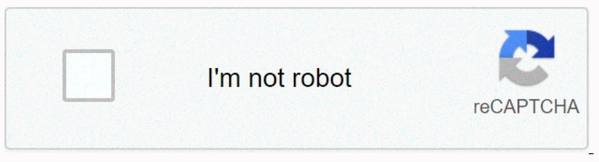

Continue

Bone marrow aspiration reporting format

George A. Fritsma * Bone Marrow Anatomy and Architecture Indications for Bone Marrow Study Bone Marrow Sampling Site Bone Marrow Aspiration and Biopsy Preparation Core Biopsy Aspiration Patient Care Management Bone Marrow Sample Direct Aspirate Smear Smears Anticoagulant Smears Imprints (Touch Preparations) Concentrate (Buff) eous coat) Smears Histologic sections (cell block) Marrow smear dye examining bone marrow aspirate or imprint low power (100×) study High power (500×) study prussian blue iron stain study examining bone marrow core biopsy sample Final bone marrow research reports After completion of this section, reader will be able to: 1. Diagram bone marrow architecture and find hematopoetic tissue. 2. Indications for bone marrow testing are listed. 3. Specify the sites of bone marrow aspirate and biopsy. 4. Collect accessories for the collection and assistance of bone marrow samples. 5. Assist the doctor in the preparation of the bone marrow sample after collection. 6. List the information obtained from bone marrow aspirates and biopsy samples. 7. Perform a bone marrow aspirate smear and a sample of the core biopsy. 8. The normal bloodmatopoetic and stromal cells of the bone marrow and their intended distribution shall be listed. 9. Perform a bone marrow differential number and calculate the myeloid-erythroid ratio. 10. Describe the properties of haematopoetic and metastatic tumour cells. 11. Prepare specimens and help with specialized confirmatory bone marrow tests. 12. Prepare a systematic written report on bone marrow testing. After ing the material in this section, the reader should be able to respond to this case study: The patient came for treatment, complaining of weakness, fatigue and malaise. Detailed blood results were as follows: HGB concentration: 7.5 gm/dL Segmented neutrophils: 21 × 109/L (70%) HCT: 21% immature neutrophils: 6 × 109/L (20%) RBC count: 2.5 × 1012/L Basophils: 1.5 × 109/L (5%) WBC Count: 30 × 109/L Eosinophils: 0.3 × 109/L (1%) Platelet count: 540 × 109/L Bone marrow was hypercellular with 90% myeloid precursors and 10% erythroid precursors. There were 15 megakaryocytes in the area of 10× microscopic goal. 1. What bone marrow findings provide information on blood cell production? 2. What is the myeloid-erythroid ratio in this patient, and what does it indicate? 3. What is the distribution of megakaryocytes usually visible in bone marrow aspirate? Bone marrow anatomy and architecture In adults, bone marrow accounts for 3.4% to 5.9% of body weight, 1600 to 3700 g or 30 to 50 ml/kg volume and produce approximately 6 billion blood cells per kilogram per day in a process called mehematopoeze.1 At birth, almost all bones contain red blood <2> <2> marrow (chapter 7). In the fifth to seventh year, adipocytes (fat cells) begin to replace the red marrow in the long bones of the arms, legs, and weapons producing yellow marrow, and in late adolescence, hematopoetic marrow is limited to the lower skull, vertebrae, shoulders, pelvic band, ribs and sternum (Figure 7-2). Although the percentage of bone space for haematopoiesis is significantly reduced, the total volume remains constant when the individual matures.2 The yellow marrow returns to hematopoiesis, increasing the volume of the red marrow in conditions such as chronic blood loss or hemolithic anemia, which raises demand. The arrangement of the red marrow and its connection with the central venous sinus are shown in Figure 7-3. Hematopoietic tissue is pinned into the sponge trabecula (bone tissue) surrounding the sinus network, which originates from the endosteum (the vascular layer just inside the bones) and stops collecting venules.3 Adipocytes occupy about 50% of the red blood marrow space for an adult 30-70 years old, fat metamorphosis increases by approximately 10% in a decade after the age of 70.4 Indications bone marrow study Because the procedure is invasive , the decision to collect and examine a bone marrow sample requires a clinical decision and application of the inclusion criteria. With the development of cytogenetic chromosomal tests, flow cytometry, immunohistochemistry and molecular diagnostics, peripheral blood can often provide information historically accessible only from the bone marrow, reducing the demand for marrow samples. On the other hand, these advanced methods also increase the diagnosis of bone marrow, thus potentially increasing the demand for bone marrow research by assessing conditions that were not previously diagnosed during a bone marrow test. the indications for the bone marrow test. In Table 17–15, bone marrow tests may be used to diagnose and stage haematological and non-haematological neoplasia, cytopenias for the cause of cytopenias and to confirm or rule out metabolic or infectious conditions suspected on the basis of clinical symptoms and peripheral blood data.6 Indications for indications for bone marrow examination Signs Neoplasia diagnosis Acute leukemia Myeloproliferative tumors such as chronic leukemia, myelofibrosis myelodysplastic tumours such as refractory anaemia Lymphoprofraural disorders such as acute lymphoblastic leukemia Immunoglobulin disorders e.g. plasma cell myeloma , macroglobulinaemia Metastatic tumors Diagnosis of neoplasia and staging Hodgkin's and non-Hodgkin's lymphoma Marrow failure: cytopenia Hypoplastic or aplastic aplasia Pure red cell aplasia Idiosyncratic drug-induced marrow suppression Myelo dysplastic syndromes, such as refractory anaemia Marrow necrosis, secondary tumour marrow necrosis, secondary to serious infections such as parvovirus B19 infection Immune and amegacaric thrombocytopenia Sickle cell crisis Megaloblastic , iron deficiency differentiation, sideroblastic , hemolithic and blood loss anemia Canning iron evaluation to assess iron deficiency processes or Metabolic disorders Gaucher disease Mast cell disease Infections Granulomatic disease Miliary tuberculosis Fungal infections Hemophagocytic syndromes Treatment monitoring After chemotherapy or radiation therapy to assess the minimal remaining disease After stem cell transplantation, to evaluate engrafment Each bone marrow procedure should be carried out after careful consideration of clinical and laboratory information. For example, a bone marrow test is most likely not necessary for anaemia, when the cause is evident from red blood cell (RBC) indices, serum iron and ferritin levels, or vitamin B12 and folic acid levels. Multilineage abnormalities, circulating blasts in adults, and unexpected pancytopenia are usually a quick marrow study. Bone marrow puncture is contraindicated in patients with coagulopathies such as hemophilia or vitamin K deficiency, although thrombocytopenia (low platelet count) is not an absolute contraindication. Special precautions may be necessary, such as treatment intervals, to avoid uncontrolled bleeding when a bone marrow procedure is performed on a patient receiving thrombotic treatment such as coumarin or heparin. The collection of bone marrow samples of bone marrow samples is a collection of samples from a medical laboratory scientist and a qualified specialty doctor, often a pathologist or hematologist.7 Before bone marrow collection, a specialist in a medical laboratory or phlebotote collects peripheral blood to carry out a full blood film test. During bone marrow collection, the laboratory scientist helps the doctor to control the samples and produce the initial preparations for the study. Red marrow is gelatin and samples may be taken. Most bone marrow samples consist of aspirate (derived from bone marrow aspiration) and a core biopsy sample (derived from a trefin biopsy), both analysed by light microscopy using 100× and 500× enhancement. Aspirate is studied to determine the types and proportions of haematological cells and to look for morphological dispersion. The main example of a biopsy demonstrates the architecture of the bone marrow: the spatial relationship of hematological cells with fat, connective tissue and bone stroma. The main biopsy sample is also used for cell evaluation. The main sample of biopsy is especially important in assessing diseases that are characterized by focal lesions, rather than dissipating the involvement of the marrow. Hodgkin's lymphoma, non-Hodgkin's lymphoma, multiple myeloma, metastatic tumors, amiloid and granulomas mainly cause focal lesions. Granuloma or granulomatic lesions are a cell accumulation that contains Langerhans cells – large, activated granular macrophages that look like epithelial cells. Granuloma signals a chronic infection. The biopsy sample also allows for morphological assessment of bone spirals, which may reveal changes related to or Paget disease.8 The bone marrow collection sites are: • • pelvic tile (spine) (Figure 17-1). For both adults and children, this site provides a suitable red marrow, which is isolated from the anatomical structures that are injured. This site is used for both aspiration and basic biopsy. • Anterior upper ridge (spine) pelvis. This site has the same advantages as the posterior top iliac ridge, but the bark bone is thicker. This site can be preferred to a patient who can only lie supine. • Sternum, below lewis angle in the second interculture. In adults, the sternum provides a lot of material for aspiration, but is only 1 cm thick and can not be used for the main biopsy. The doctor may inadvertently transfix the sternum and get into the inside of the pericardium, damaging the heart or large vessels. • The surface of the frontal media is tibia in children under 2 years of age. This site can only be used for aspiration. • Spinal process of vertebrae, ribs, or other red marrow containing bones. These locations are accessible but rarely used, unless one of them is the location of a suspicious lesion detected in the radiograph. FIGURE 17-1 The posterior superior iliac carcass is a favorable place to receive a sample of bone marrow aspirate and core biopsy because it provides a large amount of marrow and is isolated from structures that can be damaged by accidental puncture. Source: (Courtesy of Indiana Pathology Images, Indianapolis, IN.) Unwanted results are visible in less than 0.05% of marrow collections. Infections and reactions to anesthetics may occur, but the most common side effect is bleeding associated with platelet dysfunction or thrombocytopenia. Bone marrow aspiration and biopsy preparation Less than 24 hours before bone marrow collection, a medical laboratory scientist or phlebotote collects venous peripheral blood for a full blood count and blood film test using a standard collection procedure. Peripheral blood collection is often carried out immediately before the collection of bone marrow samples. A peripheral blood sample is rarely collected after bone marrow collection to prevent stress-related rise in the number of white blood cells (WBC). Most institutions buy or collect disposable sterile bone marrow sample collection trays containing: • Surgical gloves. • Shaving equipment. • Antiseptic solution and alcohol pads. • Drape material. • Local injection of anesthesia, usually 1% lidocaine, does not exceed 20 ml per patient. • No 11 scalpel knife for skin incision. • One-time Jamshidi biopsy needle (Care Fusion, McGaw Park, IL; Figure 17-2) or Westerman-Jensen needle (Becton, Dickinson and Company, Franklin Lakes, NJ; Figure 17-3). Both provide obturator, the main biopsy tool, and stylet. There is also a Snarecoil biopsy needle (Kendall Company, Mansfield, MA). Snarecoil has a coil mechanism at the tip of the needle, which allows you to bone marrow sample without needle forwarding (Figure 17-4). • Disposable Disposable up to 18 gauges of aspirational needles with an obturator. Or, an aspirational needle from the University of Illinois can be used for stern puncture. The needle at the University of Illinois provides a flange that prevents the penetration of the sternum into the pericardial. • Microscope slides or lids are washed in 70% ethanol. • Petri dishes or shallow round watch glasses. • Vials or tubes with closing closures. • Wintrobe haematocrit tubes. • Anticoagulant liquid tripotasi ethylenedinotetraacetic acid (K3EDTA). • Zenker fixation: potassium dichromate, mercury chloride, sodium sulphate and glacial acetic acid; B5 fixative: aqueous mercury chloride and sodium acetate, or 10% neutral formaline. As Zenker fixation and B5 contain toxic mercury, a controlled disposal is required. • Gauze sauces. FIGURE 17-2 Single sterile Jamshidi bone marrow biopsy and aspiration needle. An additional puncture cannula is advanced into medulla with obturator to avoid bone points. The doctor removes the obturator and slides the main biopsy needle through the cannula and into the medulla with the removal of the feather removed. The main biopsy needle is removed from the puncture needle with the location of the sample. The sample is sent using a stylus. Source: (Courtesy Care Fusion, McGaw Park, IL.) 17-3 FIG Bone marrow biopsy technique using Westerman-Jensen needle. 17-4 EIA Snarecoil bone marrow biopsy needle. The mechanism of the coil is located inside the biopsy needle, as shown in the enlarged image. The coil is rotated so that the marrow specimen is drawn into a needle. Source: (Courtesy of Tyco Healthcare/Kendall, Mansfield, MA.) The patient is asked to lie in a supine, prone, either on the right or left side decubitus position (lying on the right or left side). With regard to standard precautions, the skin is shaved, disinfected and banned if necessary. The doctor penetrates into the skin, dermis and subcutaneous tissue with a local solution of anesthetics, such as 1% or 2% lidocaine or proccaine, through a 25-gauge needle that produces 0.5 to 1.0 cm papule (bubble). The needle of the 25 gauges is replaced by the 21 gauge needle, which is inserted through the papule into the periostheum (bone surface). With the needle point periosteum, the doctor injects approximately 2 ml of anesthetic per dime-sized area, and rotates the needle, then withdraws the anesthetic needle. In addition, the doctor makes an incision of 3 mm of skin through the puncture site with a blade of no. 11 scalpel to avoid inserting the skin dots into the needle. The main biopsy A biopsy specimen is usually collected first, because the aspiration can destroy the marrow architecture. After the incision, the doctor inserts Jamshidi quoted cannula with an obturator through the skin and bark of the bones. Obturator protects against skin or bone points. Reciprocal rotation promotes the frontal progress of the canisters until the resistance is weakened, which indicates bark into the medullary cavity of the bones. The doctor removes the obturator, inserts the biopsy needle through the cannula and slowly advances the needle from 2 to 3 cm, constantly twisting along the long axis. The doctor slightly changes the angle of the needle to separate the sample of the main cylinder from the attachments of the marrow cavity, and pulls the biopsy needle and cannula from the bone, taking with them the main cylinder. The core cylinder is 1-1.5 cm long and 1-2 mm in diameter and weighs about 150 mg. The biopsy needle is placed on the ethanol-cleaned slide and the feather is pushed to push the core cylinder into the slide. Using sterile forces, the laboratory scientist prepares the imprints (touch preparations) and transfers the core cylinder to the selected fixer Zenker, B5 or formalin. When using a Westerman-Jensen needle, the doctor removes the obturator, inserts cutting knives through the cannula and pre-knives into the medullary cavity. The cutting blades are pressed into the medullary bone and the front cannula is firmly held in a stationary position. The doctor slowly pulls out the knives so that the cannula penetrates into the tissue, then pulls out the entire device. Remove the core cylinder by inserting the probe through the cutting nozzle and squeezing the herd through the needle hub into the selected slide and locking tanks. Aspiration In a separate place from the biopsy, the doctor inserts an aspiration needle of 14-18 gauges, such as a needle at the University of Illinois, with an obturator, through the skin of the bone and winter. Remove the Obturator and add a 10-20 ml syringe. The doctor pulls out the plunger to create negative pressure and pushes 1.0 to 1.5 ml of marrow into the syringe. After collecting more than 1.5 ml, the hemorrhage is diluted with sinusoidal (peripheral) blood. The doctor separates the syringe and immediately transfers it to the laboratory scientist, who removes the material into a series of clean and sterile microscopic slides or lids. The doctor may attach a second syringe to insert an additional sample for cytogenetic analysis, molecular diagnosis or immunophenotype using flow cytometry. Then the needle is removed, and the pressure is applied to the wound. In the absence of marrow, the doctor returns the obturator to the needle, advance the needle, attach a fresh syringe and try again. The syringe and needle are slightly pulled out and the process is repeated. If this test is unsuccessful, the doctor removes the needle and syringe, applies pressure and begins the procedure in a new place. If the marrow is fibrotic, cellular or packed with leukemia cells, the first and second aspiration may be unsuccessful, known as a dry tap. In this case, a biopsy is required, and the laboratory scientist can monitor the morphology of cells using the imprint of the slide or touch preparation. Patient care bone marrow biopsy or aspiration, the doctor applies a pressure dressing and advises the patient to stay in the same position for 60 minutes to avoid bleeding. Bone marrow sample management Direct aspirate smears Aspirant laboratory scientist receives an aspiration syringe from the doctor's bed and immediately transfers the drops of marrow sample to six or eight ethanol-washed microscope slides. Marrow clots quickly, so good organization is necessary. Using the dispersion slides, the scientist spreads the drop into a wedge-shaped smear from 1・2 to 3・4 slide length, similar to the peripheral blood film. Bony spirals from 0.5 to 1.0 mm in diameter and larger fat balls sequence behind the spreader and settle on the slide. In the preparation of a direct smear, the scientist avoids crushing the spirals. The scientist can easily fan smears to promote rapid drying in order to preserve cell morphology. In the syringe, the sample consists of peripheral blood with suspended bone spirals of light color and fat balls. The scientist evaluates the blood of the syringe for spirals: more spirals mean a sample with more cells to identify and categorize. If the sample contains a small number of fat globes or spirals, the scientist may warn the doctor to collect an additional sample. Anticoagulant aspirate smears Anticoagulant specimens are a more urgent alternative to direct aspiration smears. The scientist expresses aspirate from the syringe into the vial containing K3EDTA and then pipettes anticoagulant aspirate to clean the glass slides by spreading the aspirate by the same method as in the preparation of a direct smear. All anticoagulants distort cell morphology, but K3EDTA causes the least distortion. Crush the smears To prepare the crush smears, the medical laboratory scientist pushes part of the aspirate into a petri dish or clock glass covered with several millilitres of K3EDTA solution and spreads aspirate on the surface with a sterile applique. Separate bone spirals are transferred using applicators, powertrains or micropipettes (preferably) to several ethanol-washed glass slides. Scientists put additional glass slides directly on the specimens at right angles and gently press to crush the spirals. The slides are separated into the side to create two rectangular smears that the scientist can fan to encourage rapid drying. Some scientists prefer to move the aspirate directly to the slide, later tilting the slide to drain peripheral blood while maintaining the spicules. After draining, the spirals are crushed with a second slide, as described above. A scientist can add one drop of 22% albumin to the EDTA solution, especially if it is suspected that the sample contains prolymphocytes or lymphoblasts that tend to rupture. Albumin reduces the appearance of spots or basket cells, often visible in violations of lymphoid marrow. the preparation procedure can also be carried out using the use of caps instead of slides. Coverslip method requires adroit manipulation, but can yield better morphological information, since smaller caps cause less cell rupture during separation. The use of glass slides allows for automated painting, and the preparations of the lids must first be fixed on the side of the smear to the slides and then painted manually (section 16). Imprints (touch preparations) Samples of the core biopsy and clot marrow can be stored in geese and re-touched to the washed glass slide or lid to secure the cells and dry quickly. The scientist lifts directly upwards to avoid cell distortion. Imprints are valuable when the specimen is clotted or has a dry tap: cell morphology can closely replicate the morphology of aspirate, although few spirals are transmitted. Concentrate (buffalo fur) smears Buffy fur smears are useful when the direct marrow smear contains rare nuclei cells or when the number of nuclei cells should be low, as in aplastic anemia. The scientist transfers approximately 1.5 ml of the K3EDTA anticoagulant marrow sample to a narrow well glass or plastic tube, such as a Wintrobe hematocrit tube. Centrifuge the tube for 10 minutes at 2500 g and be analysed for four layers. The top layer is yellowish fat and usually takes a column of 1% to 3%. The second layer, plasma, varies in volume, depending on the peripheral amount of blood in the sample. The third layer consists of nuclei cells and is called the myeloid-erythroid (ME) layer. The ME layer is usually between 5% and 8% of the total column. The lower layer is RSB, and its volume, like the plasma layer, depends on the peripheral blood count. The scientist records the ratio of fat to ME layers using millimeter graduation in a tube. After examining the column, the scientist reaches part of the ME layer with the plasma part and transfers the suspension to a petri dish or clock glass. Marrow smears are then prepared using the technique of shredding smear. Concentrated buffalo fur smear compensates for hypocelilated marrow and allows to study a large number of nuclei cells without fat or RSB interference. On the other hand, the procedure distorts the distribution of cells. Therefore, the scientist does not assess the number of different cell types or maturation stages on the purulent fur smear. Following the preparation of aspirate smears by the scientist and the distribution of marrow aliquots for cytogenetic, molecular and immunophenotypic tests, the remaining basic biopsy sample, a sample of spirals or clots shall be submitted for histological analysis. The sample is suspended with 10% formaline, Zenker glacial acetic acid or B5 fixative for approximately 2 hours. The fixed sample is then centrifuged, the granules are decalcified and wrapped in a placement bag or lens paper and placed in paraffin Cartridge. A A a sample of hematoxine and eosin (H&E) paints in sections and a section. Marrow smear dye Marrow aspirate smears are painted with Wright or Wright-Giemsa ink using the same protocols as for the staining of peripheral blood films. Some lab managers increase the staining time to compensate for the relative thickness of marrow smears compared to peripheral blood films. Marrow aspirate smears and core biopsy samples may also be stained using an iron ferricyanide (Prussian blue) solution to detect and evaluate abnormalities in the metabolism of marrow (Section 20). In addition, some cytochemical dyes may be used for cell identification or differentiation (Table 17-2). Cytochemical dyes, used to identify bone marrow cells and maturation stages Cytochemical dye application Myeoperoxidase (MPO) Detects myelolocal cells by staining cytoplasmic granules Sudan black B (SBB) Detects myelolocal cells by staining cytoplasmic granules Periodic-Schiff (PAS) Detects lymph Led cells and certain abnormal abnormal erythrocytic cells by staining cytoplasmic glycogen Esterase Release myelocytics from the monocytic stages of puberty (multiple esterase substrates) Tartrate-resistant acid phosphatase (TRAP) Detects tartrate-resistant acid phosphatase granules in hairy cell leukemia Study bone marrow aspirate or embossed describes bone marrow aspirate or embosing the use of low and high power targets for the study of direct smears or embosseds of bone marrow aspirate. Box 17-1 Bone marrow aspirate microscopic smear test: low and high power Low power: 10x target (100x total augmentation) • Evaluate peripheral dilution • Find bone spirals and clear cell morphology areas • Observe the fat-suck ratio, cell evaluation • Tumor cell search in clusters • Investigate and evaluate megakaryocytes High power: 50x and 100x target (500x and 1000x total magnification) • Watch myelo and erythrocyte maturation • Disperse the distribution of abnormal cells or maturation stages of cells or cells • Perform differential numbers from 300 to 1000 cells • Calculate myeloid and erythramide ratio Low power (100×) study When the bone marrow reaches a direct smear or imprint, prepared and stained, the scientist or pathologist begins microscopic examination using a low-power (10×) dry lens, which, tied to 10× of the eye, provides a total of 100× augmentation. Most bone marrow studies are conducted using a training report format that employs projection or multi-headed microscopes to monitor the population, fellows, medical lab students, and attend staff. The microscope finds bone spirals, bone and blood vessels that stain dark blue (Figure 17-5). In imprints, spirals are rare or absent, and the search is in the absence of spirals. In these areas, the microscope selects intact and near-adjacent nuclei cells for analysis, avoiding morphology or areas diluted with sinusoidal blood. FIG 17-5. This is a good site for morphological evaluation and cell counting (Wright stain, 100×). In addition to sculptural cellular, cellular communication is assessed by observing the proportion of hematopoiesis in adipocytes (transparent areas of fat).9 In the case of anterior or posterior iliction carcass marrow, 50% of cells are normal in patients aged 30-70 years. In childhood, the cellular connection is 80%, and after 70 years the cells decrease. For those over 70 years of age, the rule of thumb is to deprive the patient of the age of 100% and add ±10%. Thus, at the age of 75 years, the expected cell connection is from 15% to 35%. Compared to age-related normal cell values, the microscope classifies the observed area as hypocellular, normocellular or hypercellular. If a basic biopsy sample has been collected, it provides a more accurate evaluation of cells than an aspiration smear, since aspirates always contain a slight dilution of hematopoic tissue with peripheral blood. In the absence of leukemia, lymphocytes should total less than 30% of nucleitic cells; if there are more of them, the marrow sample has been substantially diluted and should not be used to estimate the cell ratio.10 Using a 10× target microscope looking for abnormal, often formed, metastatic tumor cell groups (syncytia) or lymphoblasts. The nuclei of tumor cells are often painted dark (hyperchromatic), and vacuous are visible in the cytoplasm. Clusters of tumor cells are often found near the edges of the smear. While myelocytic cells and erythrocyte cells are best studied using 500× enhancement, they can be easier to distinguish from each other using a 10× target. Stages of erythrocyte puberty stain more intense, and their margins are more defined, more easily distinguished by a smaller increase. The microscope evaluates megakaryocytes using low power (Fig. 17-6). Megakaryocytes are the largest bone marrow cells with multilayered nuclei between 30 and 50 μm diameter (chapter 13). Although in special circumstances microscopes can differentiate the three stages of maturation of megakaryocytes megakaryoblast, promegakaryocyte and megakaryocyte (MK-I to MK-III)—the overall estimate of megakaryocytes is generally satisfactory. In a well-prepared aspirate or biopsy sample, the microscope monitors 2 to 10 megakaryocytes per low power field. Deviations give important information and are reported as decreased or increased megakaryocytes. Estimates of megakaryocytes of the bone marrow are necessary for the evaluation of peripheral blood thrombocytopenia and thrombocytosis; for example, in the case of immune thrombocytopenia, the marrow megakaryocytes multiply significantly. FIGURE 17-6 Bone marrow aspirate smear showing megakaryocyte with young platelets in the plasma membrane (Wright spot, 1000×). Megakaryocytes are counted 100×, but if there is abnormal cells are tested for 500× or 1000×. Abnormal megakaryocytes may be small, lacking in detail, or poorly lobulated or hyperlobulated nuclei. Signs of anomaly can be seen with low power; however, convincing descriptions require a total increase of 500× or even 1000×. High power (500×) With the right test site, the microscope places a drop of submersible oil on the sample and switches to the 50× target, ensuring a 500× total increase. All nuclear cells are reviewed for morphology and normal maturation. In addition to megakaryocytes, myelocytes (17-7-17-10 digits) and erythrocytes (rubrics, normoblastic; Figure 17-11) series should be accompanied by eosinophils, basophils, lymphocytes, plasma cells, monocytes and histhiocytes. Chapters 7, 8 and 12 describe in detail the descriptions of the maturation phase of cells and cells. Table 17–3 lists all normal marrow cells and presents their expected percentages. 17-7 EIA Bone marrow aspirate smear. Myelocytic stages include myeloblast (MyBl), promyelocyte (ProMy), and myelocyte (Myel). The diameter of lymphocytes (lymph) illustrates its size compared to myeloid stages. The source of lymphocytes is the blood of the sinuses (Wright spot, 1000×). 17-8 EIA Bone marrow aspirate smear. Myelocytic stages include myeloblast (MyBl), promyelocytes (ProMy), myelocyte (Myel), and metamyelocyte (Meta). There is one orthochromic normoblast (OrthoN) and one lymphocyte (lymphocyte) (Wright spot, 1000×). 17-9 EIA Bone marrow aspirate smear. Myelolocal stages include myelocytes (Myel), metamyelocyte (Meta) and neutrophic bands (Wright spots, 1000×). FIG 17-10. Bone marrow aspirate smear illustrating neutrophinal bands and segmented neutrophils (SEG) (Wright stain, 1000×). FIGURE 17–11 Bone marrow aspirate smear showing an island of erythrocyte precursors with polychromatophilic and orthochromic normoblastes (Wright stain, 1000×). Expected cell distribution and stages of maturation of cells in aspirates or imprints Cell or cell maturation stage distribution of cells or cells in myeloblasts 0%-3% Pronormoblasts/rubriblasts 0%-1% Promyelocytes 1%-5% Baso philic normoblasts/prorubricites 1%-4% Myelocytes 6%-17% Polychromatophilic normoblasts/rubricytes 10%-20% Metamyelocytes 3%-20% Orthochromic normoblasts/metarubricites 6 %–10% Neutrophilic bands 9-32% Lymphocytes 5-18% Segmented neutrophils 7%-30% Plasma cells 0%-1% Eosinophils and eosinophil precursors 0%-3% Monocytes 0-1% Baso phils and stem cells 0 %-1% Histiocytes 0%-1% Megakaryocytes 2-10 visible through the low power field Myeloid to erythroroid ratio 1,5:1–3.3 : 1 Microscope looks for maturation gaps, improper distribution of maturation stages, abnormal morphology. Although the sample is usually viewed using a 50× immersion target, the 100× oil immersion target is often used for small but significant detection of disorders. Set. nuclei and cytoplasm of suspicious cells. Many laboratory directors require a differential number of 300-1000 nuclei cells. These seemingly large amounts are quickly achieved in a well-prepared bone marrow smear with 500× increasing and statistically compensating for the expected uneven distribution of spirals and haematopoetic cells. The microscope counts the cells and maturation stages surrounding several spirals to maximize the ability to detect disease-related cells. Some lab directors overshadowed the differential for a thorough smear test. Many microscopes choose not to differentiate the stages of erythrocyte maturation of the quad nuclei, while others can combine three of the four – basophilic, polychromatophilic and orthochromic normoblasts – into one amount, counting only pronormoblasts separately. In the normal marrow, most of the erythrocyte precursors are polychromatophilic or orthochromic normoblasts, and differentiation gives little additional information. On the other hand, differentiation can be useful for megaloblastic, iron deficiency, or fire anemia. The microscope can rarely find osteoblasts and osteoclasts (Figure 17-12). Osteoblasts are responsible for the formation and reconstruction of bones, and they are obtained from endosteal (internal lining) cells. Osteoblasts are similar to plasma cells with eccentric round and oval nuclei and abundant blue, mild cytoplasm, but they lack the famous Golgi apparatus characteristic of plasma cells. Osteoblasts are usually found in clusters similar to myeloma cells. Their presence in marrow aspirates and the main biopsy specimens is accidental; they do not spread the disease, but they can cause confusion. 17-12 EIA Bone marrow aspirate smear indicating a cluster of osteoblast that superficially resembles plasma cells. Osteoblasts are round to oval eccentric nuclei and mottled blue cytoplasm that does not have secretions of pellets. They may have a clear area in the cytoplasm, but they lack a well-defined central Golgi plasma cell complex (Wright stain, 1000×). Osteoclasts are almost the diameter of megakaryocytes, but their multiple, evenly positioned nucleus separates them from the multilayered megakaryocytes nuclei (). Osteoclasts appear to originate from myeloid offspring cells and are responsible for bone resorption in combination with osteoblasts. Osteoclasts are more often recognized in the main biopsy specimens than in aspirates. Figures 17-13 A large multi-core cell near the endosteal surface is osteoclast, a cell that reabsorbed the bone. Spindle-shaped cells are fibroblasts (hematoxine and eosin stains, 500×). Adipoocytes, endothelial cells that correspond to blood vessels, and reticula-like cells of the fibroblast type complete the strom of the bone marrow (chapter 7). Stromal cells and their extracellular matrix ensure an appropriate microenvironment for maturation and proliferating rarely investigated for diagnosis of haematological or systemic disease. Finally, Langerhans cells, giant cells with palisade nuclei found in granuloma, signal chronic inflammation. Upon completion of the differential, the myeloid-erythroid (M:E) ratio is calculated from myeloid to total nucleus erythroid cell stages. Not included in the M:E ratio are lymphocytes, plasma cells, monocytes, histiocytes, non-nuclear erythrocytes and non-hematopoetic stromal cells. Prussian blue iron spots test Prussian blue (iron ferricyanide) iron spots are usually used for aspirate smear. illustrate normal iron, iron absence and increased iron reserves aspirate smears. Iron stain can be used for core biopsy samples, but decalifying substances used to soften the biopsy sample during processing can leach iron, which gives a false impression of reduced iron stocks or their absence. For this reason, aspirate is favorable for iron spots, if there are enough spirals. Figures 17-14×. A, Normal iron stores. B, Absence of iron stores. C, Increased iron stores. Bone marrow core biopsy sample test The standard dye of the main biopsy sample is H&E. Other colours and their objectives are listed. Bone marrow biopsy sample and imprint (touch preparation) tests are necessary when the aspiration procedure yields a dry tap, which can be the result of hypoplastic or aplastic anemia, fibrosis or strict packaging of the marrow cavity with leukemia cells. The main advantage of the main example of biopsy is the preservation of bone marrow architecture, so that cells, tumor groups (Table 17-4Figure 17-15) and maturation stages can be examined according to stromal elements. The disadvantage is that individual hematopoetic cell morphology is eclipsed. EIA 17-15 Bone marrow aspirate smear indicating tumour cell cluster or sincy. The nucleus is irregular and hyperchromatic, and the cytoplasm is vacuolated. Cytoplasmic margins are poorly excavated (Wright spot, 500×). Paints used in the analysis of the application of bone marrow biopsy samples to paint Hematoxin and eosin (H&E) Evaluate the distribution of cells and hematopoietic cells, find abnormal cell groups. Prussian blue (iron ferricaniside) iron stain Assess iron reserves due to shortage or excess iron. Decalcification can remove iron from fixed samples; therefore, ethylenediaminenotetraacetic acid chelate or aspirate smear is preferred for the assessment of iron storage. Retiulin and trichrome dyes Check for marrow fibrosis. Acid rapid spots to investigate acid fast bacillus, fungi, or bacterial granulomatous disease. Gram spots to investigate acid fast bacillus, fungi, or bacterial granulomatous disease. Dyspepgy for the use of the tumour surface markers Wright or Wright-Giemsa colourants Observe haematopoetic haematopoetic haematopoetic antibodies Structure. In a biopsy sample, cell identification is less accurate than an aspirate smear. The microscope first examines the main biopsy sample preparation using a 10× target (100× total magnification) to estimate the cell count. Because the sample is larger, the main biopsy sample provides a more accurate cellular estimate than the aspirate. The microscope compares cell areas with clearly developed adipoocytes using a method identical to that used to test aspiration smears for cell smears. All fields are checked because the cells are distributed unevenly. Examples of hypocellular and hypercellular core biopsy sections are presented for comparison with normocellular marrow in Figure 17-16. 17-16A EIA, Representative section of the main biopsy indicating normal cell counts, approximately 50% fat and 50% hematopoietic cells (hematoxine and eosoxin, 50×). B, A sample of hypocellular core biopsy with only fat and connective tissue cells from a patient with aplastic anemia (hematoxine and eosin stains, 100×). C, a sample of hypercellular core biopsy from a patient with chronic myelogenous leukaemia. There are almost 100% fat-free cells visible (hematoxine and eosin spots, 100×). Source: (Bcourtesy Dennis P. O'Malley, MD, Director, Immunohistochemistry Laboratory, Indiana University School of Medicine, Indianapolis, IN.) Megakaryocytes are easily recognizable by their outsized diameter and even distribution throughout the biopsy. They show the characteristic lobe nucleus, although the core of the more mature megakaryocytes is smaller and darker painted in H&E preparations than in Wright-painted aspirate. Their cytoplasm ranges from pale pink younger cells to dark pink in older cells (). Due to the larger volume of the sample, microscopes more accurately estimate the number of megakaryocytes, examining the main part of the biopsy than the aspiration smear. There are usually 2 to 10 megakaryocytes per 10× area, like an aspirate smear or imprint. Figure 17-17 FIG 17-17×. Using the 50× oil immersion target, the microscope monitors the distribution of cells in the bone marrow stroma. For example, in people over 70 years of age, normal lymphocytes can form small aggregates in non-paratrabeculous regions, and groups of malignant lymphoma cells are often paratrabecular. In addition, normal lymphocytes remain as separate cells, and lymphoma cells are pleomorphic and synth. If aspirate or imprint smears cannot be prepared, the core biopsy sample may be painted with Wright, Giemsa or Wright-Giemsa ink to limit cell morphology observations. In the biopsy sections painted by Wright- or Giemsa, myelolasts and promyelocytes have oval or round nuclei with cytoplasm that stain blue (). Neutrophinic myelocytes and metamyelocytes are pale pink cytoplasm. Mature segmented neutrophils and neutrophilic bands (BAND) are recognised as smaller diameters and dark-coloured C-shaped kernels (BAND) or nuclear segments (SEG). The cytoplasm of BAND and SEG may be pale pink or may appear unpinned (Fig. 17-18, Fig. 17-1×8 FIG. 17-19× EIA. The cytoplasm of eosinophils paints red or orange, so they are the most pronounced marrow cells. Basophils can not be identified on marrow biopsy samples attached with Zenker glacial acetic acid solution. The microscope may find it difficult to distinguish myeloid cells from erythrocyte cells in biopsy samples, except to note that the latter tend to cluster with more mature normoblastes and often surround histiocytes. Polychromatophilic and orthochromic normoblasts, the two most common stages of erythrocyte maturation, have a centralized, round core that spots intensively (). Their cytoplasm is not noticeably painted, but a significant margin of the plasma membrane is clearly observed, which gives the cells of these stages the appearance of a fried egg. Since erythrocyte cells tend to cluster in small groups, they are more easily recognizable using a 10× goal, although their individual morphology cannot be seen. Figures 17-20 The appearance of late-stage normoblasts is often fried (Giemsa stain, 400×). Lymphocytes are one of the most difficult cells identified in this biopsy sample, unless they occur in clusters. Mature lymphocytes are characterized by barbed nuclear chromatin in a small, round nucleus, accompanied by a small amount of blue cytoplasm (). Immature lymphocytes (prolymphocytes) have larger round or lobulating nuclei, but still only a small rim of blue cytoplasm. Figures 17-21 Several of them are immature with larger nuclei, which contain one pronounced nucleolus (Giemsa, 400×). Plasma cells are difficult to distinguish from siocytes in H&E painted sections, but they are identified by the wright-giemsa dye as cells with eccentric dark nuclei and blue cytoplasm and a prominent light central Golgi apparatus (Figures 17-22). Characteristically, plasma cells are located near blood vessels. FIG 17-22. The nucleus is eccentric, the cytoplasm is blue with a famous central unsponched Golgi apparatus (Giemsa stain, 400×). Final bone marrow tests Although in most cases the aspirate smear and biopsy sample are diagnostic, additional tests may be required. Such studies and their applications shall be submitted . These tests require an additional bone marrow volume and a specialised collection of samples. Information on Prussian blue iron stains and submitted earlier. Each study is described in the section in Table 17-5 in Table 17-5. Final studies carried out on selected bone marrow samples bone marrow test application Example Iron stain iron deficiency detection, iron overload Fresh marrow aspirate 20 Cytochemical tests diagnosis of leukemia and lymphomas Fresh marrow aspirate 29, 33, 35, 36 Cytogenetic studies Diagnosis of acute leukemia by removal, translocation and polyomyoso; remission tests 1 ml marrow heparin 30 Molecular tests Polymerase chain reaction for diagnostic point mutations; minimal residual disease studies 1 ml marrow EDTA 31 Fluorescence in situ hybridization For diagnostic mutations; minimal residual disease tests Fresh marrow aspirant 31 Flow cytometry Immunophenotyping, usually malignant haematopoetic cells, clonanum; minimal residual disease tests per ml of marrow heparin, EDTA or ACD 32 ACD, acid citrate dextrose; EDTA, ethylenedinotetraacetic acid. Bone marrow study reports The components of the Bone Marrow Report should be generated systematically and presented. An example of a bone marrow test report is given in Table 17-6, Table 17-23. FIG 17-23. PB,Peripheral blood; BMA, bone marrow aspirate; BMBX, bone marrow biopsy sample; DX, diagnosis. Components of the bone marrow study report component Description Patient's history Patient identity and age, storytelling of symptoms, physical data, Findings, kindred, treatment complete blood count (CBC) peripheral blood CBC collected no more than 24 hours before bone marrow puncture, includes hemogram and peripheral blood film test Cell hypocellulose, normcellular, or hypercellular classification based on the ratio of blood top cells to adipocyte Megakaryocytes invert on using 10× goal (100× , compare with the reference range and comment on the narrative of maturation of morphology describing the puberty of myelocytic and erythrocyte (normoblastic, rubricytic) series of supplementary haematological cells Narrative, describing the number and morphology of eosinophils, basophils, mast cells, lymphocytes, plasma cells, monocytes and histhioocytes, where appropriate, with stromal cells Narrative describing the number

and morphology of osteoblasts, osteoclasts, bone trabeculae, fibroblasts, adipocytes and endothelial cells; the appearance of sinuses; presence of amyloid, granuloma, fibrosis, necrosis differential number The number of all cell and cell stages observed in differential numbers from 300 to 1000 cells and comparing the results with the reference intervals The ratio of myoids to erythroroids is calculated by the breakdown into categories of demepatometer hematological cells, less lymphocytes, plasma cells, monocytes and histiocytes Iron findings Normal or decreased iron store Diagnostic story Summary of recorded data and additional laboratory chemical, microbiological and immunological studies Summary • Adult haematopoetic tissue is found in flat bones and at the ends of long bones. Hematopoiesis occurs through spongy trabeculae bones near the vascular sinuses. • Bone marrow collection is a safe but invasive procedure performed by a pathologist or hematologist in cooperation with a medical laboratory scientist to obtain samples used to diagnose and monitor haematological and systemic diseases. • The necessity of a bone marrow test should be assessed in the light of all clinical and laboratory information. In anemia, the cause of which is evident from rbc indices, bone marrow study is not required. Examples of indications for bone marrow testing include multi-line abnormalities in peripheral blood, pancytopenia, circulating explosions and stopping lymphomas and carcinomas. • A peripheral blood sample is collected for a full blood count no more than 24 hours before bone marrow collection and the CBC results are presented together with the results of the bone marrow test. • Bone marrow may be collected from the posterior or anterior iliac ridge or sternum using sterile single biopsy and aspirational needles and cantons. The location and equipment depends on how old the patient is and whether both aspirate and biopsy sample are desired. • The medical laboratory scientist receives a bone marrow sample and prepares aspirate smears, crushed preparations, imprints, anticoagulant bone marrow smears and fixed parts of the biopsy and samples for confirmatory tests. • The scientist and pathologist of the medical laboratory cooperates with residents, buddies, visiting doctors and students of medical laboratory science to paint and review bone marrow aspirate smears, biopsy departments and the results of the confirmatory procedure. • Confirmatory procedures include cytochemistry, cytogenetics and immunophenotyping flow cytometry; fluorescence in situ hybridisation; molecular diagnostics. • The scientist and pathologist of the medical laboratory determine the distribution of cells and megakaryocytes, then perform a differential number from 300 to 1000 bone marrow bloodthropist cells and calculate the ratio of M:E by comparing the results with the reference intervals. • The pathologist describes the features of haematopoetic disease, metastatic tumour cells and bone marrow stromal abnormalities and prepares a systematic written report on bone marrow testing, including diagnostic narrative. Now that you have completed this section, go back and read the case study again at the beginning and answer the questions below. See the answers to the questions can be found in the Appendix. 1. Where is the most hematopoetic tissue found in adults? a. Liver b. Lungs c. Spleen d. Long bones 2. What is desired in bone marrow collection Adults? A. A. intercultural space on the sternum b. Front or posterior iliac ridge c. Any thoracic vertebra on Dec. Front head of the femur 3. Aspirate should be tested with little power to evaluate all of the following except: a. Cell b. Megakaryocyte numbers c. Abnormal cell morphology d. Presence of tumour cell groups 4. What is the normal M:E ratio range for adults? a. 1.5:1 to 3.3:1 b. 5.1:1 to 6.2:1 c. 8.6:1 to 10.2:1 d. 10:1 to 12:1 5. Which are the most common stages of erythrocytes found in normal marrow? a. Pronormoblasts b. Pronormoblasts and basophilic normoblasts c. Basophilic and polychromatophilic normoblasts Polychromatophilic and orthochromic normoblasts 6. What cells, sometimes seen in bone marrow biopsy samples, are responsible for bone formation? a. Macrophages b. Plasma cells c. Osteoblasts d. Osteoclasts 7. What is the largest hematopoetic cell found in normal bone marrow aspirate? a. Osteoblast b. Myeloblast c. Pronormoblast d. Megakaryocyte 8. Which of these signs is not an indication for bone marrow testing? A. Pancytopenia (decreased number of RSB, PBCs and platelets in peripheral blood) b. Anaemia with RBC indices corresponding to low serum iron and low ferritin c. Detection of blasts in peripheral blood on Dec. Need for hodgkin's lymphoma stop 9. In a bone marrow biopsy sample, RBC precursors are estimated to account for 40% of marrow cells, while the other 60% were granulocyte precursors. What is the M&E ratio? a. 4:6 b. 1.5:1 c. 1:1.5 d. 3:1 10. Several large cells with multiple nuclei were marked in a bone marrow biopsy sample. They were located near the endosteum, and their nucleus was evenly positioned throughout the cell. What are these cells? a. Megakaryocytes b. Osteoclasts c. Adipocytes d. Fibroblasts 11. The advantage of the underlying biopsy bone marrow sample against aspirate is that the main biopsy sample: a. It is possible to purchase a less invasive collection technique b. Allows you to evaluate the architecture and cellular layout c. Retains basophil staining properties due to Zenker fixation on Dec. There are better bone marrow iron stores with Prussian blue spots for links 1 rating. Koury M.J, Lichtman M.A. Marrow structure and haematopoetic microenvironment. In: Kaushansky K, Lichtman M, Beutler E, et al. Williams hematology. 8th ed. New York: McGraw Hill 2010; 41-74. 2. Farhi D.C. Pathology of bone marrow and blood cells. 2nd ed. Philadelphia: Lippincott Williams & Wilkins 2008. 3. Foucar K, Reichard K, Czuchlewski D. Bone marrow pathology. 3rd Ed. Chicago: ASCP Press 2010. 4. Silberstein L, Scadden D. Hematopoetic microenvironment. In: Hoffman R, Benz E.J, Silberstein L, et al. Basic principles and practices of haematology. 6th ed. St. Louis: Elsevier 2013. 5. Reddy V, Marques M.B, Fritsma G.A. Rapid Hematology Testing Guide. 2nd ed. Washington, DC: AACC Press 2013. 6. Perkins L. Study Study blood and bone marrow. In: Greer J.P., Arber D. A, Glader B, et al. Wintrobe clinical hematology. 13. Philadelphia: Wolters Kluwer Health/Lippincott Williams & Wilkins 2013. 7. Ryan D. H. Marrow Study. In: Kaushansky K, Lichtman M, Beutler E, et al. Williams hematology. 8th ed. New York: McGraw Hill 2010. 8. Krause J.R. Bone marrow biopsy. New York: Churchill Livingstone in 1981. 9. Hartsock R.J. Smith E.B, Pett C.S. Normal variants with aging hemopoetic tissue content in the bone marrow from the anterior iliac ridge. Am J Clin Pathol; 1965; 43:326-331. 10. Abrahamson J.F, Lund Johansen F, Laerum O.D. et al. Cytometric assessment of peripheral blood contamination and proliferative activity in human bone marrow cell populations. Cytometry; 1995; 19:77-85. *The author thanks Lynne Shaw, MT (ASCP), director of the Bone Marrow Pathology Laboratory at the University of Birmingham, for her essential contribution to this department.