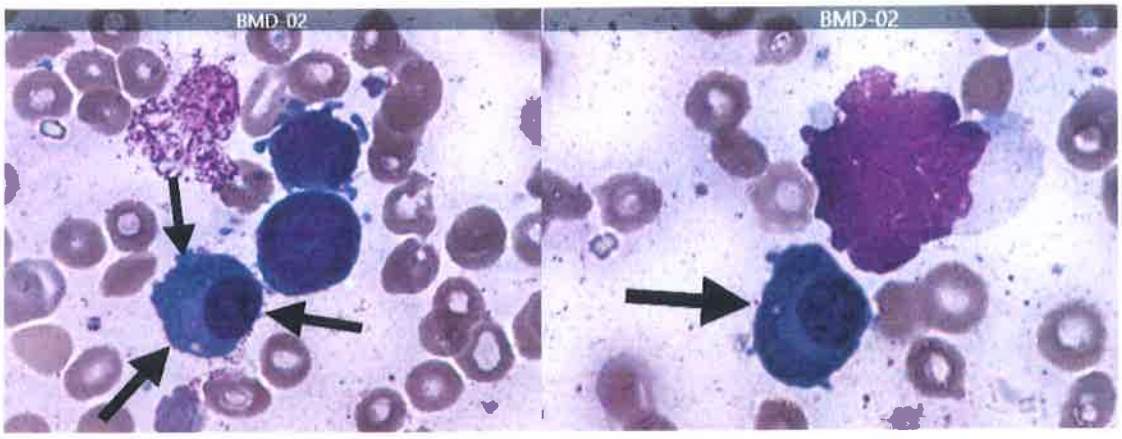
Cell Identification

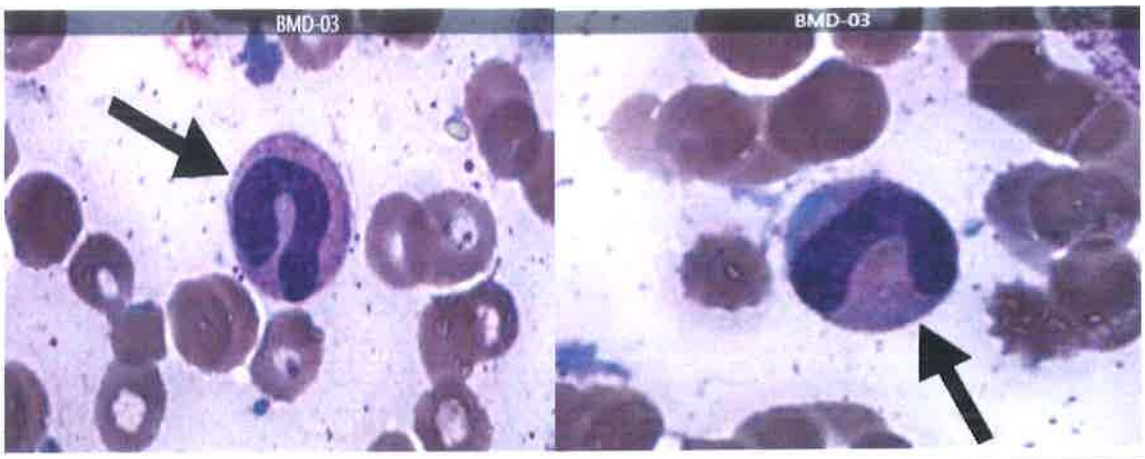
BMD-02



Identification	Participants Freq.	%	Evaluation
Plasma cell, morphologically mature/abnormal/containing inclusion (eg, Dutcher body, Russell body)	352	99.7	Educational
Erythrocyte precursor with vacuolated cytoplasm	1	0.3	Educational

The arrowed cells are mature plasma cells, as correctly identified by 99.7% of participants. Plasma cells represent terminally differentiated B-lymphocytes and are a normal constituent of the bone marrow where they usually comprise less than 5% of the cellularity. They range in size from 10 to 20 µm, and they are often oval shaped with relatively abundant cytoplasm and eccentrically located nuclei. The N:C ratio is 1:2. Their nuclei are usually round to ovoid with prominently coarse and clumped chromatin that is often arranged in a cartwheel-like or clock-face pattern. The cytoplasm stains gray blue to deeply basophilic. A prominent hof or perinuclear zone of pale or lighter staining cytoplasm is typically seen adjacent to one side of the nucleus.

BMD-03

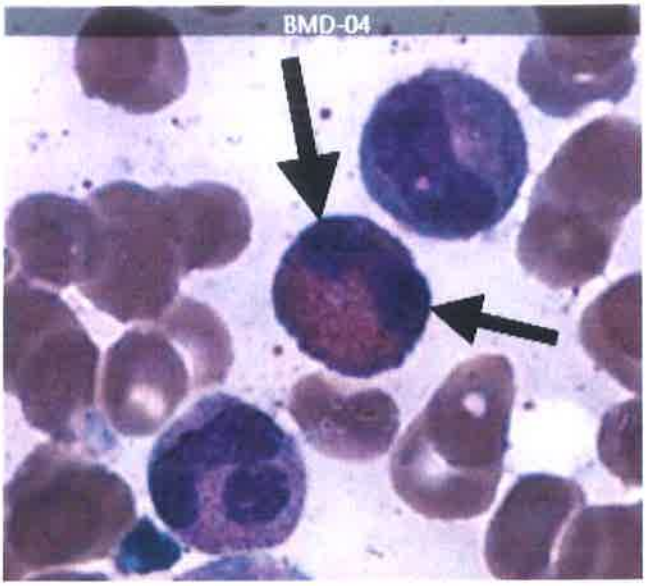


Identification	Participants Freq.	%	Evaluation
Neutrophil, segmented or band	340	96.3	Educational
Neutrophil, giant band or giant metamyelocyte	6	1.7	Educational
Neutrophil with dysplastic nucleus and/or hypogranular cytoplasm	3	0.9	Educational
Neutrophil, toxic (to include toxic granulation and/or Döhle bodies, and/or toxic vacuolization)	3	0.9	Educational
Eosinophil, any stage	1	0.3	Educational

The arrowed cells are neutrophil, band forms, as correctly identified by 96.3% of participants. Segmented neutrophils and their immediate precursors, bands, constitute 12% to 25% of the nucleated cells in the bone marrow. The band is round to oval and 10 to 18 µm in diameter. The nuclear-to-cytoplasmic ratio is 1:1.5 to 1:2 and the nuclear chromatin is condensed. The nucleus is indented to more than half the distance to the farthest nuclear margin, but the chromatin is not condensed to a single filament (as is the defining feature of the fully mature neutrophil). The nucleus can assume many shapes: it can be band- or sausage-like; S-, C-, or U-shaped; and twisted or folded on itself. The cytoplasm is similar to that of other post-mitotic neutrophilic cells, with specific granules predominating in the pale cytoplasm.

1.7% of participants incorrectly identified the arrowed cells as neutrophils, giant bands. Giant bands may be 3 or more times the size when compared to normal segmented neutrophils, with decreased chromatin clumping. The nucleus seems to be too big for the cytoplasm, and is often irregular in contour, folded back on itself, and twisted into bizarre S and C shapes. Neither of the arrowed images show these characteristics.

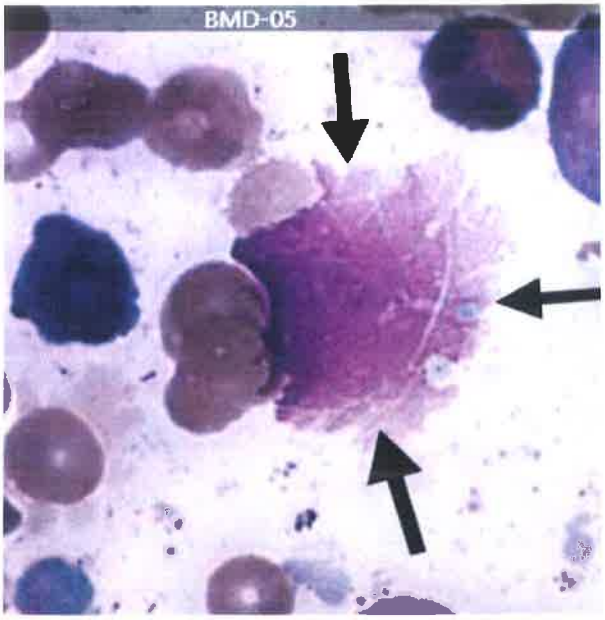
BMD-04



Identification	Participants Freq.	%	Evaluation
Eosinophil, any stage	353	100.0	Educational

The arrowed cell is an eosinophil, as correctly identified by 100.0% of participants. Eosinophils are round to oval leukocytes that are present in the blood, bone marrow, and tissues of normal individuals. They are generally easily recognized due to their characteristic coarse orange-red granulation. They are comparable in size to neutrophils, ie, 10 to 15 µm in diameter in their mature forms, and 10 to 18 µm in diameter in their immature forms. In the most mature eosinophilic form, the nucleus is segmented into two or more lobes connected by a thin filament. About 80% of segmented eosinophils will have the classic two-lobed appearance. The remainder of segmented eosinophils will have three lobes and an occasional cell will exhibit four to five lobes. Eosinophils exhibit the same nuclear characteristics and the same stages of development as neutrophils. The earliest recognizable eosinophilic form by light microscopy is the eosinophilic myelocyte.

BMD-05

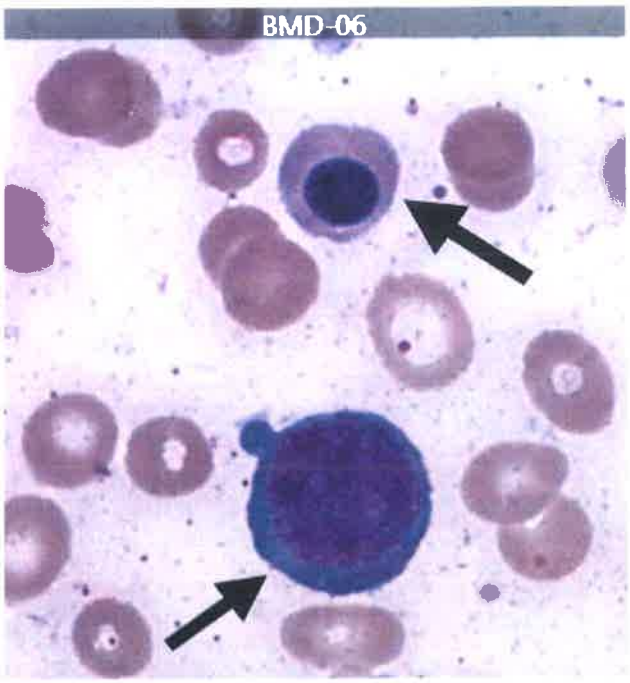


Identification	Participants Freq.	%	Evaluation
Basket cell/smudge cell	338	95.8	Educational
Stain precipitate	12	3.4	Educational
Macrophage (histiocyte)	1	0.3	Educational
Squamous epithelial cell/endothelial cell	1	0.3	Educational
Stromal cell	1	0.3	Educational

The arrowed cell is a basket cell/smudge cell, as correctly identified by 95.8% of participants. A basket cell or smudge cell is most commonly associated with cells that are fragile and easily damaged in the process of making a smear. The nucleus may either be a nondescript chromatin mass, or the chromatin strands may spread out from a condensed nuclear remnant, giving the appearance of a basket. Cytoplasm is either absent or indistinct. Smudge cells are usually lymphocytes, but there is no recognizable cytoplasm to give a clue to the origin of the cell. They are seen most commonly in disorders characterized by lymphocyte fragility, such as infectious mononucleosis and chronic lymphocytic leukemia. Basket cells should not be confused with necrobiotic neutrophils, which have enough cytoplasm to allow the cell to be classified.

3.4% of participants incorrectly identified the image as stain precipitate which is usually due to unclean slides or improper drying of the stain on the smear. Unlike smudge cells, stain precipitate typically appears as metachromatic red, pink, or purple granular deposits on and between cells.

BMD-06



Identification	Participants Freq.	%	Evaluation
Erythrocyte precursor, normal (includes pronormoblast, basophilic, polychromatophilic normoblast, and orthochromic normoblasts)	345	97.7	Educational
Erythrocyte precursor, abnormal/dysplastic nuclear features (includes pronormoblast, basophilic, polychromatophilic normoblast, and orthochromic normoblasts)	3	0.9	Educational
Erythrocyte precursor with megaloblastic changes/maturation	3	0.9	Educational
Basket cell/smudge cell	1	0.3	Educational
Neutrophil, giant band or giant metamyelocyte	1	0.3	Educational

The arrowed cells are normal erythrocyte precursors, as correctly identified by 97.7% of participants. The cell on the top corresponds to orthochromic normoblast and the cell on the bottom corresponds to basophilic normoblast.

Basophilic normoblasts are 10 to 17 µm in diameter, slightly smaller than their precursors, pronormoblasts, but similar in cellular and nuclear shape. The chromatin is coarse-trabecular and "beady" in appearance. The nuclei of large or early basophilic normoblasts may reveal single nucleoli, but those of small or late basophilic normoblasts lack nucleoli. A perinuclear halo is often visible. The cytoplasm is intensely basophilic, imparting a royal blue color. The N:C ratio is approximately 6:1.

Orthochromic normoblasts are round or ovoid cells and are smaller than their precursors polychromatophilic normoblasts (8 to 12 µm in diameter). The nucleus is also very small, often pyknotic. It is often eccentrically placed and at times may be extruding or fragmented. The cytoplasm usually stains uniformly pinkish orange with little or no basophilia. The N:C ratio is approximately 1:2.

Case Presentation:

This bone marrow aspirate smear is from a 24-year-old woman with a history of infantile nephrotic cystinosis, status-post 2 renal transplants. Laboratory peripheral blood data includes: WBC = 1.7 x 10E9/L; RBC = 2.32 x 10E12/L; HGB = 6.8 g/dL; HCT = 20.8%; MCV = 83 fL; PLT = 62 x 10E9/L; and RDW = 15%.

(BONE MARROW, WRIGHT-GIEMSA)

Case Discussion: Hypocellular bone marrow with decreased granulopoiesis
This hypocellular aspirate smear is from a post-transplant patient, and it shows overall decreased cellularity including decreased granulopoiesis. Granulocytes include neutrophils, eosinophils, basophils, and their precursors. Decreased granulopoiesis in the bone marrow is most commonly manifested in the peripheral blood as leukopenia (total WBC count < 3 - 4 x 10E9/L). The most common form of leukopenia is neutropenia [absolute neutrophil count (ANC) < 2.5 x 10E9/L in infants and < 1.5 x 10E9/L in all other age groups] or agranulocytosis (ANC < 0.5 x 10E9/L for any age). Neutropenia can occur in isolation or with other cytopenias and can lead to life-threatening infections, especially when severe and sustained.

Causes of neutropenia can be categorized as congenital and acquired. Congenital neutropenias are most commonly of intrinsic etiology and usually diagnosed at birth or at very young ages, while acquired neutropenias are most commonly of extrinsic etiology and can present at any age (see Table 1).

Table 1. Most common causes of neutropenia

Congenital (intrinsic)	Acquired (extrinsic)
Benign ethnic neutropenia	Infection (hepatitis, HIV, EBV, bacterial, parasitic, and rickettsial)
Familial neutropenia	Medications (cytotoxic or immunosuppressive agents) or post-transplant (BM or solid organs)
Severe congenital neutropenia	Nutritional deficiencies (vitamin B12, folate, copper)
Fanconi anemia	Hematologic malignancies (MDS, LPD, LGL)
Dyskeratosis congenita	Autoimmune disorders (RA, SLE)
Schwachman-Diamond syndrome	Aplastic anemia
Chronic idiopathic neutropenia	

HIV – human immunodeficiency virus; EBV – Epstein-Barr virus; BM – bone marrow; LGL – large granular lymphocytic leukemia; MDS – myelodysplastic syndrome; LPD – lymphoproliferative disorder; RA – rheumatoid arthritis; SLE – systemic lupus erythematosus

Question 1: Which of the following is an acquired (extrinsic) cause of neutropenia?

A. Dyskeratosis congenita
B. Fanconi anemia
C. Large granular lymphocytic leukemia
D. Schwachman-Diamond syndrome

Leukopenia/neutropenia is common following organ transplantation and this discussion focuses on acquired isolated neutropenia in this particular setting. About 20 - 63% of kidney recipients will experience at least one episode of leukopenia/neutropenia. It typically occurs around day 100 after transplantation and can last for 1 to 4 weeks. The cause of leukopenia/neutropenia is usually multifactorial, but immunosuppressive agents/drugs are one of the most common factors. The most predictable immunosuppressive agent to cause leukopenia/neutropenia is azathioprine (AZA), with 50% of renal transplant recipients developing leukopenia/neutropenia due to AZA. Other immunosuppressive agents/drugs include T-cell depleting antibody therapies, mycophenolate mofetil, anti-cytomegalovirus (CMV) myelosuppressive agents (valganciclovir and ganciclovir), calcineurin inhibitors, and several

commonly used antibiotics (such as trimethoprim-sulfamethoxazole, beta-lactam antibiotics, and piperacillin).

Drug-induced neutropenia also occurs outside of transplantation, and well over 100 drugs currently in clinical use have been associated with neutropenia. The most frequently implicated drugs include analgesics (eg, ibuprofen), antiarrhythmics (eg, procainamide and quinidine), antibiotics (eg, ampicillin, cefotaxime, oxacillin, penicillin G, quinidine), anticonvulsants (eg, phenytoin), gastrointestinal (eg, cimetidine), and psychotropic (eg, clozapine and chlorpromazine). Clinically, the level of myelosuppression induced by the drug may be quite variable. Certain drugs, such as clozapine, are so strongly associated with neutropenia that proactive monitoring of the ANC during therapy is standard of care. Deficiencies of some essential nutrients, such as folic acid, vitamin B12, zinc, and copper, may also lead to leukopenia/neutropenia.

Question 2: Which one of the following is the most predictable immunosuppressive agent to cause leukopenia/neutropenia in a post-kidney transplant patient?

A. Azathioprine
B. Cimetidine
C. Ibuprofen
D. Mycophenolate mofetil

Viral and bacterial infections are also a well-known cause of leukopenia/neutropenia following organ transplantation, and they can have marked myelosuppressive effects in renal transplant recipients. Common infections include CMV, parvovirus B19, human herpesvirus-6 (HHV6), influenza, and ehrlichiosis. Infection can produce neutropenia outside of transplant as well and is a relatively frequent cause of neutropenia in children. The mechanisms by which this occurs include direct infection of hematopoietic progenitor cells, increased utilization of neutrophils at sites of infection, sequestration of neutrophils due to splenomegaly or generalized neutrophilic activation, increased binding of neutrophils to infected or altered endothelial cells, immune attack via molecular mimicry of foreign antigens, production of bone marrow suppressive inflammatory cytokines, and even hemophagocytosis due to systemic macrophage activation. Infection by virtually any organism can produce neutropenia by some combination of these mechanisms. However, transient neutropenia in children is consistently produced by viral infections, including non-specific viral gastroenteritis and respiratory tract infections.

Question 3: Which of the following is one of the common infectious causes of leukopenia/neutropenia following organ transplantation?

A. *Babesia*
B. Cytomegalovirus
C. *E.Coli*
D. Human immunodefiency virus

Bone marrow morphology in neutropenia in post-transplant setting depends on the etiology. A majority of bone marrows associated with drug/immunosuppressive-induced neutropenia exhibit granulocytic hypoplasia due to decreased proliferation. Other cases are characterized by left-shifted granulocytic hyperplasia due to a survival defect, with a decrease in mature forms and/or arrested maturation at the myelocyte stage (eg, drug-induced immune destruction). Prominent ingestion of neutrophils by macrophages (hemophagocytosis) is also possible in immune-mediated or viral neutropenia. AZA may affect cells other than granulocytes and cause striking multinucleation and megaloblastic changes in the erythroid lineage. Some infections will suppress progenitor cells, inducing hypoplasia. Viral inclusions

may be seen in CMV and parvovirus B19 infections, the latter infecting the erythroid precursor cells. Notably, myeloid dysplasia is not commonly seen in infections.

There are no ideal therapies for leukopenia/neutropenia after renal transplantation. The most effective way to attenuate leukopenia/neutropenia is to discontinue or decrease the dose of the potentially offending drugs. However, this strategy is frequently linked to increased risk of organ transplant rejection. Recombinant granulocyte colony stimulating factor (G-CSF), such as filgrastim (Neupogen), may be effective in treating leukopenia/neutropenia in the setting of chemotherapy. G-CSF can be considered second-line therapy after adjustment of other medications. In extreme cases, hematopoietic stem cell transplantation may be needed to treat severe leukopenia/neutropenia, including those caused by myelosuppressive agents, if other maneuvers fail.

Question 4: What is the most effective way to treat leukopenia/neutropenia in the post-transplant setting, but comes with increased risk of rejection?

A. Discontinue or decreased the dose of the most likely offending drug
B. Hematopoietic stem cell transplantation
C. Recombinant G-CSF
D. Wait for neutropenia to recover by itself

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References:

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2. Kjeldsberg CR, Perkins SL. *Practical Diagnosis of Hematologic Disorders*. 5th ed. ASCP; 2010.
3. Yang Yu, Yu B, Chen Y. Blood disorders typically associated with renal transplantation. *Front Cell Dev Biol*. March 2015.
4. Mason E, Pozdnyakova O. Aplasia. In: Van der Walt J, Orazi A, Arber D, eds. *Diagnostic Bone Marrow Hematopathology*. 1st ed. Cambridge University Press; 2021

Answers to Questions:

Question 1: C. Large granular lymphocytic leukemia
Causes of neutropenia can be categorized as congenital and acquired. Congenital neutropenias are most commonly of intrinsic etiology and usually diagnosed at birth or very young ages, while acquired neutropenias are most commonly of extrinsic etiology and can present at any age. Large granular lymphocytic leukemia is an example of an acquired (extrinsic) cause of neutropenia, while the other choices are examples of congenital (intrinsic) etiologies.

Question 2: A. Azathioprine
Azathioprine (AZA) is the most predictable immunosuppressive agent to cause leukopenia/neutropenia, occuring in approximately 50% of renal transplant recipients. However, leukopenia/neutropenia due to AZA is most often reversible with decrease or discontinuation of the drug. Mycophenolate mofetil (MMF) causes leukopenia/neutropenia in 13 - 35% of renal transplant recipients. The bone marrow effects of MMF are correlated with its active metabolite, mycophenolic acid (MPA). Ibuprofen and cimetidine are typically not used in the renal transplant setting.

Question 3: B. Cytomegalovirus
Cytomegalovirus (CMV), parvovirus B19, human herpesvirus-6 (HHV6), influenza and ehrlichiosis are the most common infections causing leukopenia/neutropenia following organ transplantation. The other answer choices cause infections, but they are not common causes of leukopenia/neutropenia following organ transplantation. HIV (human immunodeficiency virus) attacks the body's immune system. If HIV is not treated, it can lead to AIDS (acquired immunodeficiency syndrome). *E. coli* (*Escherichia coli*) is a type of bacteria that can cause diarrhea, pneumonia, and urinary tract infections. *Babesia* is a parasite that infects red blood cells and is usually transmitted by a tick bite.

Question 4: A. Discontinue or decrease the dose of the most likely offending drug
There are no ideal therapies for leukopenia/neutropenia after renal transplantation, but the most effective way to attenuate leukopenia/neutropenia is to discontinue or decrease the dose of the most likely offending drugs. Recombinant granulocyte colony stimulating factor (G-CSF), such as filgrastim (Neupogen), may be effective in treating leukopenia/neutropenia in the setting of chemotherapy. G-CSF can be considered second-line therapy after adjustment of other medications. In extreme cases, hematopoietic stem cell transplantation may be needed to treat severe leukopenia/neutropenia, including those caused by myelosuppressive agents, if other maneuvers fail. Neutropenia, especially when severe, can lead to life-threatening infections, and waiting for it to recover by itself is not a viable option.

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