablative

or reduced intensity transplant preparative regimens demonstrate similar morphologic features, although their severity is dependent on the intensity of the radiochemotherapy regimen.

Several years are required to fully restore the hematopoietic and immune systems to a healthy state because of the toxicity of agents used in preparing the marrow for transplantation. The myeloablative and conditioning regimens that purge malignant progenitors from the bone marrow may also remove or damage nonmalignant hematopoietic and stromal progenitor cells, resulting in a diminished capacity for transplanted and native stem cell renewal (45). This deficit is not always apparent from examination of a posttransplant peripheral smear or marrow biopsy. A severe prolonged deficiency of erythroid and megakaryocyte marrow progenitors may persist for many years after bone marrow transplantation, even though peripheral blood cell counts and marrow cellularity may reach pretransplant levels. Colony forming units-fibroblasts (CFU-f), the precursor stromal compartment for cells of the osteogenic lineage, are critical to hematopoietic cell survival, proliferation, and differentiation. CFU-f reconstitution may take as long as 12 years to reach pretransplant numbers and is solely of host origin (46).

Morphologic Features of Bone Marrow Reconstitution

The immediate postchemotherapy or posttransplant period is usually followed by 1 to 2 weeks of marked marrow aplasia. Fat cell regeneration provides the first morphologic evidence of bone marrow regeneration, followed at day 6 to day 14 by the appearance of minute clusters of immature, monotypic hematopoietic cells that gradually mature and enlarge (Fig. 4; Table 2) (39,41,47). These colonies are comprised of cells of a single hematopoietic lineage (“monophyletic”), usually myeloid or erythroid, and presumably arise from committed stem cells in the bone marrow transplant patient (38,43). The regenerating colonies tend to be paratrabecular in patients receiving myeloablative therapy only and interstitial following stem cell transplantation (39). Very early posttransplant hematopoiesis is usually polyclonal but may be monoclonal (48). Early erythropoietic islands are usually dominated by large basophilic normoblasts that may exhibit dyserythropoietic features (41). As hematopoietic reconstitution continues, the distribution of hematopoietic cells in the bone marrow is often atypical, such that clusters of myeloid precursors are abnormally localized in the intratrabecular areas and erythroid precursors occur near the endosteum (43). Megakaryocytes are normally the last to engraft. They are usually localized in the

Fig. 3. Effect of chemotherapy and bone marrow reconstitution on bone marrow histopathology. Left: bone marrow aspirates (Wright-Giemsa stain, $1,000\times$). Right: bone marrow biopsies (H&E stain, $400\times$). **A:** Acute myelogenous leukemia, pretreatment. Blast cells comprise $>90\%$ of bone marrow cellularity. **B:** Status: postchemotherapy, day 14. There is a marked reduction in cellularity, with scattered residual cells consisting primarily of macrophages, plasma cells, and lymphocytes. **C:** Status: postchemotherapy, day 28. Normal trilineage hematopoiesis has been restored, with normal bone marrow cellularity for the age of the patient.

central part of the intertrabecular areas, and may appear in clusters, rather than in their normal scattered distribution (43). Macrophages, pseudo-Gaucher cells, and sheets of regenerating promyelocytes may also appear (39,49). Macrophages are believed to have a functional role in erythropoietic regeneration, and there

is a significant correlation between the numbers of activated macrophages and normoblasts (49,50). In acute myeloid leukemia (AML) patients, regenerating promyelocytes can be mistaken for recurrent disease, but usually show brisk mitotic activity and a prominent paranuclear Golgi zone in contrast to their neoplastic

Fig. 4. Histopathology of early bone marrow reconstitution, status post-bone marrow transplantation. **A:** Virtually acellular bone marrow status post-bone marrow transplantation for acute leukemia, day 14 (H&E stain, 200 $\times$). **B:** Colonies of early regenerating bone marrow cells, day 28 (H&E stain, 200 $\times$). **C:** Close-up of regenerating bone marrow cells showing early myeloid and erythroid cells (H&E stain, 400 $\times$).

TABLE 2. Histopathologic features of myeloablation and bone marrow reconstitution

Time period	Histopathologic features
Day 0–7	Cell death and necrosis Fibrinoid necrosis with homogenous, PAS+ proteinaceous transudate Hemosiderin-laden macrophages Edema and focal hemorrhage vascular congestion and sinus ectasia Transient reticulin fibrosis Small noncaseating granulomas Osteocyte necrosis Adipocyte proliferation
Day 7–14	Small foci of immature hematopoietic cells Frequent hemosiderin-laden macrophages
Day 14–28	Expansion of hematopoietic islands with complete myeloid and erythroid maturation Resolution of fibrosis and fibrinoid necrosis Megakaryocyte regeneration

counterparts (39). In some AML patients, therapy may be complicated by persistent myelodyspoietic features, rebound blastosis, bone marrow necrosis, or the abrupt development of hyperleukocytosis (39).

The gradual return to normal bone marrow cellularity is accompanied by resolution of the edema, reticulin fibrosis, and fibrinoid necrosis (39). The marrow should be approximately 50% normocellular by the third posttransplantation week and normocellular by 8 to 12 weeks (Fig. 5).

Patients with acute promyelocytic leukemia (AML, FAB-M3) are usually treated with all-transretinoic acid (ATRA) followed by conventional anthracycline-containing chemotherapy. Since ATRA induces maturation of the neoplastic cells, rather than their destruction, morphologic remission can often be achieved without significant bone marrow hypoplasia (51,52).

The time course of the progression from early hematopoiesis to normal marrow cellularity for age is extremely variable; some patients may achieve normal cellularity in as little as 14 days, while others require several months. However, 28 days is typical (42). The kinetics of engraftment depend on the source of donor cells (i.e., peripheral blood stem cells, cord blood, bone marrow), the dose of infused CD34⁺ cells, the type and dose of exogenous hematopoietic growth factors (i.e., G-CSF, rhGM-CSF, erythropoietin), and HLA cross-matching (53). The rate of marrow recovery is affected by the homing efficiency and clonogenic potential of the transplanted cells and whether the infused cells were expanded in vitro prior to infusion (54).

The effects of granulocyte colony stimulating factor (G-CSF) or granulocyte-macrophage colony stimulating factor (GM-CSF) are particularly evident

Fig. 5. Schematic illustration of serial morphologic changes during bone marrow reconstitution.

in reconstituting bone marrow and peripheral blood and must be differentiated from other causes. In the peripheral blood, these agents cause an increase in the total white blood cell count and the absolute number of neutrophils, monocytes, and eosinophils. Their effects on the bone marrow include eosinophilic hyperplasia and increases in cellularity and the myeloid:erythroid (M:E) ratio. In addition, prominently granulated and/or vacuolated neutrophils and neutrophilic precursors appear in both the peripheral blood and bone marrow (55).

Clinical Pathology of Bone Marrow Transplantation

Bone marrow pathology should be correlated with peripheral blood counts, peripheral smear examination, reticulocyte count, and other data. Adequate bone marrow cellularity with leukopenia and/or thrombocytopenia may indicate peripheral cell utilization or destruction, particularly if the immature reticulocyte fraction is increased and giant/large platelets are seen. An immune cause for pancytopenia should be suspected once infection and other causes have been excluded. The correlation of marrow and peripheral blood findings is especially crucial in leukemic patients. Serial bone marrow evaluation is often necessary in the early post-BMTx period to differentiate between leukemia disease relapse and hematopoietic reconstitution, particularly in patients receiving high doses of cytokines. In addition,

other clinical laboratory procedures are often performed to assure graft quality, monitor the progress of bone marrow reconstitution, confirm engraftment, and evaluate special clinical problems that may develop.

The identification of molecular markers of disease, such as aberrant antigens or specific chromosomal deletions, permits the early identification of relapse and disease recurrence. The analysis of bone marrow and peripheral blood by immunophenotypic analysis, fluorescence in situ hybridization (FISH), polymerase chain reaction (PCR), and standard cytogenetic analysis has become standard in posttransplantation monitoring.

Monitoring Graft Quality

The efficacy of hematopoietic recovery, the incidence of posttransplant infection, and overall survival patient survival are dependent upon infusion of an adequate number of CD34⁺ cells (i.e., $2-5 \times 10^6/\text{kg}$ of body weight), as well as an appropriate functional bone marrow microenvironment (56–58). Therefore, there is great interest in standardized procedures to quantitate the number of hematopoietic progenitor cells in a bone marrow harvest, estimate the potential marrow-repopulating ability, calculate the dose of a harvest needed for hematopoietic reconstitution, and follow the process of bone marrow reconstitution. The quantitation of CD3⁺ cells in the final graft product is also necessary to assess the adequacy of T cell depletion, while B cell enumeration is also performed if B cell depletion is used as a

Fig. 6. Schematic illustration of CFU-GM colony assay.

measure to prevent Epstein-Barr virus (EBV)-associated posttransplant lymphoproliferative disease (16).

The granulocyte-macrophage colony forming cell (myeloid progenitor cells, CFU-GM) colony assay is the reference method for stem cell enumeration. This assay provides an indirect measure of the concentration of marrow-repopulating pluripotent stem cells through the detection of the committed progenitor CFU-GM (59,60). In the CFU-GM colony assay, cells are cultured at 37°C in a medium containing fetal calf serum (FCS),

methylcellulose, 2-mercaptoethanol, growth factors, and other components for 1 to 2 weeks in a humidified 5% CO₂ atmosphere (Fig. 6). Since each hematopoietic precursor forms a distinct colony, the number of colonies is determined and appropriate dilution factors are applied for the initial cell suspension. With this assay, various investigators have found that a transplant threshold dose of $>2.5 \times 10^6$ CD34⁺ cells/kg or $>8.0 \times 10^4$ CFU-GM/kg (61–63). Although these cells are present at a very low concentration (approximately

1,000 cells/mL of whole blood, 2–3/1,000 nucleated marrow cells) in the normal steady state situation, their numbers greatly increase during recovery from chemotherapy-induced pancytopenia, particularly in patients receiving human hematopoietic growth factors (60). However, measurement of the outburst of these cells with the CFU-GM colony assay is not practical in a clinical setting, since 2 weeks are required for the completion of the assay, and its complex nature precludes interlaboratory standardization. Unfortunately, this CFU-GM colony assay is also technically difficult and time consuming to perform, poorly standardized, and provides only retrospective information. Under these circumstances, flow cytometric analysis of the CD34 antigen is usually used for the quantitation of hemopoietic stem cells. Surrogate measurements of viability, such as the measurement of methylene blue dye exclusion are technically simple, but are less sensitive and specific than the CFU assay.

The discovery of the CD34 antigen by Civin et al. (64,65) in the mid-1980s led to a practical means of real-time enumeration of stem cells. The CD34 antigen is a highly acidic, single-chain transmembrane sialoglycoprotein produced by a gene mapped to human chromosome 1 and expressed on hematopoietic precursor cells and capillary endothelial cells (66,67). Functionally, the CD34 molecule serves as a phosphorylation target for activated protein kinase C and may be involved in signal transduction during early lymphohematopoiesis (66). Flow cytometric enumeration of CD34⁺ cells is used for the detection of the progenitor cell outburst, establishing the optimal time for stem cell harvest, and determining the number of leukapheresis procedures that should be carried out (Fig. 7) (68,69). In addition, flow cytometric analysis of CD3⁺, CD33⁺, and CD34⁺ cells has been used to follow immune reconstitution in patients receiving autologous or allogeneic stem cell transplants.

The technique developed by Seina and collaborators in the early 1990s and subsequently known as the Milan method was the first widely used procedure for flow cytometric hematopoietic stem cell (HSC) enumeration (16,69). The original Milan method uses direct immunofluorescence staining of peripheral blood or bone marrow with fluorescein isothiocyanate (FITC)-labeled anti-CD34 with erythrocyte lysis (Fig. 7). At least 10,000 events are acquired during flow cytometric analysis and displayed as a two-dimensional histogram of side scatter (X-axis) vs. CD34 fluorescence intensity (Y-axis). CD34⁺ cells appear as a discrete cluster showing low side-scatter and bright FITC (CD34) expression (16). Although the utilization of phycoerythrin (PE)-labeled anti-CD34 and the acquisition of 50,000 events was shown to improve the accuracy of the Milan method, poor interlaboratory reproducibility remained a pro-

Fig. 7. The Milan method for the determination of CD34 percentage in a well-mobilized patient. A two-dimensional plot of CD34 vs. side scatter is constructed and CD34⁺ cells are defined by region 1.

blem (16). In the mid-1990s, the International Society of Hemotherapy and Graft Engineering (ISHAGE) adopted a new method developed by Sutherland that used multicolor labeling (CD34-PE and CD45-PerCP) and sequential gating to improve accuracy and reproducibility (16) (Fig. 8). Commercial kits using standardized microspheres for absolute counting are presently available with analytic software and prepackaged standardized reagents to perform semiautomated enumeration of relative and absolute CD34⁺ counts. The number of infused CD34⁺ cells required for adequate hematopoietic reconstitution is a matter of continuing study, but there is evidence that a cell dose of $>5 \times 10^6$ CD34⁺ cells/kg is associated with rapid engraftment and a reduced risk of graft failure and infectious complications (61,70,71).

Monitoring Bone Marrow Reconstitution

A period of weeks is required for the restoration of normal levels of red blood cells, platelets, and granulocytes after myeloablative therapy, with or without stem cell rescue, while complete functional reconstitution of the hematopoietic and immune systems takes place over a period of years. The peripheral blood white blood cell count usually reaches a nadir by day 8, followed by a gradually increasing absolute granulocyte count, which may reach 2,000/ μ L by day 20, and normal levels by day 28 (41).

The early prediction of bone marrow engraftment is essential for the efficient utilization of blood products, as well as identifying patients with delayed or failed

Fig. 8. The ISHAGE method for the determination of CD34 percentage from the same listmode data as for Fig. 6. **A:** Note the presence of counting microspheres at the right of the plot. CD45⁺ cells are defined by region 1 in A. **B:** The cells defined by region 1 are displayed, and the CD45⁺ total PE⁺ population is further defined by region 2. **C:** Plot is displayed as a sum of regions 1 and 2 and is used to further define a CD34⁺CD45⁺ low SSC population by region 3. **D:** The final plot is employed as a sum of region 1, 2, and 3, and defines the FSC and SSC characteristics of hematopoietic stem cells. Note that the percentage is increased over that shown in the Milan method, as debris is excluded.

bone marrow reconstitution. Among the many laboratory parameters studied, present interest has centered on the reticulocyte, since red blood cell regeneration precedes white blood cell and platelet regeneration by several days. Although conventional light microscopy-based reticulocyte enumeration does not provide the accuracy or sensitivity needed for this application, modern flow cytometric-based automated hematology analyzers provide both an accurate total reticulocyte count and permit delineation of the population of the immature reticulocytes that first appear in the peripheral blood during early engraftment. In this technique, peripheral blood is stained with an RNA-specific fluorescent dye that binds to the RNA found in reticulocytes but not in mature erythrocytes. The relative amount of cellular RNA, and the intensity of the fluorescence signal, correlates with the age of the

reticulocyte, because “young” reticulocytes possess the most RNA. During chemotherapy induction, the reticulocyte count reaches an extreme nadir and consists only of cells with a low RNA content. The early reticulocyte fraction (highly fluorescent reticulocytes [HFR]) normally begins to recover approximately 2 weeks postchemotherapy, followed by granulocytes several days later (72).

Many studies of patients have found the mean fluorescence of the reticulocyte fraction (RMI), the HFR, and other parameters to be significant predictors of bone marrow engraftment (73–77). Using combined data from serial platelet, neutrophil, and reticulocyte counts, Lazarus et al. (78) determined that the mean day of recovery in bone marrow transplantation patients was day 12.1. The failure of the mean reticulocyte volume (MRV) or reticulocyte mean fluorescence

intensity (MFI) values to increase early after bone marrow transplantation may indicate primary graft failure and identify a group of patients who might benefit from early rescue (79). Furthermore, in patients mobilized with chemotherapy and growth factors for peripheral blood stem cell harvest, an increase in immature reticulocyte fractions was reported to precede the presence of circulating CD34⁺ cells by about 2 days, suggesting that flow cytometric reticulocyte enumeration might be helpful in monitoring the timing of stem cell harvesting (80).

In spite of the relatively rapid restoration of peripheral blood cell counts following myeloablative chemotherapy or bone marrow transplantation, there is a continuing severe cellular and humoral immunodeficiency for months to years. The first flow cytometric studies of post-bone marrow transplantation immune recovery by Ault et al. (81), Lum (83), and other investigators (82,84) revealed an early numerical recovery of natural killer (NK) and CD8⁺ cells, but a persistent deficit in CD4⁺ cells until 6 or 8 months posttransplantation. Not surprisingly, posttransplantation immune function studies revealed intact NK-cell function, reduced clonogenic potential, and a decreased proliferative response to mitogens, antigens, and allogeneic cells during this period. Generally, cytotoxic cell function requires about 3 months post-BMT to recuperate, while more specialized immune functions may require a year or more. The reasons for posttransplant defects in cell-mediated immunity are complex and include decreased interleukin-2 (IL-2) synthesis, defective expression of the IL-2 receptor, and metabolic abnormalities (including deficient protein kinase C-dependent Na/H⁺ exchange) (81–84). B-cell function during the first posttransplantation year is similar to that of human cord blood cells, with proliferation and IgM production in response to stimulation, but absent secretion of IgM. Posttransplantation immunoglobulin secretion also follows a pattern similar to that seen in young children, with normalization of serum IgG₁, IgG₃, and IgM levels within the first year, but more persistent deficiencies of IgA, IgG₂, and IgG₄ cells. Such an intrinsic B-cell defect may be clinically important after the first posttransplant year, since many of the T-cell defects have normalized by this time and provide *in vitro* B-cell help signals. Patients developing graft-vs.-host disease following allogeneic transplantation may have even more severe defects in both cellular and humoral immunity that prolong immune reconstitution. Telomere attrition has recently been added as a contributing factor to immune dysfunction in long-term allogeneic transplant recipients (85).

Nonmyeloablative treatment before allogeneic transplantation permits near normal T cell-dependent

non-MHC restricted mitogenic responses, even in the early period after transplant in patients with hematologic malignancies, while myeloablative conditioning results in decreased T cell mitogenic capacity and slower immune recovery (86). Nonmyeloablative conditioning may also permit faster development of immune response to residual host malignant cells and nonmalignant abnormal hematopoietic cells. Peripheral blood B-cell numbers reach pretransplant levels by the second month and reach normal levels in 6 to 7 months.

Confirmation of Engraftment

Chimerism, in modern transplantation science, refers to the presence of donor lymphohematopoietic cells in a transplant recipient. These cells are most commonly derived incidentally from fetomaternal or blood transfusion, but are more purposefully the result of hematopoietic cell transplantation. Full donor (“complete”) chimerism defines a state in which all hematopoietic cells in the recipient are derived from the donor, whereas mixed chimerism describes the persistence of host and donor cells together or the coexistence of host and donor cells within a given cellular compartment, such as lymphocytes. Mixed chimerism occurs during bone marrow reconstitution and reflects a dynamic interplay between host and donor cells such that hemopoietic cells of both recipient and donor origin are present. Mixed chimerism is usually a transient state, ultimately leading to complete chimerism or a reversion to autologous hemopoiesis (87,88). The term microchimerism has been used to indicate the presence of minimal numbers of donor cells detectable only with very sensitive techniques (89).

Several investigators have demonstrated the induction of complete donor chimerism by reduced intensity conditioning and allogeneic stem cell transplantation from matched, unrelated donors (90–94). Since these procedures may also be associated with an increased risk of graft failure and disease recurrence, careful sequential monitoring of the dynamics of chimerism has become especially important for the documentation of marrow engraftment, the follow-up of allogeneic transplant recipients, the detection of minimal residual disease, and the scheduling therapeutic intervention (Fig. 9) (95–99).

The sensitivity and accuracy of the techniques used to detect chimerism exert an important influence on interpretations of its clinical significance after bone marrow transplantation. Several methods have been reported for this purpose, including cytogenetic, phenotypic, and molecular procedures (Table 3) (89,100,101). However, PCR-based methods are usually preferred because they are independent of gender mismatch,

Fig. 9. Serial determinations of chimerism status post-bone marrow transplantation. The patient was a 13-year-old male with precursor B-cell lymphoblastic leukemia who developed relapse disease at 46 months. **A:** Bone marrow aspirate, 46 months status post-bone marrow transplantation. Blast cells morphologically comprise 11% of bone marrow cellularity (Wright-Giemsa stain, 1,000 $\times$). **B:** Bone marrow biopsy, 46 months status post-bone marrow transplantation, showing clusters of immature cells (H&E stain, 400 $\times$). **C:** Flow cytometric study of bone marrow aspirate, 46 months status post-bone marrow transplantation. A two-dimensional histogram (CD10 vs. CD19) of CD45-dim cells (9% of total cell population) is shown. The majority of the cells exhibit bright dual expression of CD10 and CD19. **D:** Serial engraftment study of bone marrow aspirate specimens by PCR. DNA was extracted from the specimens using a standard silica-based procedure. DNA was amplified by the polymerase chain reaction using fluorescent-labeled primers to D3S1358vWA31, D8S1179, D21S11, D5S818, and D13S317 polymorphic markers. The PCR products were analyzed on an ABI 310 Capillary Genetic Analyzer using GeneScan software. The results are reported as the percentage of recipient genotype in the posttransplant sample and calculated as the mean of the peak height and peak area for each marker in the sample run in triplicate. The lower limit of detection of the technique is 0.5–1.0%. At 46 months, 24.6% recipient cells were detected in the patient's bone marrow.

TABLE 3. Methods for the detection of chimerism

Assay/Marker	Sensitivity %	Quantitative Accuracy	Comments
Sex-mismatched transplant			
Cytogenetics	10	Low	Cumbersome, slow turn-around time, technically difficult
FISH			
Y chromosome marker	1–2.5	High	
Simultaneous X/Y markers	0.3–0.6	High	Rapid turn-around
PCR assay Amelogenin gene	0.0001	Semiquantative	
Sex-matched transplant			
RFLP	1–10	Semiquantative	Cumbersome, slow turn-around time, technically difficult
PCR STR/VNTR	1–5	Semiquantative	
Multiplex PCR STR	<1	High	Rapid, technically easy amplification of 9 STR markers
Multiplex PCR FACS-sorted cell subset	0.001–0.01	High	Cells sorted by phenotype e.g., CD34
SNP	<1	High	Minisequencing

FISH, fluorescent in situ hybridization; RFLP, restriction fragment length polymorphism; STR, short tandem repeats; VNTR, variable number tandem repeats; SNP, single nucleotide polymorphism.

highly sensitive, and require only minute amounts of biologic material. Since Lawler et al. (102) first reported the use of PCR for the amplification of highly polymorphic short tandem repeat (STR) sequences for chimerism analysis, numerous assays based on variable number of tandem repeat (VNTR) or STR markers have been reported (102–104). A recently established multiplex quantitative PCR procedure originally designed and validated for forensic purposes is used by many laboratories (105–107). Before transplantation, the best STR is selected for each donor-recipient combination based on informative markers determined prior to the transplant. Although informativeness in the context of a chimerism study is substantially different from forensic analysis, a recent study by Thiede et al. (108) supports the use of these assays in chimerism testing even though they may include a number of markers with low informativeness for chimerism analysis.

An alternative technical approach is based on genotyping and quantitative analysis of single nucleotide polymorphisms (SNPs) (109,110). Since SNPs are the most common type of genetic variation, this technique is an important addition to the spectrum of methods applicable to chimerism analysis as it offers accurate quantitative assessment and sensitivity superior to most other approaches. Since the introduction of more sensitive techniques, the persistence or reappearance of autologous cells has been observed more frequently particularly in patients with T cell depleted grafts (111–113).

The following guidelines for chimerism detection were recommended by a workshop sponsored by the National Marrow Donor Program and the International Bone Marrow Transplant Registry (89).

- Chimerism analysis should be undertaken using sensitive techniques (<1%) with STR or VNTR

analysis being the approach most likely to give reproducible informative data.

- Peripheral blood cells are generally more useful than bone marrow cells for chimerism analysis.
- Lineage-specific chimerism should be considered the assay of choice in the setting of myeloablative and reduced-intensity conditioning.
- In nonmyeloablative, T-cell depleted transplants, and novel graft-vs.-host disease prophylactic regimens, chimerism analysis should be performed at 1, 3, 6, and 12 months because therapeutic interventions may depend on chimerism status.
- In nonmalignant disorders, chimerism testing should be performed at 1, 2, and 3 months as early patterns of chimerism may determine the need for more frequent testing and therapeutic intervention.

The analysis of cellular subsets in patients carrying disease-associated chromosomal aberrations is obviously important in the diagnosis of minimal residual disease and the choice of adoptive immunotherapy after stem cell transplant (SCT) (113,114). T-cell mixed chimerism clearly contributes to graft tolerance and resistance against GVHD, and mixed myeloid cell chimerism in acute and chronic myeloid leukemia patients may reflect residual disease or be an early indicator of imminent relapse (115–120). These studies clearly indicate that chimerism kinetics within specific cell subsets would be very informative about immune reactivity, disease course, optimal therapeutic strategies, and prognosis.

The role of chimerism testing in the clinical management of bone marrow transplantation recipients differs between the various conditioning regimens used and at different time points after transplantation. The

post-bone marrow transplantation phases: transplantation to hematological engraftment, engraftment to establishment of complete chimerism, and late post-transplant period, all display different chimerism kinetics with different therapeutic implications. The optimal timing of chimerism testing and inferred therapeutic options continues to be debated. Current efforts to standardize the technical approaches will provide greater diagnostic support for clinical decision-making and a basis for comparison of outcomes between various centers (121,122).

ABERRANT BONE MARROW RECONSTITUTION

There are numerous immune and hematologic complications that can limit the success of hematopoietic stem cell transplantation or successful bone marrow reconstitution following myeloablative therapy. These include absent or suboptimal growth of infused stem cells, residual disease cells, relapse of the original disease, myelofibrosis, and the development of new hematopoietic malignancies (Table 4).

TABLE 4. Adverse hematologic consequences of myeloablative therapy and/or bone marrow transplantation

Problem	Diagnostic features
Engraftment failure and delayed engraftment	Marked marrow hypocellularity
Residual bone marrow disease	Diffuse histiocytic proliferation Careful review of bone marrow aspirate, biopsy, and clot section supplemented by flow cytometry and/or immunoperoxidase staining
Minimal residual disease	Advanced diagnostic technique with detection limit of at least 10^{-3} cells
Hematogones	Flow cytometric immunophenotypic analysis
Graft-versus-host disease	Proliferation of macrophages, myelofibrosis, bone marrow hypoplasia
Myelofibrosis	Reticulin fibrosis, usually in patients with CML
Therapy-related acute leukemia (t-AML)	Morphology, immunophenotypic analysis, and cytogenetic analysis
Post-transplant lymphoproliferative disorders (PTLD)	Morphology, immunophenotypic analysis, molecular analysis, EBV analysis
Toxic myelopathy	Cytopenias with bone marrow hypocellularity, stromal damage, edema, perivascular plasmacytosis, necrobiosis of neutrophilic granulocytes, and cellular debris

Engraftment Failure, Acute Graft Rejection, and Delayed Engraftment

Many factors may cause a poor outcome of bone marrow transplantation ("graft failure") or the loss of recently engrafted marrow tissue ("graft rejection"). Generally, failure of initial engraftment (primary graft failure) is due to genetic differences between the donor and recipient, damaged stem cells or an insufficient numbers of stem cells, inadequate immunosuppression or pretransplant conditioning, alloimmunization due to previous multiple blood transfusions, excessive T-cell depletion of the engrafted material, an abnormal microenvironment in the bone marrow of the host, an abnormal donor marrow, drug toxicity, or viral infections (22,123,124). Failure of the graft after engraftment (secondary graft failure) occurs as a result of drug toxicity, infections, fibrosis, or cell-mediated immune reactions.

Immunologically-mediated bone marrow acute graft rejection is particularly common in three situations: 1) multiply-transfused patients with aplastic anemia, 2) patients receiving bone marrow from an MHC-mismatched donor, and 3) patients receiving T-cell depleted bone marrow (16). The incidence of acute rejection is only about 1% in patients receiving an immunologically unmanipulated, HLA-matched graft from a sibling, but increases to 8–15% in patients receiving T-cell depleted-phenotypically matched grafts (56,125). Graft rejection is primarily caused by host T lymphocytes that survive the pretransplant conditioning regimen, proliferate in the allografted bone marrow, then suppress the growth of donor cells, and initiate cell-mediated responses against donor targets. Slightly different mechanisms of rejection may be involved in different patient populations, since suppressor T lymphocytes ($CD3^{+}CD8^{+}CD57^{+}$) predominate in HLA-matched siblings undergoing allograft rejection, while cytotoxic T lymphocytes ($CD3^{+}CD8^{+}CD57^{-}$) are found in rejecting marrow transplant patients receiving an HLA-matched allograft from an unrelated donor (56).

Severe thrombocytopenia after primary posttransplant recovery of platelets ("secondary failure of platelet recovery" [SFPR]) is associated with serious complications, a poor clinical outcome, or even death (126). Thrombocytopenia occurs in as many as 20% of patients undergoing allogeneic transplantation, but has a much lower incidence (8%) in autologous transplantation (126). Cytomegalovirus infection has been implicated as a significant risk factor for the development of SFPR by several groups of investigators (126).

Morphologically, bone marrow aspirate smears from patients with engraftment failure or delayed engraftment

Fig. 10. Histopathology of residual disease and delayed bone marrow reconstitution. Left: Bone marrow aspirates (Wright-Giemsa stain, $1,000\times$). Right: Bone marrow biopsies (H&E stain, $400\times$). **A:** Acute myelogenous leukemia, pretreatment. Blast cells comprise $>60\%$ of bone marrow cellularity. **B:** Status: postchemotherapy, day 14. Blast cells continue to comprise the majority of bone marrow cellularity. Flow cytometric analysis (data not shown) revealed a pattern of antigenic expression similar to the original lesion. **C:** Status postreinduction chemotherapy, day 28. Small bone marrow spicules are populated primarily by plasma cells and lymphocytes. There is a virtual absence of active hematopoiesis.

are markedly hypocellular, with a predominance of stromal cells, while core biopsies and clot sections often show diffuse histiocytic proliferation (Fig. 10) (127,128). In CML, delayed marrow regeneration is associated with the presence of pretransplant reticulin fibrosis,

increased megakaryocytes, increased $CD68^+$ macrophages, a decreased number of erythroid precursors, and an elevated platelet count (129,130). Clinically, early graft failure (>50 days posttransplantation) is manifested by host T lymphocytosis ($CD3^+$, $CD8^+$, DR^+),

while late graft failure (≥ 50 days posttransplantation) is associated with a syndrome of delayed granulopoietic regeneration, fever of unknown origin, and abdominal symptomatology (131). Graft rejection is often heralded by progressive lymphocytosis and a sudden drop in the absolute neutrophil count (16). The prognosis for continued successful engraftment is poor once lymphocytosis occurs, although the infusion of donor lymphocytes has been successful in a few patients. Preventive therapy in high-risk patients is directed at the inclusion of increased total body irradiation, total lymphoid irradiation, or immunosuppressive agents into the preconditioning regimen (16).

Residual Bone Marrow Disease

The early detection of residual or relapse bone marrow disease in patients receiving myeloablative therapy is for future therapeutic planning, including early intensification therapy. Therefore, it is a widespread clinical practice to perform a bone marrow study 6 to 14 days after the start of remission induction to document marrow hypoplasia or detect residual leukemia cells (132,133). Generally, $<5\%$ blasts is considered indicative of remission (134).

In childhood acute lymphoblastic leukemia (ALL), the importance of a simultaneous bone marrow biopsy or clot section to supplement the bone marrow aspirate has been emphasized (135). In childhood patients with ALL in remission at day 28, there is little correlation between the cellularity of the aspirate and biopsy at day 7, and the biopsy usually shows a higher proportion of blasts than the aspirate (136). Immunohistopathologic examination of the bone marrow biopsy can supplement morphologic evaluation. In this regard, Rimsza et al. (137) found that clusters of more than five $CD34^+$ and/or TdT^+ marrow cells were predictive of imminent relapse, even in children with ALL in complete remission for as long as 12 months. Hematogones were differentiated from lymphoblasts by this technique, and only 1 in 10 children who remained in complete continuous remission had $CD34^+$ or TdT^+ clusters. The presence of $>5\%$ blasts has also been associated with significantly worse overall survival ($P<0.0001$) and worse disease-free survival ($P=0.02$) in adult ALL (138).

Several groups of investigators have evaluated the prognostic significance of postchemotherapy and post-BMTx bone marrow studies in AML. In one study, the presence of $>40\%$ abnormal cells remaining at day 4 of a 7-day induction chemotherapy regimen correlated with the presence of resistant disease in patients treated with daunorubicin, cytosine-arabioside, and 6-thioguanine (139). At day 6 of a 7-day course of

cytosine-arabioside/daunorubicin/vincristine or cytosine-arabioside/amsacrine, the presence of $>20\%$ malignant cells in a bone marrow aspirate significantly predicted the failure to achieve complete remission (140). Others have used a bone marrow cellularity of $>62.5\%$, a blast count of $>40\%$, a reduction of $<50\%$ of blasts, or marrow cellularity at day 6 as evidence of resistant disease (141–143). However, doubt has been raised about the value of early marrow studies in AML. In one study, a bone marrow examination at day 6 of induction chemotherapy identified only 50% of AML patients with resistant disease and falsely included 13% of patients who achieved remission (144). In another study, routine bone marrow evaluation at day 14 was found to provide useful information only in patients with circulating leukemia cells or the presence of an unexpectedly high absolute number of nonlymphocytic cells (145).

In acute promyelocytic leukemia (APL), neither the persistence of promyelocytes nor the presence of dysplastic myelocytes after induction therapy is an indication of chemotherapy failure or a poor ultimate prognosis (146).

Relapse of CML posttransplantation is difficult to determine morphologically, and requires determination of $t(9;22)$ by cytogenetic analysis or PCR. However, the appearance of atypical dwarf megakaryocytes after transplantation was found to be a sign of disease relapse (49,130). Histopathologic evaluation of the bone marrow in CML patients receiving chemotherapy include granulocytic, predominantly megakaryocytic, and myelofibrotic patterns (147). Myelofibrotic changes are typically associated with interferon (IFN) and busulfan (BU) therapy and regressed with hydroxyurea treatment (147).

Minimal Residual Disease

Minimal residual disease (MRD) is the persistence of leukemic cells in the bone marrow of other tissues after remission induction below the limit of detection by conventional morphologic assessment. Presently, it is believed that these residual leukemic cells are the possible source of disease relapse in many patients who achieve “complete” morphologic remission from ALL and other forms of leukemia. Unfortunately, the clinically relevant level of sensitivity for MRD detection has not been established, nor has it been documented that additional therapy to eradicate very small numbers of residual cells improves survival for patients in clinical and morphologic remission.

Laboratory techniques for the detection of minimal residual disease must meet four criteria, sensitivity (detection limit of at least 10^{-3} cells), specificity (ability

to differentiate normal and malignant cells), reproducibility, and applicability (easy standardization and rapid collection of results) (148,149). Morphologic evaluation, with an overall detection limit of approximately 5%, is clearly not suitable for the detection of minimal residual disease (150–152). Therefore, immunophenotypic analysis, cytogenetics, FISH, Southern blotting, PCR, and other techniques with detection limits of 10^{-2} to 10^{-6} cells have been applied, as well as the clonogenic assay, which has a detection limit of $\leq 10^{-4}$ (149). The techniques and application of minimal residual disease have recently been reviewed by several authors (149,153–159).

Flow cytometric analysis is the most widely utilized technique for the detection of MRD. Although it is less sensitive than molecular techniques, it is simple to perform, rapidly provides quantitative data, and has adequate sensitivity in many leukemia cases. The detection of minimal residual disease in leukemia by flow cytometric analysis is based on the presence of aberrant immunophenotypic features that are not characteristic of normal cell populations in the specimen under study. For example, the expression of TdT, cytoplasmic CD3, CD1a, or the dual phenotype $CD4^{+}/CD8^{+}$ by bone marrow cells is diagnostic of residual MRD in T-ALL, since cells with these phenotypes are normally confined to the thymus. The detection of B-ALL MRD is more difficult, as small numbers of immature B-cells are normally present in the bone marrow. However, the majority of B-ALL cases have aberrant antigenic features, including cross-lineage antigen expression (i.e., TdT, T-cell, or myeloid antigens), asynchronous antigen expression, or changes in the level of antigen expression (i.e., “dropped” or overexpressed antigens). In this regard, the discovery of $CD10^{+}$, TdT^{+} , or $CD34^{+}$ cells in the cerebrospinal fluid (CSF) is diagnostic of MRD, since immature leukocytes with these markers are not normally present in the CSF.

Hematogones

Hematogones are B-lineage lymphoid precursor cell populations in the bone marrow that may simulate ALL or lymphoblastic lymphoma. These precursor lymphoid cells are homogenous with condensed chromatin and scant cytoplasm and may account for greater than 50% of bone marrow cells (Fig. 11). They are found in large numbers in normal infants and occasionally in adults with a variety of hematologic and nonhematologic disorders (160,161). In ALL patients undergoing myeloablative therapy, the differentiation of hematogones from small foci of blast cells is critical, since residual blast cells usually herald disease relapse. Like-

wise, transient peripheral blood neoplastic blastemia following induction chemotherapy is a poor prognostic indicator, even in the absence of bone marrow blasts (39).

The distinction of leukemic blasts from hematogones can be difficult by morphologic examination alone. Even though hematogones express CD34 and TdT, albeit in small quantities, it is usually in a nonclustered fashion (162). Clusters of more than five CD34 and/or TdT-positive cells usually denote a malignant lymphoblastic population (137). Immunophenotypic analysis by flow cytometry is widely used to supplement morphologic and immunohistochemical evaluation, since hematogones display a maturational spectrum of B-lymphoid differentiation antigens, whereas neoplastic lymphoblasts express an incomplete maturational spectrum with aberrant antigen expression (Table 5). Thus, hematogones express many of the same antigens as neoplastic lymphoblasts (e.g., TdT, CD34, CD10, and various pan B-cell antigens) but the population as a whole shows a spectrum of B-lymphoid differentiation antigen expression with a predominance of intermediate differentiated cells ($CD19^{+}$, $CD10^{+}$) and mature B-lineage cells (surface immunoglobulin) and heterogeneity of adhesion molecule expression (CD44, CD54) (160,162). Furthermore, $CD34^{+}TdT^{+}$ cells form the smallest subset in a hematogone population, there is no evidence of clonality by either cytogenetic or immunogenetic analysis, and the DNA content is normal (162).

Several groups of investigators have found that immature B-lymphocytes cells in the bone marrow of patients with ALL in remission following chemotherapy or bone marrow transplantation do not necessarily signify relapse (163–166). The largest study of hematogones to date was reported by McKenna et al. (167), who used four-color flow cytometry in 662 consecutive bone marrow specimens from patients with a wide variety of clinical disorders. Overall, hematogones were found in nearly 80% of the marrow specimens, but were most common in patients with lymphoma, marrow regenerative states, immune cytopenias, and acquired immunodeficiency syndrome (167). Hematogones always exhibited a normal antigenic profile, while lymphoblasts in precursor B-ALL showed 1 to 11 immunophenotypic aberrancies. However, Kallakury et al. (168) reported that flow cytometric monitoring of posttherapy bone marrow specimens from patients with precursor B-cell acute lymphoblastic leukemia, is inferior to PCR and concluded that PCR for antigen receptor gene rearrangements is a valuable and specific tool, helpful in differentiating hematogones from minimal residual disease in patients with treated ALL whose bone marrow harbors increased precursor B-cells (hematogones).

Fig. 11. Histopathologic features of hematogones. **A:** Bone marrow aspirate, acute myelogenous leukemia, status post-bone marrow transplantation, week 46 (Wright-Giemsa stain, 1,000 $\times$). Scattered blast cells with lymphoid features are present. **B:** Bone marrow biopsy showing active trilineage hematopoiesis (H&E stain, 400 $\times$). **C:** Flow cytometric study of bone marrow aspirate. A two-dimensional histogram (side scatter vs. CD45 expression) shows a small, nondiscrete population of cells with decreased CD45 expression, comprising approximately 5% of the total cell population. **D:** Gated flow cytometric analysis. The CD45-dim population has been gated for further study. **E:** Gated analysis of CD45-dim cells for CD10 and CD19 expression. The majority of the gated cells exhibit moderately bright dual expression of CD10 and CD19, suggesting an immature B-cell lineage. **F:** Gated flow cytometric analysis of CD45-dim cells for CD5 and CD20 expression. The majority of the cells lack CD20 expression.

TABLE 5. Differential Diagnosis of Hematogones

Feature	Hematogones	Precursor B-Cell leukemia/lymphoma
Light microscopy	Nonclustered, homogenous small blastoid cells with condensed chromatin and scant cytoplasm.	Variable, usually small clusters to sheets of homogenous small blastoid cells.
Flow cytometry ^a	Non-clonal cell population with a maturational spectrum of B-lineage differentiation antigen expression from early B-cell precursors to intermediately differentiated cells to mature B-cell cells. Cells with an intermediate and mature immunophenotype predominate. Heterogenous expression of adhesion molecules (CD44, CD54).	Homogenous cell population with clonal antigen expression. Incomplete maturational spectrum, usually with predominance of early B-cell precursors. Frequent aberrant expression of lymphoid and/or myeloid antigens.
Immunostaining	Scattered CD34 ⁺ and/or TdT ⁺ cells without significant clustering. CD20 ⁺ cells exceed CD34 ⁺ and/or TdT ⁺ cells.	Clusters of more than five CD34 ⁺ and/or TdT ⁺ cells.
Cytogenetic and molecular analysis	No evidence of clonal abnormalities by cytogenetic analysis or immunoglobulin/T-cell receptor (TCR) gene rearrangement studies.	Frequent cytogenetic anomalies. Monoclonally rearranged antigen receptor genes.
DNA content	Diploid	Diploid or aneuploid

^aCharacteristic immunophenotypes - Early B-cell precursors - CD10⁺CD19⁺CD34⁺Tdt⁺CD45^{dim}, intermediately differentiated B-cells - CD10⁺CD19⁺CD20^{dim}CD34⁻Tdt⁻CD45^{dim}, mature B-cell cells - CD19⁺CD20⁺CD45^{bright}SIg^{positive}.

Graft-vs.-Host Disease

GVHD is the major cause of morbidity and mortality following allogeneic bone marrow transplantation. GVHD occurs in approximately 50% of cases with histocompatible marrow transfusions, and in nearly all cases of bone marrow transplantation with an HLA-mismatched marrow. When moderate to severe, GVHD has a significant mortality rate (40–80%). GVHD is a complex immunologic phenomenon that is poorly understood. However, GVHD is usually a T-cell mediated process occurring in an environment of lymphocyte subset imbalance, alloantigen presentation, and abnormal production or increased responsiveness to cytokines (16,169,170). Both acute and chronic forms of GVHD are recognized.

Acute GVHD (aGVHD) follows lymphocyte reactivity to disparities of “minor” histocompatibility antigens in the skin, gastrointestinal tract, and liver (171–174). An increased likelihood of GVHD is associated with HLA disparity between donor and host, the older age of donor and host, allosensitization of the donor, sex mismatch between donor and recipient, increased intensity of the preparative regimen, and donor T cell dose. The clinical symptomatology varies from mild skin rashes, gastrointestinal (GI) disturbances (nausea, vomiting, diarrhea), and impaired liver function tests, to life-threatening disease, with skin destruction, liver failure, bloody diarrhea, and severe immunosuppression. Approximately 5–10% of marrow-transfused patients die of aGVHD. A variety of immunosuppressive drugs (methotrexate, cyclosporine, prednisone,

ATG, etc.) have been used with a success rate of 35–50% in these patients. More recent attempts have been directed at deleting T cells from the marrow inoculum prior to transfusion by the use of ATG, anti-T cell monoclonal antibodies, lectin agglutinin sedimentation, or sheep red blood cell (RBC) rosetting. Unfortunately, T-cell depletion has been associated with increased graft rejection, presumably because of a poorly understood graft enhancing effect of T cells. Immunological studies conducted over nearly two decades have failed to discover an accurate means of predicting the onset or severity of acute GVHD, although recent evidence suggests that the expression of the T-cell activation antigen CD134 (OX-40) on CD4⁺ cells may be useful (16).

Chronic GVHD (cGVHD) may follow the acute process or develop de novo. It occurs in 25–65% of bone marrow transplant recipients. The platelet count is a predictor of survival; platelet counts <100,000/μL are associated with an overall mortality >50% (175,176). cGVHD is believed to represent an immunodysregulatory state characterized by autoimmune phenomena, and the clinical picture resembles autoimmune disease, with involvement of the skin, GI tract, and liver. Circulating autoantibodies are present, and deposits of complement and immunoglobulins have been identified at the dermal-epidermal junction. Risk factors for cGVHD include prior aGVHD, older donor or recipient age, HLA mismatch, use of an unrelated donor, viral infection, splenectomy, donor lymphocyte infusion (DLI), and use of peripheral blood stem cells (175). Early combination therapy with prednisone, cyclosporine A, and azathioprine has conventionally been used

to treat cGVHD, but treatment with pentostatin, monoclonal antibodies, extracorporeal photopheresis, and an IL-2 receptor fusion toxin (Ontak) is gaining acceptance (177).

There are no pathognomonic morphologic features of GVHD. However, the proliferation of CD68⁺ macrophages has accompanied acute and chronic GVHD, while bone marrow hypoplasia and myelofibrosis has been reported in chronic GVHD (178,179). Long-term marrow cultures in patients with GVHD have shown decreased numbers of myeloid, erythroid, and multipotent progenitor cells, as well as reduced numbers of fibroblast colony-forming cells and adherent stromal cells, with the most severe deficiencies in patients with acute GVHD (180,181). B cell precursors are also decreased in patients with cGVHD (182).

Myelofibrosis

Mild transient reticulin fibrosis is not uncommon after chemotherapy, but severe collagen fibrosis is more typical of CML. In pre-BMTx bone marrow biopsies from CML patients, there is an association between increased reticulin fiber density, an increased number of CD61⁺ megakaryopoiesis, increased CD68⁺ macrophages, a decreased number of erythroid precursors, and an elevated platelet count (49,129,130). Studies utilizing morphometric quantitation of fiber density in pre-BMTx bone marrow biopsies revealed significant myelofibrosis (>3 times the normal fiber content) in about one-fourth of patients with CML. An increase in reticulin fibers and the development of myelofibrosis is also seen in patients treated with busulfan, and particularly interferon-alfa (IFN-alfa), while a regression of myelofibrosis occurs with hydroxyurea treatment (50,147). There is usually an initial regression of myelofibrosis after BMTx, but it often recurs in the areas of regenerating hematopoiesis, and is associated with the presence of atypical dwarf megakaryocytes, severe acute GVHD, and a significant delay in the time to achieve transfusion independence (183). De novo post-BMTx myelofibrosis also develops in a few patients. Both auto and allogeneic bone marrow transplantation have made a significant impact in the management and survival of the clinical ramifications of primary myelofibrosis.

Therapy-Related Acute Leukemia

Therapy-related acute myeloid leukemia (t-AML) is a form of secondary leukemia arising from cytotoxic chemotherapy and/or radiation therapy (184). The incidence of t-AML following high-dose chemotherapy for a prior malignancy is progressively increasing, (185,186) and t-AMLs are among the most common

second malignancies in both pediatric and adult populations (187,188). Polymorphism or homozygous gene deletions of glutathione S-transferases P1, M1, and T1 may play a role in the increased incidence of t-AML due to insufficient detoxification of the chemotherapeutic drugs (189,190). Patients treated for Hodgkin's lymphoma, non-Hodgkin's lymphoma (NHL), myeloma, polycythemia vera, breast cancer, ovarian carcinoma, testicular carcinoma, or de novo ALL are at the greatest risk of developing t-AML, and more than 50% of patients with secondary AML have breast cancer, NHL, and Hodgkin's lymphoma (188,191,192). In contrast to t-AML, the incidence of therapy-related ALL is rare, and Pagano et al. (193,194) reported that in almost all cases of secondary ALL that they studied, the role of previous drugs does not appear to be relevant.

There are two well defined groups of therapy-related AML (195):

- Leukemia secondary to the use of alkylating agents
- Leukemia secondary to the use of topoisomerase inhibitors

Leukemias secondary to the use of alkylating agents (e.g., cyclophosphamide, nitrosoureas, chlorambucil, etc.) usually occur 5 to 6 years following primary tumor and exposure to the mutagenic agent, with the reported range being approximately 10–192 months (196,197). The risk for occurrence is related to the total cumulative dose of the alkylating agent and the age of the patient (184). This type of t-AML initially presents as a myelodysplastic syndrome (MDS) with evidence of bone marrow failure. The MDS phase may evolve to AML or a higher grade MDS (184). Notably, a substantial number of patients succumb to the disease in the myelodysplastic phase, while a few progress to t-AML (184). The clonal cytogenetics abnormalities observed are similar to those seen in AML with multilineage dysplasia and de novo MDS, refractory cytopenia with multilineage dysplasia (RCMD), or refractory anemia with excess blasts (RAEB) (184,195). Unbalanced and complex chromosomal aberrations are common, including deletions of chromosome 13 and loss of the entire or part of chromosome 5 and/or 7. The deletion of the long arm of chromosome 5 usually includes bands q23 to q32.13. Other chromosomes frequently involved in a nonrandom manner include 1, 4, 12, 14, and 18.1. A particularly unfavorable event is the loss of the coding regions for tumor suppressor genes from hematopoietic progenitor cells, since the remaining allele becomes susceptible to inactivating mutations leading to leukemic transformation (188). Christiansen et al. (198) noted that mutations with the loss of function of p53 are significantly associated with deletion or loss of 5q in

t-MDS and t-AML after previous treatment with alkylating agents and are associated with genetic instability. Orazi et al. (199) reported frequent over-expression of p53 in therapy-related MDS and t-AML and observed that p53 expression in MDS and AML was associated with shorter survival. This type of therapy-related leukemia is generally refractory to antileukemia therapy and is associated with short survival.

Leukemia due to DNA-topoisomerase II inhibitors occurs in patients of all ages treated with topoisomerase II inhibitors and usually has a shorter latency period compared to t-AML due to an alkylating agent (184,200). The reported interval is approximately 12–130 months, with a median of 33–34 months (184). Leone et al. (188), however, suggested that younger age and a mean latency period of 2 years are characteristic features of this type of leukemia. The tumorigenic action of topoisomerase inhibitors is due to the formation of multiple DNA strand breaks with balanced chromosomal translocations. This is most commonly associated with epipodophyllotoxins (e.g., etoposide, etc.) and anthracyclines (e.g., daunorubicin, doxorubicin, etc.) (195, 201,202). This type of therapy-related leukemia usually, but not invariably, presents without a myelodysplastic phase (184).

Morphologically, t-AML due to topoisomerase inhibitors characteristically has a significant monocytic component (184,188), although acute promyelocytic leukemia (203,207), myelodysplastic syndrome, megakaryoblastic leukemia or ALL (200,204) morphologies have been reported. The predominant genetic abnormality is on chromosome 11q23, where the mixed lineage leukemia (MLL) gene is located, with the following common translocations: t(9;11), t(19;11), t(4;11), t(6;11) (188,204,205). Other observed abnormalities include t(8;21) and t(3;21) involving band 21q22, inv(16), t(8;16) and t(6;9) (201,206–208). There are reports that t-AML with inv(16) and t(15;17) is often associated with prior therapy with topoisomerase II inhibitors, but a similar finding is observed in patients treated with only radiotherapy (209,210). In the uncommon cases where ALL is observed, the most frequent cytogenetic abnormality is t(4;11)(q21;q23) (204). The following are regarded as good risk cytogenetic features: t(15;17); t(18;21) and inv(16) (211). Although present data suggests that the initial response rates of most cases of this type of therapy-related leukemia to intensive initial chemotherapy is comparable to those of primary acute leukemia of the corresponding type, the long-term follow-up data to predict the ultimate survival in these patients is insufficient (184,206,208). de Witte et al. (211) suggested that patients who attain a polyclonal and/or a cytogenetic complete remission may be candidates for autologous stem cell transplantation, but for the

remaining patients allogeneic stem cell transplantation from a histocompatible sibling or an alternative donor is the best curative option at present (211,212).

In view of the fact that the incidence of therapy-related AMLs is increasing, close follow-up of patients in the first few years following successful treatment of primary malignancies should be considered and any peripheral blood cytopenia should be considered suspicious (188,213). Exclusion of drugs known to be leukemogenic from the treatment schedule should be considered in patients where the standard of care of primary malignancies would not be compromised (188).

Posttransplant Lymphoproliferative Disorders

Posttransplant lymphoproliferative disorders (PTLDs) are lymphoid neoplasms that develop as a consequence of immunosuppressive therapy in patients receiving bone marrow or solid organ transplants (214,215). The spectrum of posttransplant lymphoproliferative disease ranges from benign to malignant monoclonal or polyclonal lymphoid proliferations, and it occurs in about 2% of solid organ transplant recipients, approximately 1% of autologous bone marrow transplant recipients, and up to 20% of patients with multiple risk factors, including receipt of an HLA-mismatched allogeneic marrow transplant and immunosuppressive therapy for GVHD, such as anti-CD3 monoclonal OKT3, cyclosporine A, and FK506 (216–220). EBV, either primary or reactivated, is strongly associated with development of PTLD (215,221–225). However, approximately 20% of PTLDs are EBV-negative, especially in the setting of autologous bone marrow transplantation (226). Impaired immune surveillance, chronic antigenic stimulation from the allograft, and the oncogenic effects of immunosuppressive therapy are additional factors that lead to PTLDs.

In contrast to the typical extranodal involvement of solid organ transplant recipients with PTLD, bone marrow allograft recipients with PTLD often have widespread disease involving both nodal and extranodal sites (214,227,228). In particular, PTLDs occurring in T-cell depleted (TCD) allogeneic bone marrow transplant recipients tend to be clinically aggressive monoclonal neoplasms with a high incidence of extensive dissemination at presentation (229). In children, PTLDs often present with otolaryngologic symptoms, including tonsillar necrosis, tonsillitis, airway obstruction, lymphadenitis, sinusitis, and otitis media (230). The bone marrow is involved in approximately 25% of cases (214). The histopathologic and immunophenotypic features of different forms of PTLD are shown in Table 6 (214).

TABLE 6. Classification and Histopathology of PTLD

Type of PTLD	Histopathologic, immunophenotypic, and genetic features
Early lesions	
Reactive plasmacytic hyperplasia	Preserved nodal architecture with plasmacytic hyperplasia. Frequent plasma cells and rare immunoblasts. Usually show polyclonal immunoglobulin gene rearrangement.
Infectious-mononucleosis-like	Preserved nodal architecture with paracortical expansion and frequent immunoblasts. Usually show polyclonal immunoglobulin gene rearrangement.
Polymorphic PTLD	Mixture of lymphoid cells at various stages of differentiation, including small lymphocytes, plasma cells, centroblasts, and immunoblasts. Mitotic figures and individual cell necrosis may be present. Geographic areas of necrosis are rarely found. Usually show c and EBV genomes.
Monomorphic PTLD	
B-cell neoplasms	
Diffuse large B-cell lymphoma	Destructive infiltrates of large lymphoid cells that fulfill the histologic criteria for large cell lymphoma. Nearly all cases show clonal rearrangement of immunoglobulin genes.
Burkitt/Burkitt-like lymphoma	B-cell neoplasms with monotonous cells with multiple nucleoli and basophilic cytoplasm.
Plasma cell myeloma	Rare neoplasms with features of plasma cell myeloma. Often aggressive.
Plasmacytoma-like lesions	Rare neoplasms that resemble extramedullary plasmacytomas.
T-cell neoplasms	Nodal effacement by monomorphic lymphoid infiltrate showing expression of pan-T cell antigens and variable expression of other antigens. Classified using general criteria for T-cell neoplasms. Variable EBV expression. Show clonal T-cell receptor gene rearrangement.
Hodgkin lymphoma and Hodgkin lymphoma-like PTLD	Morphologic and immunophenotypic features of classical Hodgkin lymphoma. HL-like cases have RS-like cells with an atypical B-cell immunophenotype. Usually EBV-positive.

The diagnosis of monomorphic PTLDs should follow the recognized classification of NHL, although specified in the report as PTLD. Pathologic evaluation of these lesions should include routine morphology, immunophenotyping on fresh tissue (flow cytometry or frozen section), and preservation of tissue for molecular genetic analysis (Fig. 12) (214). Tissue analysis for EBV is helpful in the analysis of early or equivocal lesions. Polymorphic lesions should be evaluated for clonality by immunophenotyping, and the antigen-receptor and EBV genomes, if necessary (214). Prompt recognition, diagnosis, and intervention with reduction in immunosuppression and antiviral therapy are essential to reduce the mortality of PTLD.

Toxic Myelopathy

Toxic myelopathy is a rare bone marrow lesion caused by toxic damage to the stromal and mesenchymal components of the bone marrow. Persistent cytopenia is the clinical hallmark of toxic myelopathy, while the bone marrow is hypocellular with prominent stromal damage, including edema, perivascular plasmacytosis, necrobiosis of neutrophilic granulocytes, and cellular debris (231). Toxic myelopathy occurs in <1% of patients receiving chemotherapy or radiotherapy.

SUMMARY

Bone marrow transplantation is a curative therapy for patients with selected diseases that are usually incurable

by other therapeutic methods. It involves the infusion of hematopoietic stem cells from the bone marrow or peripheral blood into a patient, effectively replacing a diseased hematopoietic or immune system with that of the donor. The stem cells may be obtained from a relative or unrelated individual (allogeneic transplantation) or obtained from the patient after temporary disease remission is obtained. In conventional bone marrow transplantation, intensive chemoradiotherapy is given prior to the bone marrow transplantation to eradicate the bone marrow containing the diseased cells. Successful growth of the stem cells (engraftment) normally occurs within 2–3 weeks by the appearance of the progeny of donor stem cells in the circulation of the recipient. The patient is profoundly ill during this period of time and requires intensive medical support. Nonmyeloablative bone marrow transplantation is a recently developed, less hazardous form of bone marrow transplantation that circumvents the relatively high morbidity and mortality of conventional bone marrow transplantation. In nonmyeloablative bone marrow transplantation, a chimeric hematopoietic system is formed that provides an excellent environment for a “graft vs. malignancy effect.” A successful bone marrow transplant requires a functional bone marrow microenvironment and the infusion of an adequate number of stem cells. The quality of the graft is determined prior to transplantation by flow cytometric analysis for CD34 antigen using a modification of the Milan technique. Rapid engraftment and a reduced risk of graft failure and infectious complications is associated with a cell dose of $>5 \times 10^6$ CD34⁺ cells/kg.

Fig. 12. Histopathologic features of posttransplant lymphoproliferative disease, polymorphic type; left lung, lower lobe, superior segment. **A:** Portion of lung showing effacement by atypical lymphoid infiltrate (H&E stain, 400 $\times$). **B:** Immunostain stain of lung for CD3 (400 $\times$); frequent scattered cells are positive. **C:** Immunostain stain of lung for CD20 (400 $\times$); numerous scattered cells show immunoreactivity.

Repeated bone marrow examination is critical to ensure adequate preparation of a patient for bone marrow transplantation and to monitor the posttransplantation course of the patient. The bone marrow is profoundly hypocellular following chemotherapy or a conventional preparative regimen for bone marrow transplantation. Regrowth of the bone marrow (bone marrow reconstitution) is usually evident after 2 weeks, from the appearance of small, scattered islands of immature, monotypic hematopoietic precursors, followed by clusters of developing erythroid cells, myeloid cells, and finally, megakaryocytes. Bone marrow reconstitution is accompanied by progressive increase in the erythrocytes, leukocytes, and platelets. Since erythroblasts are the first hematopoietic precursors to regenerate, measurement of the early reticulocyte fraction is presently the best clinical laboratory monitor of early engraftment. Normalization of the white blood count is achieved by day 28 in most patients, although complete functional reconstitution of the hematopoietic and immune systems requires several years. Unfortunately, there are many problems that may hamper bone marrow reconstitution or pose other medical problems for the bone marrow transplant patient. These include engraftment failure and delayed engraftment, infection, residual bone marrow disease, GVHD, myelofibrosis, therapy-related acute leukemia, posttransplant lymphoproliferative disorders, and toxic myelopathy. There have been many developments in the use of multiparametric flow cytometry and molecular techniques for the early detection and monitoring of these complications.

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