Morphology: Bone marrow morphology should not be undervalued.

The bone marrow aspirate: The nucleated differential count

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The nucleated differential count

- To assess haemopoietic activity
- To compare the proportion of the different cell lineages with known reference ranges
- To quantify abnormal cells, if present
- <u>Should be performed</u>
 - In the cellular trails of the BM smear behind the particles (minimally diluted with PB)
 - In an area where the cells are well dispersed with good cytological detail
 - Where there is the least number of smudged cells

Bone Marrow evaluation

골수 흡인 도말 소견

particle crush preparation







2017년도 대한진단혈액학회 학술대회 인하대학교 문연숙 선생님 자료

골수 흡인 도말 소견 Direct film



2017년도 대한진단혈액학회 학술대회 인하대학교 문연숙 선생님 자료

ICSH guidelines for the standardization of bone marrow specimens and reports

- One first scans the marrow film under low power (100X or 200X)
- \rightarrow looking for irregularities in cell distribution
- number of megakaryocytes
- the presence of abnormal cells
- The differential count is performed at 400X or 1000X.
- Percentage of an abnormal cell type is required for diagnosis
- \rightarrow <u>At least 500 cells</u> should be counted in <u>at least 2 smears</u>
- Not essential to the diagnosis
- \rightarrow <u>At least 300 cells</u> should be counted

Int. Jnl. Lab. Hem. 2008, 30, 349-364

The nucleated differential count

Should comprise		Should not include
Blast cells	Promonocytes	Megakaryocytes
Promyelocytes	Monocytes	Macrophages
Myelocytes	Lymphocytes	Osteoblasts
Metamyelocytes	Plasma cells	Osteoclasts
Band forms	Erythroblasts	Stromal cells
Segmented neutrophils		Smudged cells
Eosinophils		Non-haemopoietic cells
Basophils		Metastatic tumour cells
Mast cells		Lymphoid aggregates

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골수검사 보고서 표준안 (제안)

4. Differential Count		
Total cell count		
M:E ratio		
Blasts	Eosinophils	Lymphocytes
Promyelocytes	Basophils	Monocytes
Myelocytes	Pronormoblasts	Plasma Cells
Metamyelocytes	Basophilic normoblasts	Others ()
Band form neutrophils	Polychromatophilic normoblasts	Others ()
Segmented neutrophils	Orthochromatophilic normoblasts	

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Hematopoiesis



f1.3 Stages of hematopoietic cell development starting at hematopoietic stem cell through common lymphoid and common myeloid progenitor cell stages illustrated. Note that dendritic cells may be derived from either common lymphoid or granulocyte/monocyte progenitors. [Metcalf 2007b, Kierszenbaum 2002a, Ishkawa 2007]

Granulopoiesis

t1.8 Granulopoiesis			10 M	
Stage of Maturation	Morphology	% in BM	Cytochemistry	Immunophenotype
Myeloblast	High nuclear-to-cytoplasmic ratio Blastic, dispersed chromatin Agranular/minimally granular cytoplasm	0-3%	MPO – to weakly + (up to 20 granules) α-naphthol chloroacetate esterase – to weakly +	CD34+, HLA-DR+, myeloid ag+
Promyelocyte	Eccentric nucleus with prominent paranuclear hof (pale zone) Sparse, concentrated primary granules	1%-4%	MPO+, lysozyme+, α-naphthol chloroacetate+	CD34–, HLA-DR–, myeloid ag+
Neutrophilic myelocyte	Round nucleus with condensed chromatin Moderate to abundant secondary (specific) granules, which produce a finely granular pink cytoplasm	12%-22%	MPO+, lysozyme+, α-naphthol chloroacetate+, leukocyte alkaline phosphatase+	CD34–, HLA-DR–, myeloid ag+
Neutrophilic metamyelocyte	Indented nucleus, condensed chromatin Cytoplasm packed with granules with predominance of secondary granules	12%-22%	MPO+, lysozyme+, α-naphthol chloroacetate+, leukocyte alkaline phosphatase+	CD34–, HLA-DR–, myeloid ag+
Band neutrophil	Horseshoe-shaped mature nucleus lacking discrete indentations Cytoplasm packed mostly with secondary granules; gelatinous (tertiary) granules also present	5%-14%	MPO+, lysozyme+, α-naphthol chloroacetate+, leukocyte alkaline phosphatase+	CD34–, HLA-DR–, myeloid ag+
Neutrophil	 3-5 discrete nuclear lobes (joined by a thin chromatin strand) Highly condensed chromatin Cytoplasm packed with granules with predominance of secondary granules; gelatinous (tertiary) granules also present 	9%-20%	MPO+, lysozyme+, α-naphthol chloroacetate+, leukocyte alkaline phosphatase+	CD34–, HLA-DR–, myeloid ag+

Normal neutrophils (2-5 lobed)



Normal band form neutrophils



Normal **metamyelocytes**



236 to 239 Normal metamyelocytes in adults

Normal myelocytes



264 to 272 Normal promyelocytes in adults

a newborn

Normal **blasts**





Mature eosinophils





Secondary eosinophilic granules: Orange-red color







Immature eosinophils

Mature basophils



Secondary basophilic granules:

Course, dark blue-black, and often overlay the segmented nucleus

Immature basophil → basophil로 감별계산!



Mast cell production



Mast cell precursor -> Mast cell, Basophil

Mast cell: reside in tissues

play a role in initiation of allergic responses, activating during parasitic infections, protective immune responses to microbial pathogens

nuclei are round,

granules impart a dense purple-black appearance to the cytoplasm often obscuring the nucleus

Monopoiesis



Monocyte

 Gradual nuclear folding
 the acquisition of cytoplasmic granules (Esterase stains +)



Promonocyte





Monoblast

Monocyte & histiocytes (macrophages and dendritic cells)

- Phagocytic activity Diff. count X

- Produce a plethora of cytokines (regulatory activities in hematopoiesis, inflammatory states, and diverse immune reactions)

-> migrate to all organs of the body

Macrophages

– Within bone marrow, macrophages are in particles on BM aspirate smears



i1.21 2 mature macrophages demonstrate the voluminous cytoplasm with ingested material that characterizes these cells on a bone marrow aspirate smear. Note the mature nuclear chromatin pattern of the nuclei of these 2 macrophages. (Wright)



i1.22 Immunoperoxidase for CD68 shows numerous macrophages with stellate cytoplasmic processes in bone marrow core biopsy section. (immunoperoxidase)

Dendritic cells

- Potent antigen-presenting cells (APCs), capable of initiating a primary innate immune response
- Inconspicuous on morphologic review
- Immunohistochemical techniques are required for cell ID (CD1a, S100, CD123, CD23, and CD35)



Dendritic cells



i1.23 Very rare S100+ dendritic cells present on core biopsy section. (immunoperoxidase)



Plasmacytoid dendritic cells

Erythropoiesis

- The earliest morphologically recognizable cell in the erythroid lineage → Pronormoblast (Erythroblast)
- Nucleus
- Progressive nuclear condensation
- Extrusion of the pyknotic nucleus at the end of the orthochromic normoblast stage
- Extruded nuclei \rightarrow subsequently undergo phagocytosis (macrophages)

Cytoplasm

- Adequate hemoglobin production
- Gradually changes from deeply basophilic to pinkish



11.25 Bone marrow aspirate smear illustrates a cluster of developing erythroid precursors, including basophilic normoblasts, polychromatophilic normoblasts, and orthochromic normoblasts. Note the progressive decrease in overall nuclear size, and the gradual change from deeply basophilic to pink cytoplasm that characterizes gradual hemoglobin production within these cells. (Wright)





Greater increases in cytoplasm

Cytoplasmic blebbing may be evident

ID of immature megakaryocytes generally requires immunophenotyping CD31, CD41, CD42b, CD61

Lymphopoiesis

 The stage of maturation of both B and T lymphocytes
 Generally defined by <u>the surface antigen profile</u> rather than by <u>morphologic features</u>





i1.35 Abundant lymphoid cells with features of hematogones present on be marrow aspirate smear from 13-day-old male infant. (Wright)

Normal lymphoid precursors (Hematogones):

- ✓ Highly condensed nuclear chromatin
- ✓ Inconspicuous nucleoli
- ✓ Scant cytoplasm
- Account for a very low percentage in adults

Normal lymphocytes





Granular lymphocytes

Prolymphocytes & lymphoblasts



A variety of lymphoblasts (lineage determined by other means)

Not recognized in normal bone marrow

Lymphoblast, Prolymphocyte -> neoplastic cell Lymphocyte precursor cell -> non-neoplastic cell

Plasma cells



Humoral immune response

<5% of BM cells

Eccentric round nuclei, abundant basophilic cytoplasm, a paranuclear hof

Reside adjacent to vessels & dispersed in the medullary space



Myeloma cells (501 and 503 are flame cells)



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Myeloma cells

Bone marrow cellularity and differential counts

Age-related normal values in bone marrow

Age	% Cellularity	% Granulocyte	% Erythroid	% Lymphocytes	Comments
Newborn	80-100%	40-50%	40%	10-20%	High EPO birth Hematogones* may be prominent
1-3 months	80-100%	50-60%	5-10%	30-50%	Erythroid precusors ↓ Abundant lymphocytes with many hematogones*
Child	60-80%	50-60%	20%	20-30%	
Adult (30-70 years)	40-70%	50-70%	20-25%	10-15%	Cellularity fairly stable
Elderly (>70 years)	≤25%	50-70%	20-25%	10-15%	Cellularity ↓

* Hematogones are normal B-lymphocyte precursor cells

Bone marrow pathology 3rd, Vol. 1, p32

The nucleated differential count (Adult)

t2.3 Normal Adult Values for Bone Marrow Differential Cell Counts

Cell Type	Normal Range (%)		
Myeloblasts	0%-3%*		
Promyelocytes	1%-8%		
Myelocytes	10%-15%		
Metamyelocytes	10%-15%		
Band/neutrophils	12%-25%		
Eosinophils and precursors	1%-5%		
Basophils and precursors	0-1%		
Monocytes	0-2%		
Erythroblasts	0-2%		
Other erythroid cells	15%-25%		
Lymphocytes	10%-15% [†]		
Plasma cells	0-1%		

*Percent myeloblasts higher in pediatric specimens (3%-4%), lower in specimens from adults, especially elderly (0-1%) *Percent lymphocytes higher in specimens from young children due to abundant hematogones (benign lymphocyte precursor cells)

References: [Bain 1996, Wickramasinghe 2007]

Bone marrow pathology 3rd, Vol. 1, p34

The nucleated differential count

Differential Cell Counts of Bone Marrow in Percent of Total Nucleated Cells						
		ROSSE (1977)		MAUER (1969)	JANDL (1987) Adult (mean, range)	
Cell types	Birth (mean, SD)	1 month (mean, SD)	18 months (mean, SD)	Childhood (mean, range)		
Normoblasts, total	14.48 ± 7.24	8.04 ± 5.00	8.21 ± 3.71	23.1	21.5 (14.2-30.4)	
Pronormoblasts	0.02 ± 0.06	0.10 ± 0.14	0.08 ± 0.13	0.5 (0.0-1.5)	0.6 (0.2-1.4)	
Basophilic n.	0.24 ± 0.25	0.34 ± 0.33	0.50 ± 0.34	1.7 (0.2-4.8)	20(0.7.3.7)	
polychromatophilic n.	13.06 ± 6.78	6.90 ± 4.45	6.97 ± 3.56	18.2 (4.8-34.0)	12.4 (12.2-24.2	
Orthochromatic n.	0.69 ± 0.73	0.54 ± 1.88	0.44 ± 0.49	2.7 (0.0-7.8)	6.5 (2.0-22.7)	
Neutrophils, total	60.37 ± 8.66	32.35 ± 7.68	36.06 ± 7.40	57.1	56.0 (45.1-66.5)	
Myeloblasts	0.31 ± 0.31	0.62 ± 0.50	0.06 ± 0.08	1.2 (0.0-3.2)	1.0 (0.5-1.8)	
Promyelocytes	0.79 ± 0.91	0.76 ± 0.65	0.64 ± 0.59	1.4 (0.0-4.0)	3.4 (2.6-4.6)	
Myelocytes	3.95 ± 2.93	2.50 ± 1.48	2.49 ± 1.39	18.3 (8.5-29.7)	11.9 (8.1-16.9)	
Metamyelocytes	19.37 ± 4.84	11.30 ± 3.59	12.42 ± 4.15	23.3 (14.0-34.2)	18.0 (9.8-25.3)	
Bands	28.89 ± 7.56	14.10 ± 4.63	14.20 ± 5.23		11.0 (8.5-20.8)	
Segmented	7.37 ± 4.64	3.64 ± 2.97	6.31 ± 3.91	12.9 (4.5-29.0)	10.7 (8.0-16.0)	
Eosinophils	2.70 ± 1.27	2.61 ± 1.40	2.70 ± 2.16	3.6 (1.0-9.0)	3.2 (1.2-6.2)	
Basophils	0.12 ± 0.20	0.07 ± 0.16	0.10 ± 0.12	0.06 (0.0-0.8)	<0.1 (0.0-0.2)	
Lymphocytes, total	15.6	49.0	45.5	16.0 (4.8-35.8)	15.8 (10.8-22.7	
Transitional	1.18 ± 1.13	1.95 ± 0.94	1.99 ± 1.00			
Small	14.42 ± 5.54	47.05 ± 9.24	43.55 ± 8.56			
Plasma cells	0.00 ± 0.02	0.02 ± 0.06	0.06 ± 0.08	0.4 (0.2-0.6)	1.8 (0.2-2.2)	
Monocytes	0.88 ± 0.85	1.01 ± 0.89	2.12 ± 1.59		1.8 (0.2-2.8)	
Megakaryocytes	0.06 ± 0.15	0.05 ± 0.09	0.07 ± 0.12		<1.0 (0.0-0.2)	
Reticulum cells					0.3 (0.0-0.5)	
M/E ratio	4.2	4.0	4.4	2.9 (1.2-5.2)	2.5 (1.2-5.0)	

Data are from Rosse (1977), Mauer (1969), and Jandi (1987).

M/E, Myeloid/erythroid ratio.

Myeloid: granulocyte, monocyte와 그 precursor cells

Henry's 23rd, p537

Other cell types within bone marrow stroma and adjacent to bone

Diff. count X



i2.52 Both macrophages and fat cells are more prominent within bone marro particles on aspirate smears following potent chemotherapy. (Wright)

Macrophages & **fat cells** (adipocytes)



i2.53 Bone marrow aspirate smear illustrates 2 osteoblasts exhibiting markedly eccentric nuclei and a Golgi region that is separate from the nucleus. (Wright)

Osteoblasts:

A single, markedly eccentric nucleus and abundant blue-gray cytoplasm Can be seen from young children

i2.54 Bone marrow aspirate smears illustrate 2 osteoclasts, highlighting the range in cytoplasmic characteristics of these cells. Less mature osteoclasts have basophilic cytoplasm **a**, while brightly eosinophilic cytoplasm with coarse eosimphilic granules characterizes mature osteoclasts **b**. (Wright)

Osteoclasts:

Multiple discrete small, round nuclei and abundant cytoplasm containing finely granular, variably eosinophilic material Pediatric samples

Artifacts



i2.66 Bone marrow aspirate particle crush smears illustrate excessive cell crushing with streaking of nuclear material. (Wright)



Excessive pressure → Excessively crushed!

i2.67 Virtually no intact cells are present on this excessively crushed bone marrow aspirate particle crush preparation. Note prominent nuclear streaking. (Wright)



i2.68 Low a and high b magnification of bone marrow aspirate direct smears illustrate several clots. Note marked cellular distortion. (Wright)



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12.69 On bone marrow aspirate smears, either prominent normoblast clumping from specimen clotting **a** or marked platelet clumping in a patient wit essential thrombocythemia **b** can mimic particles and interfere with differential counting. (Wright)

Overstaining or understaining of smears Water contamination Aspirate smears too thick

Small clots on aspirate smears

Slide QC is very important!

References

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