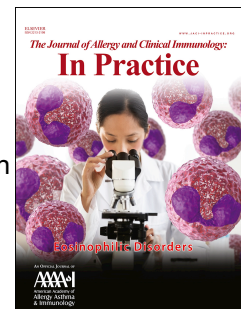


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The role of bone marrow evaluation in clinical allergy and immunology practice: When and why

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ABSTRACT

Allergists and Immunologists rely on other specialists for higher risk procedures such as biopsies of the lung or gastrointestinal tract. However, we perform and interpret a handful of procedures ourselves. Training programs have historically required competency for prescribing immunoglobulin infusions, patch testing, rhino laryngoscopy, lung function testing, and provocation testing for airway hyperreactivity even though other specialists often perform them.

Bone marrow aspirations and biopsies are not included in fellowship training assessments despite a significant number of marrow evaluations being requested by Allergists and Immunologists. For example, nearly one marrow assessment per month has been requested over two years for patients in the Allergy Immunology clinic at Walter Reed National Military Medical Center. Marrow assessments are often required for diagnosis, monitoring, and treatment related toxicities. Interpretive and procedural competency would benefit the field given the range of diseases in clinical immunology practice that requires marrow assessment.

We have generated a comprehensive list of the major conditions that might require bone marrow assessments in any Allergy and Immunology practice. We then summarize the specific tests that must be ordered and show how to determine sample quality. Finally, some providers may desire procedural competency and for those individuals we discuss tips for the procedure.

INTRODUCTION

The bone marrow biopsy procedure has its roots in the trepanning of bone and is considered the oldest medical procedure (1). The cellular marrow inside the cortex is also a central lymphoid organ where pluripotent stem cells generate common lymphoid as well as multipotent myeloid stem cells. These progenitors undergo a maturational process to develop into all cellular components of the blood. The marrow is also a destination for mature plasma cells that return from peripheral lymphoid tissue. Early stem cells are morphologically indistinct but have identifying markers and are able to differentiate into specific lineages *ex vivo*. Examination of bone marrow can reveal clonality as well as defects in leukocyte maturation, proliferation, and function. A marrow examination can also be used to support the diagnosis of an autoimmune cytopenia where there is peripheral destruction of blood cells in the reticuloendothelial system.

INDICATIONS

Bone marrow analysis is indicated in a variety of immunological and allergic diseases including: immunodeficiencies, hemophagocytic lymphohistiocytosis (HLH), mast cell disease, hypereosinophilic syndromes, acquired C1 inhibitor deficiency, and for hematopoietic bone marrow transplant purposes (Table I). In several of these disorders, the underlying diagnosis can guide if, when, and how frequently to assess the marrow. However, novel disorders of the immune system continue to be defined (or remain yet to be defined) and the possibility of a bone marrow assessment should be carefully considered in each case. A detailed summary of each disorder is outside the scope of this article but groups of disorders are summarized below to highlight important points.

Immunodeficiency

Patients with immunodeficiencies are at increased risk of three diseases that can necessitate longitudinal marrow evaluations. These include bone marrow failure, lymphoma, and myelodysplasia. Additionally, infections can occupy the marrow and, therefore bone marrow cultures are indicated for subjects with immunodeficiency (Table II). It is important to note that a well-defined clinical and cellular phenotype commensurate with a novel genetic diagnosis can take years to be established. The presence of marrow failure, myelodysplasia, or lymphoma can help a clinician narrow the differential diagnosis prior to obtaining or elucidating a known or novel genetic diagnosis. Many immunodeficiency diseases can have distinct marrow phenotypes even beyond marrow failure, myelodysplasia, and lymphoma. These unique phenotypes have been described in diseases such as CTLA4 haploinsufficiency (2), GATA2 deficiency (3), WHIM syndrome (4), PIK3CD gain of function (5), and recombination activating gene 1 or 2 (RAG1 or RAG2) deficiency (6,7). For example, bone marrow B-cell immunophenotyping can reveal hyperplasia of precursor B-cells known as hematogones with nearly absent mature B cells in patients with PIK3CD gain of function. B-cell immunophenotyping of marrow has also been used to demonstrate arrest of B-cell development at the pre-B cell stage leading to an increase in immature pro-B cells in patients with X-linked agammaglobulinemia (8).

Marrow failure describes any degree of marrow hypocellularity readily observed in the bone marrow biopsy (not necessarily in the marrow aspirate) in the context of a peripheral cytopenia (Figure 1). Marrow failure is termed “aplastic anemia” when the biopsy

cellularity falls below 25%(9). Myelofibrosis, lymphoid infiltrates, and granulomas may contribute to marrow failure in patients with infections or autoimmune cytopenia and many can improve with immunosuppressive therapy(10,11). Normal ranges of marrow cellularity are also based on age. Cellularity is 100% at birth and declines 10% each decade of life. A relevant cytopenia includes neutropenia, thrombocytopenia, or anemia. A single cytopenia is likely to develop after the development of marrow failure and the presence of a cytopenia is frequently identified in patients with immunodeficiency disease(12). A patient with a single or multiple lineage cytopenias would have a hypoplastic marrow if marrow failure is present or a normocellular or even hypercellular marrow if there is peripheral destruction by the reticuloendothelial system. Thrombocytopenia, anemia, and neutropenia are each an indication for bone marrow evaluation even in the absence of immunodeficiency disease. Each type of cytopenia is evaluated in a similar manner and standard clinical practices have been published for each(13–15). A marrow analysis can also help diagnose myelosuppression secondary to infections, medications, toxins, and nutritional deficiencies(16,17).

Marrow failure is associated with most categories of immunodeficiencies(18) including severe combined immunodeficiencies, combined immunodeficiencies, hypogammaglobulinemia, defects in immune tolerance and regulation, defects in phagocyte function, and type I interferonopathies. Examples of severe combined immunodeficiencies associated with marrow failure include: CERNUNNOS/XLF deficiency (19) and LIG4 deficiency (18). Examples of combined immunodeficiencies associated with marrow failure include: PGM3 deficiency (20), Dyskeratosis congenita (2), Schimke syndrome (21),

Cartilage-hair hypoplasia(22), SAMD9 gain of function (18), and SAMD9L gain of function (18). IKAROS deficiency is found in some patients with hypogammaglobulinemia and may progress to marrow failure (23). Fanconi anemia is a well-known congenital bone marrow failure syndrome that has recently been associated with immune dysregulation often described as common variable immunodeficiency (24). CTLA4 haploinsufficiency is an example of a defect in immune regulation and tolerance and has been linked with marrow failure (2). Marrow failure is associated with defects in phagocyte function including GATA2 deficiency (2) and Shwachman-Diamond syndrome (2). Investigators of a French cohort of patients with marrow failure found that certain germline variants associated with immunodeficiency are found at increased frequency including SAMD9L, SAMD9, TERC (dyskeratosis congenita), GATA2, and SBDS2 (Shwachman-Diamond syndrome) (18). DADA2 syndrome is both an autoinflammatory and immunodeficiency disease and has been associated with marrow failure (25).

Myelodysplastic syndrome (MDS) or myelodysplasia is seen at increased frequency in patients with immunodeficiency. Several groups at increased risk are patients with severe congenital neutropenia such as elastase deficiency, HAX1 deficiency, and Shwachman-Diamond syndrome; patients with reduced phagocyte function such as GATA2 deficiency and patients with combined immunodeficiency including patients with gain of function variants in SAMD9 and SAMD9L as well as MYSM1 deficiency (26,27)²². Myelodysplasia often results in hypercellularity of the marrow though normocellular and hypocellular forms exist. A blast percentage less than 20% is characteristic of MDS and greater than 20% is seen in acute myeloid leukemia.

137

138 Chronic severe neutropenia (ANC < 500) in isolation will often present with oropharyngeal,
139 soft tissue, and respiratory infections. Bone marrow assessment is indicated to evaluate for
140 marrow failure and myelodysplasia (14,28,29). The assessment of myeloid maturation of a
141 patient with neutropenia could reveal early myeloid arrest at the promyelocyte stage. This
142 finding would support a diagnosis of severe congenital neutropenia. A variety of genetic
143 determinants underlies severe congenital neutropenia as previously described
144 (4,14,28,29). A hypercellular bone marrow with increased mature neutrophils is a
145 distinctive feature in the syndrome known as warts, hypogammaglobulinemia, infections,
146 and myelokathexis syndrome where there is retention of marrow neutrophils due to
147 hyper-responsiveness of the chemokine receptor CXCR4 (4).

148

149 Lymphoma accounts for approximately two thirds of all malignancies identified in patients
150 with immunodeficiency (30). Lymphomas are thought to invade the bone marrow in up to
151 50% of cases (31). Patients with common variable immunodeficiency, combined syndromic
152 and non-syndromic immunodeficiency, susceptibility to EBV (32), immune dysregulation
153 (33), and defects in innate immunity are at increased risk of lymphoma (34). Nearly 10% of
154 patients with common variable immune deficiency developed lymphoma in one study over
155 forty years (35) suggesting an even higher lifetime risk. B cell lymphoma is thought to be
156 more common overall in patients with immunodeficiency, likely related to chronic EBV
157 infection. Infection and lymphoma may mimic one another. A bone marrow analysis is
158 indicated in the work up of a patient with immunodeficiency if an infection source is not
159 identified or if antibiotic treatment does not improve symptoms. Routine monitoring of

EBV is important in many immunodeficiency diseases since increasing concentration of EBV by quantitative PCR would raise the concern for a developing lymphoma. Determination of EBV genome load in T, B, and NK subsets may be useful in people with genetic susceptibility to EBV such as those with X-linked lymphoproliferative disease and people with chronic active EBV without a known genetic cause including individuals from Asia as well as Central and South America (36,37).

Hemophagocytic lymphohistiocytosis

HLH is characterized by uncontrolled immune activation often driven by infections such as EBV. It is typically associated with an underlying immunodeficiency, malignancy, or rheumatologic disease. Acute illness often necessitates urgent bone marrow evaluation. Bone marrow evaluation is indicated to evaluate for hemophagocytosis and to rule out leukemia and lymphoma (Figure 1). Diagnostic criteria for HLH include: fever, splenomegaly, cytopenias of two cell types (red cells, platelets, and neutrophils), hypertriglyceridemia, hypofibrinogenemia, hyperferritinemia, elevated soluble CD25, hemophagocytosis (bone marrow, lymph node, or spleen), and low or absent NK cell cytotoxicity. Familial HLH is often characterized by narrow downstream defects of the cytolytic immune synapse whereas other immunodeficiency diseases associated with HLH expression occur with forms of albinism or diseases with more widespread immune dysfunction. The broad array of diseases and genetic defects associated with HLH supports the development of competency in marrow analysis (38–40).

Acquired C1-esterase inhibitor deficiency

Acquired C1-esterase inhibitor deficiency is an indication for a marrow assessment. The disease is diagnosed by recurrent angioedema without hives or itch and by low C4, C1 inhibitor, and C1q. These patients present as older adults without a family history of angioedema. A majority will have underlying disease including monoclonal gammopathy with or without multiple myeloma or an indolent lymphoma. The likelihood of underlying myeloma with a high-risk monoclonal gammopathy (41) and bone marrow involvement with lymphoma(42) is high and suggests that most patients with acquired C1-esterase inhibitor deficiency should have at least one bone marrow assessment.

Mast cell disease and hypereosinophilic syndromes

The management of diseases of type 2 immunity including: systemic mastocytosis, idiopathic anaphylaxis, mast-cell activation syndrome as well as hypereosinophilic syndrome typically includes a bone marrow assessment (Figure 1). Bone marrow analysis is needed to help rule out indolent systemic mastocytosis in patients with elevated baseline tryptase > 15 ng/mL, anaphylaxis to Hymenoptera sting and baseline tryptase > 11.4 ng/mL, idiopathic anaphylaxis, or mast-cell activation syndrome (43). Criteria have been proposed to guide when a bone marrow analysis is indicated in patients with idiopathic anaphylaxis (44). Additionally, a bone marrow analysis is needed to differentiate between indolent systemic mastocytosis, smoldering systemic mastocytosis, aggressive systemic mastocytosis, aggressive systemic mastocytosis with an associated hematologic neoplasm, and mast cell leukemia. Bone marrow molecular testing for KIT D816V by allele-specific PCR is required in the evaluation of systemic mastocytosis (Table II). Progression from indolent systemic mastocytosis to aggressive disease does occur and changes in clinical

parameters could prompt a repeat marrow evaluation (45,46). Marrow assessment is essential to evaluate hypereosinophilic syndrome to diagnose the myeloid variant and to discriminate this from systemic mastocytosis with eosinophilia (47). A marrow assessment is also important in patients with lymphocytic variant hypereosinophilic syndrome to help rule out angioimmunoblastic T-cell lymphoma (48,49). Bone marrow molecular testing for hypereosinophilic syndrome includes: KIT D816V allele-specific PCR, CHIC2 (4q12) Deletion (FIP1L1 and PDGFRA Fusion) RT-PCR, BCR/ABL1, and JAK2 V617F (Table II).

Transplant

Bone marrow assessment may be performed before and after a bone marrow transplant by protocol depending on the institution. Bone marrow biopsy cellularity can be a useful parameter to compare before and after transplant especially if poor initial engraftment of donor cells or loss of donor cells after initial engraftment is suspected. The pre-transplant conditioning regimen and immunosuppressive medications may damage stromal cells in the marrow and ultimately affect differentiation and function of hematopoietic progenitors and cellularity (50). Chimerism evaluation during hematopoietic bone marrow transplantation is another possible indication for marrow assessment although peripheral blood analysis is also often adequate (51).

STUDIES TO ORDER BEFORE THE PROCEDURE BEGINS

The “bone marrow” order will direct the hematopathologist to complete a set of standard studies using the first milliliter of aspirate as well as the two-centimeter biopsy (Table II). A bone marrow technician from hematopathology is typically available to accept the aspirate

and biopsy and prepare the basic studies at the bedside. The first bone marrow aspirate is used to make a series of smears. These are processed in hematopathology with special stains for viewing under light microscopy. A few of these stains include Perls Prussian Blue to assess marrow iron, myeloperoxidase for myeloid maturation, and periodic acid-Schiff (PAS) to view a variety of cells. Routine stains of the biopsy include Wright-Giemsa and hematoxylin and eosin for cellularity, reticulin staining to detect fibrosis, and Perls stain for iron. Biopsy sections are viewed under light microscopy after being stained or following immunohistochemistry. Aspirate is viewed under light microscopy after being stained and cells in the aspirate are also assessed by flow cytometry. Inspection of the marrow biopsy is important to look for: cellularity; lymphoid aggregates; granulomas; dysplasia; mast cell morphology and clustering; eosinophilia; EBV-encoded RNA (EBER) expression; fibrosis; maturation; iron storage; and, blast percentage.

Most patients require additional orders in addition to the basic bone marrow order needed by the hematopathologist. A second aspirate is used for the remaining studies shown in Table II. Cytogenetics and clonality studies (immunoglobulin receptor and TCR rearrangements) as well as EBV/CMV PCR are studies that are included in almost all bone marrow assessments to help evaluate for neoplasia. Immunodeficiency disease, HLH, hypereosinophilic syndrome, and mast cell disorders each require special studies of the aspirate.

SAMPLE QUALITY

There are several methods to determine marrow aspirate quality and hemodilution as well as biopsy quality during the procedure and several days later after sample processing and analysis by hematopathologists. The presence of multiple cellular particles containing bone and fat called spicules in the aspirate ensures sample adequacy. Immediately after aspiration, 0.1 mL of aspirate is viewed grossly for the presence of spicules in a small petri dish followed by spreading them on slides (Figure 2). If 5 or more spicules are present, then additional aspirate volume can be collected from the same site for analysis. It may be prudent to reenter the bone marrow at a different site and recollect samples if there are fewer than 5 spicules seen on the first aspirate. The initial 1 mL of aspirate is typically the least hemo-dilute bone marrow sample and is used to generate slides for morphological assessment. A good quality biopsy specimen will be at least 2 cm in length and will appear red in coloration. Sections of collagen or cortical bone within the sample may appear white and can reduce the amount of biopsy material suitable for microscopic analysis by the pathologist. Although 1 cm or less may be needed by the pathologist for establishing a diagnosis, a larger more generous specimen will often obviate the need to return for a second sample (Figure 2).

The quality of remaining aspirate samples can be determined by microscopic evaluation of morphology and flow cytometry by the hematopathologist and provided in his or her report or during reviews by "tumor board" several days later. Peripheral blood does not typically contain substantial numbers of plasma cells, megakaryocytes, or hematopoietic stem cells but contains large numbers of platelets and CD10+ granulocytes. Platelets are not found in large numbers in the bone marrow. Flow cytometry can be used to assess

aspirate sample quality by detection of CD38++ CD138+ plasma cells, CD34+ hematopoietic stem cells, lymphocytes, monocytes and a low percentage of CD10+ granulocytes (52). Assessment of morphology by smears as well as surface markers by flow cytometry of the aspirate should show erythroid, myeloid, and lymphoid progenitors (53). Precursor B-cells known as hematogones should be identified by a characteristic pattern of antigen expression including CD10, CD20, CD34, and CD38 using flow cytometry. These populations can be mistaken for leukemia clones. Gating strategies for flow cytometry-based immunophenotyping of marrow aspirate will vary based on the underlying disease being evaluated.

PREPARING THE PATIENT

A bone marrow aspiration and biopsy are procedures with relatively low risk of major adverse events. The procedure has been outlined in detail elsewhere (54). There are several points worth mentioning before discussing or performing a biopsy. First, a patient may have negative perceptions of the procedure based on previous biopsies they may have undergone or from others they have known who have had a biopsy. A bone marrow aspirate and biopsy should not cause excessive pain when using appropriate reassurance and local analgesia technique. The vast majority of adults requires only local analgesia though pediatric patients will require conscious sedation. Second, a review of the patient's chart is important to note details that may increase the risk of complications such as anticoagulant use, blood counts including hemoglobin and platelet count, hardware implants, as well as the presence or risk of osteoporosis, soft tissue infection, or presence of subclinical coagulopathies. Very little pressure is needed to obtain good quality samples

since the needles are designed to enter bone by rotation. Osteoporosis in particular is not uncommon in patients seen in the immunology clinic due to long periods of corticosteroid use. Marrow aspiration and biopsy of a patient with osteoporosis should be executed with particular care since depth of entry through the bone cortex may be subtle. A scalpel is used to create a skin incision for ease of entry of the marrow biopsy needle, to prevent skin pinching by the needle, and to improve healing. Adverse events are quite rare but can include pain, soft tissue infection, osteomyelitis, and retroperitoneal hemorrhage (55).

THE PROCEDURE

Bone marrow aspiration and biopsy procedures are usually performed on the posterior iliac crest. Once the procedure has begun after a time out, local anesthetic directed both at the skin and the periosteum should be used liberally to prevent pain. 5mL and 10 mL of lidocaine is sufficient volume for young pediatric and older pediatric and adult patients, respectively. If more local anesthetic is needed or if an additional site is attempted, no more than 4.5 mg/kg or 300 mg (30 mL of 1% solution) should be used. The high gauge needle used to deliver local analgesia is the best guide for placement of the larger lower gauge bone marrow aspiration and biopsy needles, especially in obese patients (where longer spinal and marrow needles may be called for). Gentle tapping of the thin needle to the bone confirms a good entry point for the marrow needle. Finally, after entering the marrow, two aspirates from a single-entry point in the bone are collected sequentially. The needle is removed and cleared of clot or a new needle is obtained. Entry into the same skin incision at a different bony entry point site is then performed. A bone marrow biopsy is then obtained. It is desirable to avoid using the same bone puncture site for both aspiration and

biopsy in order to avoid hemorrhagic trephine biopsies. A complete blood count with differential and peripheral smear is needed around the same time as the bone marrow sample collection for comparative evaluation of different cell types.

CONCLUSION

Reasonable providers might debate if and when to obtain a bone marrow assessment in patients seen in an allergy and immunology clinic. The bone marrow aspiration and biopsy for patients in practice are usually not needed on an urgent basis compared to frank hematologic malignancies like acute leukemia, although HLH can be one notable exception. However, marrow assessment is generally not indicated in some well-known immunodeficiency diseases such as severe combined immunodeficiency diagnosed at birth. Nonetheless, there are many cases where the bone marrow assessment can help establish a diagnosis and provide a pathophysiological explanation, such as lymphocyte differentiation status, in order to elucidate a patient's presentation and ultimately guide treatments. Furthermore, the risks associated with bone marrow aspiration and biopsy are arguably lower and less severe than liver, kidney, or lung biopsies especially in the absence of procedural sedation involving general anesthesia. What is clear is that Allergists and Immunologist as well as their patients would benefit from awareness and competency in bone marrow procedures and interpretation. Establishing collaborative training programs with hematologists and hematopathologists with procedural and interpretive expertise is a good starting point.

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FIGURE LEGENDS

Figure 1

A biopsy with normal cellularity is shown in (a). Profound marrow failure and lymphoid aggregates are shown in (b) from a subject with CTLA4 haploinsufficiency. Tryptase immunohistochemistry of a biopsy from a subject with indolent systemic mastocytosis (c) demonstrates mast cells in an aggregate surrounding a central core of lymphocytes. EBV RNA expression in a subset of lymphocytes (arrows) is demonstrated by in situ hybridization in a subject with EBV-driven hemophagocytic lymphohistiocytosis (d). Inset in (d) shows the corresponding aspirate with erythrophagocytosis.

(a), (c), and (d) from Hematopathology, second edition 2017. Elaine S Jaffe et al. published by Elsevier, ISBN: 978-0-323-29613-7. (a) from Figure 10-3A (c) from Figure 49-6B (d) from Figure 30-1B and 30-1C. (b) from reference (2) Figure 6a published by Elsevier.

Figure 2

Numerous spicules (a) are present in a high-quality aspirate and appear as granular white flecks on a petri dish. A second aspirate is required if spicules are not present on the initial sample. A good quality biopsy (b) should be approximately 2 cm in length and is red in coloration with minimal white cartilaginous regions. A good quality biopsy in a person with advanced marrow failure or fibrosis might also appear white.

TABLE I: Immunological disorders where a marrow examination is indicated.**Immunodeficiency***Cytopenias and bone marrow failure*

Severe combined immunodeficiency

Combined immunodeficiency

Common variable immunodeficiency

Defects in peripheral tolerance

Phagocyte defects

Autoinflammatory disease

Secondary causes: lymphocytic infiltrates, nutritional deficiency, infections, toxins

Myelodysplastic syndrome or acute myeloid leukemia

Combined immunodeficiency

Phagocyte defects

Rule out lymphoma

Common variable immunodeficiency

Combined immunodeficiency

Susceptibility to EBV disease

DNA repair defects

Immune dysregulation diseases

Hemophagocytic lymphohistiocytosis

Immunodeficiency disease

Familial (cytotoxic machinery defect)

Autoimmune disease

Mast cell disease

Systemic mastocytosis

Idiopathic anaphylaxis

Hymenoptera allergy

Tryptasemia

Hypereosinophilic syndrome

Idiopathic HES

Overlap HES

Lymphoid-variant HES

Myeloid-variant HES

Acquired C1 esterase inhibitor deficiency

Monoclonal gammopathy of uncertain significance

Rule out lymphoma

Workup towards bone marrow transplantation

Pre-transplant evaluation

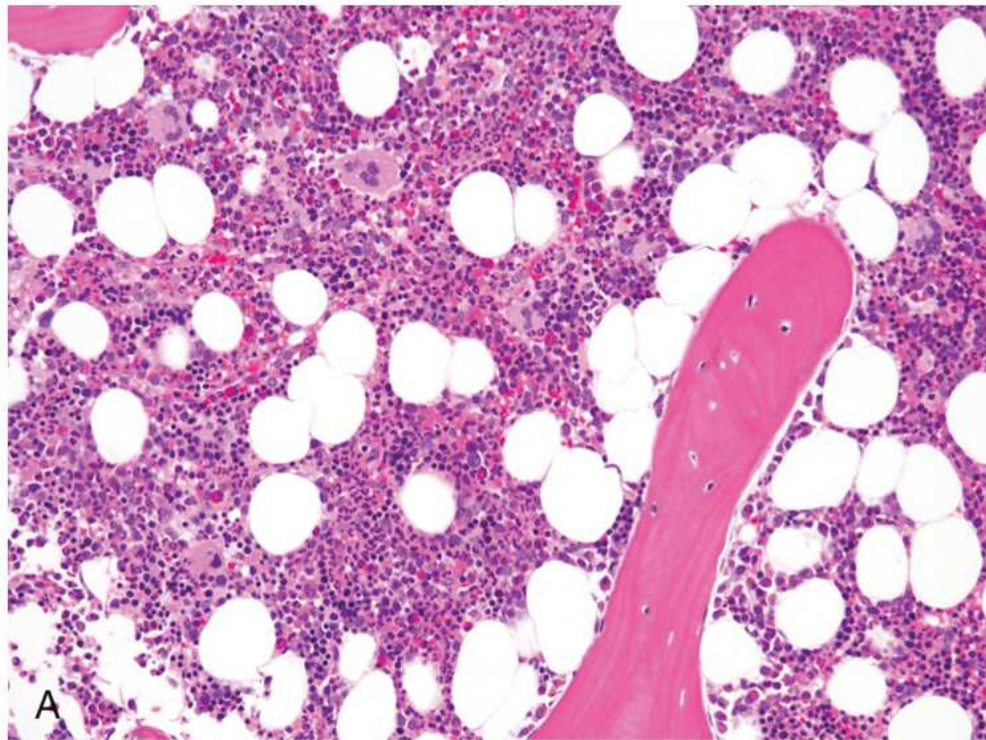
Post-transplant evaluation (can include chimerism study)

Table II: Marrow tests to order in Allergy and Immunology

Samples	Tests	When to order
Biopsy and aspirate	Bone marrow order *	Standard testing
Aspirate	Cytogenetics chromosome analysis by FISH or karyotyping ** T-cell receptor gene rearrangement PCR Immunoglobulin gene rearrangement PCR CMV PCR EBV PCR Bone marrow cultures (fungal, mycobacterial, bacterial) KIT D816V allele-specific PCR CHIC2 (4q12) Deletion (FIP1L1 and PDGFRA Fusion) RT-PCR BCR/ABL1 RT-PCR and FISH JAK2 V617F PCR Chimerism microsatellite study for engraftment	Standard testing Immunodeficiency Mast cell disorders and HES HES Transplant
Peripheral blood	Complete blood count with differential	Standard testing

* Pathologist performs various assays of the biopsy (immunohistochemistry and special stains) and aspirate (microscopy and immunophenotyping)

** can detect PDGFRB and FGFR1 translocation as well as large chromosomal translocations seen in other malignancies

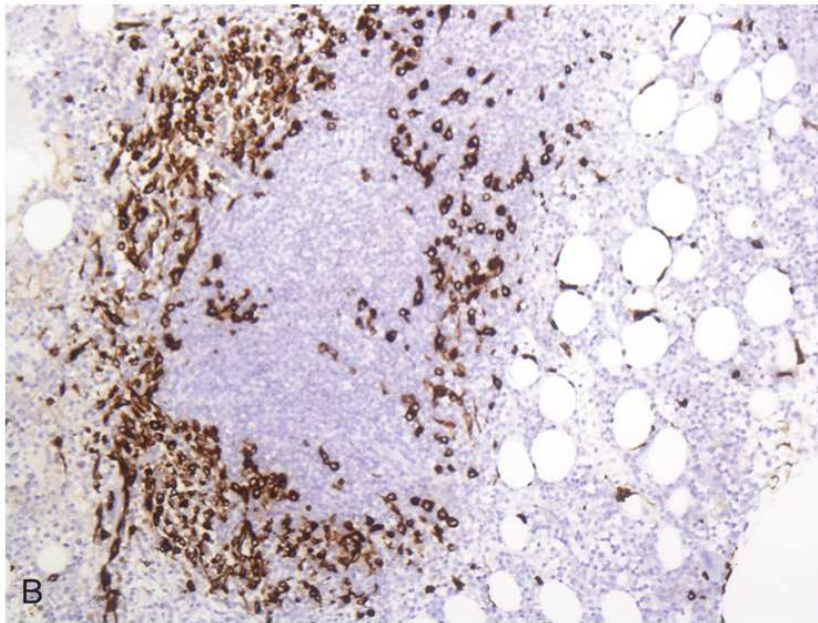


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Figure 1a

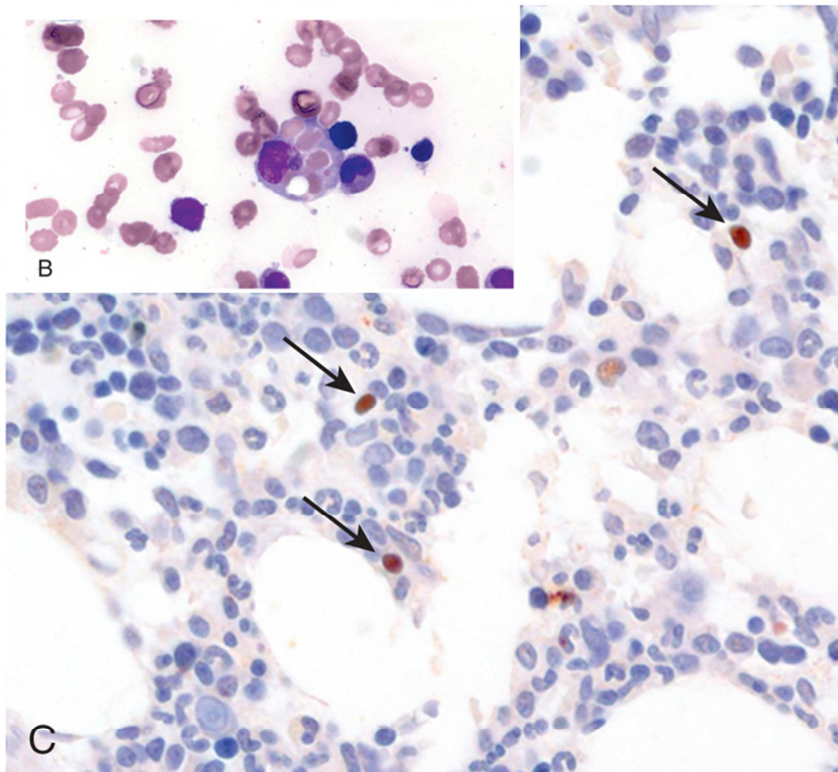


Figure 1b



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Figure 1c



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Figure 1d



Figure 2a



Figure 2b