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Review: ABC of CBC (Cell Blood Count)

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Introduction

A complete blood cell count (CBC) is one of the most common laboratory tests in clinical practice. More than 10% to 20% of CBC is reported as abnormal. Therefore, it is in every doctors' interest to have some understanding of test basics and have a structured action plan for their interpretation.

Automated Cell Counters like the coulter counter can electronically count circulating blood cells like red blood cells (RBCs), white blood cells (WBCs), and platelets.

Principles of Coulter counter

It generates an electrical pulse when a blood cell passes through a small aperture surrounded by electrodes.

Each electrical pulse represents an individual cell, and the pulse height indicates the cell volume.

It can therefore register the total cell count as well estimate the average cell volume and the variation in cell size. For example, it can measure RBC numbers, measure RBC volume i.e. the mean corpuscular volume (MCV) and the RBC distribution width (RDW), respectively.

They are also capable of measuring cell content of WBCs and provide automated differential count (ie, 5-part differential). The granulocyte count is fairly accurate and it can also provide absolute values for all blood counts i.e Absolute Neutrophil count (ANC), Absolute Lymphocyte count (ALC), Absolute Eosinophil count (AEC), and Absolute Monocyte Count (AMC).

Hemoglobin and hematocrit are the other measured variables by the coulter.

From this one can calculate the MCH, MCHC

Limitations of coulter counter:

Standardization of Machine is necessary to ensure consistent results

When accepting the platelet count and the automated WBC differential count it is always necessary to cross check it by the human eye on direct Peripheral Smear

For example, it is possible that the machine may show a low platelet count and the direct peripheral smear show a normal platelet count. This condition is known as Pseudothrombocytopenia. This may occur because the anticoagulant EDTA may clump the platelets and hence the coulter would record a decreased platelet count. Thus every low platelet count must be crosschecked by peripheral smear before an elaborate investigation for thrombocytopenia is undertaken. In our country people from Bihar, Bengal, Assam & the Northeast have Macro platelets, which very often is the cause of Pseudothrombocytopenia.

EDTA may clump platelets causing the coulter to record a decreased platelet count

Evaluation of a CBC Report

When interpreting a CBC report a practitioner will have to focus on the following variables:

- Hb: As a general indicator of anemia or polycythemia
- MCV (Mean Corpuscular Volume): A key parameter for the classification of anemias
- RDW (RBC distribution width): A relatively useful parameter in the differential diagnosis of anemia
- RBC count: An increased RBC count associated with anemia is characteristic of ineffective erythropoiesis seen classically in beta thalassemia trait
- Platelet count: To detect either thrombocytopenia or thrombocytosis
- WBC with differential count: Usually gives important clues for the diagnosis of infections, hematological disorders like acute leukemia as well as for the presence of leukopenia and neutropenia

For abnormal WBC counts, the practitioner should immediately ask which WBC type is affected: Neutrophils, Lymphocytes, Monocytes, Eosinophils, or Basophils as this may provide important clue to a patient's clinical condition and aetiology.

Normal Variables of CBC

Normal values of individual parameters vary with age

While interpreting a CBC a pediatrician must note the normal values of individual parameter for age as they vary with age. This is very important as many gross errors are made when this is not done. Many Pathologists have preprinted normal values for adult in their CBC report hence a pediatrician is well advised to have normal values of CBC parameters in their clinic and refer to it frequently.

Table of normal values in children

Finally, an “abnormal” CBC should be interpreted within the context of a child’s baseline value because up to 5% of the general population without disease may display laboratory values outside the statistically assigned “normal” reference range

Differences in the CBC based on race and sex should be considered when interpreting results. Hence it is very important to have normal values for Indian Children available.

RBC-associated parameters are lower and platelet counts higher in women compared with men

As a generalization, RBC-associated parameters are lower and platelet counts are higher in women compared with men.

Table 1: Normal CBC values for different age groups

	Hb	HCT	MCV	MCHC	Ratio	WBC ($10^3/\mu l$)	Platelets ($10^3/\mu l$)
26-30 Weeks	13.4	41.5	118.2	37.9	-	4.4	254
28 Week	14.5	45	120	31	5-10		275
3 Week	15	47	118	32	3-10		290
Term (Cord)	16.5	51	108	33	3-7	18.1	290
1-3 day	18.5	56	108	33	1.8-4.6	18.9	192
2 Week	16.6	53	105	31.4		11.4	252
1 Month	13.9	44	101	31.8	0.1-1.7	10.8	
2 Month	11.2	35	95	31.8			
6 Months	12.6	36	76	35		11.9	
6 Months-2 Yrs.	12.0	36	78	33	0.7-2.3	10.6	150-350
2 -6 Yrs.	12.5	37	81	34	0.5-1.0	8.5	150-350
6 – 12 Yrs.	13.5	40	76	34	0.5-1.0	8.1	150-350
12 – 18 Yrs.							
Male	14.5	43	88	34	0.5-1.0	7.8	150-350
Female	14.0	41	90	34	0.5-1.0	7.8	150-350
Adult	15.5	47	90	34	0.8-2.5		
Male	14	41	90	34	0.8-4.1	7.4	150-350
Female	14.0	41	90	34	0.5-1.0	7.4	150-350

Evaluation of CBC in a patient with Anemia

Important principles of interpretation of CBC in ANEMIA are outlined:

- Be sure anemia is present and that the seemingly low Hb is not just a normal variation of Hb for age.
- Vice versa many women believe their Hb is always on the lower side and that that is normal for them. This concept needs to be changed do as to evaluate & treat large no of women with mildly low Hb who are definitely affected by mild anemia.
- Classify anemia on Basis of MCV
  - Microcytic (MCV, <80 fL or normal for age)
  - Normocytic (MCV, 80-100 fL or normal for age)
  - Macrocytic (MCV, >100 fL or normal for age).
- This will help narrow the differential diagnosis in each patient with anemia
- Peripheral blood smear evaluation during the initial evaluation of anemia is a great asset in all subtypes of anemia. It helps substantially in the differential diagnosis and provides guidance for further testing. It can help narrow down the investigations in a child and substantially economize the investigations.

Microcytic anemia

The differential diagnoses for microcytic anemia are:

- Iron deficiency anemia (IDA)
- Anemia of chronic disease (ACD)
- Thalassemia
- Sideroblastic anemia
- Lead (Pb) poisoning
- Copper (Cu) poisoning
- Zinc (Zn) ingestion

It is worth noting here that all the causes of microcytic hypochromic anemia affect the hemoglobin synthesis in some way.

The most common cause of the microcytic anemias is IDA.

Doing a peripheral smear and it will give you an important clue.

Serum ferritin may be done to confirm iron deficiency. A low serum ferritin level is diagnostic of IDA. Diagnosis of IDA is unlikely in the presence of a persistently normal or elevated serum ferritin level.

Diagnosis of IDA is unlikely in presence of persistently normal or elevated serum ferritin level

It is important to remember serum ferritin is an acute phase reactant and it is elevated in association with infection. Caution is necessary while interpreting serum ferritin value in-patient of suspected IDA with infection.

Thalassemia should be considered in patients with chronic microcytosis and normal serum ferritin levels, especially if they belong to Lohana, Khoja, Agarwal, Bhanushali, Bania, Neobuddhist, Sindhi and Jain community

If the serum ferritin level is normal in patients with chronic microcytosis, especially if they belong to a community in whom thalassaemia is more common for e.g. Lohana, Khoja, Agarwal, Bhanushali, Bania, Neobuddhist, Sindhi, Jain community; a diagnosis of thalassemia should be considered, and hemoglobin electrophoresis should bedone.

- Also look at RBC count; if RBC count is > 5.0 x 10^9 / L it favours possibility of Thalassaemia Minor.
- Various other computed parameters might help one suspect - Thalassaemia Minor. One such useful parameter is if the MCV/RBC ratio is < 11 it favours possibility of beta Thalassaemia Minor while a ratio > 11 favors IDA.
- RDW may also help differentiate IDA from Beta Thalassaemia Minor. RDW is elevated in IDA while it is normal in beta thalassaemia minor.
- **For interpretation of Hb electrophoresis you may require Hematologist help.**

Hemoglobin electrophoresis results are normal in the -thalassemia trait and abnormal in the -thalassemia trait as well as in other thalassemic syndromes. Concomitant IDA or Megaloblastic anemia may mask the typical abnormality seen in the -thalassemia trait. HbA_2 ($_2$ $_2$) level are increased in megaloblastic anemia & sometimes the values can be increased from the normal value of 2% to a value of > 3.5% & give a false diagnosis of beta thalassemia minor. All borderline HbA_2 values must be rechecked after correction of anemia.

HbA_2 ($_2$ $_2$) levels are increased in megaloblastic anemia & may give a false diagnosis of beta thalassemia minor

Acquired microcytic anemia that is not IDA suggests the possibility of Anemia of chronic disease (ACD). A child with disseminated Koch or RA having mild microcytic anemia not responding to iron supplement therapy is a classical example of anemia of chronic disease. These children would benefit from Erythropoietin therapy.

Diabetic patients on Ayurvedic medications are prone to develop Pb poisoning

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Fig. 1 Algorthm for investigation of Mycrocytic Hypochromic anemia

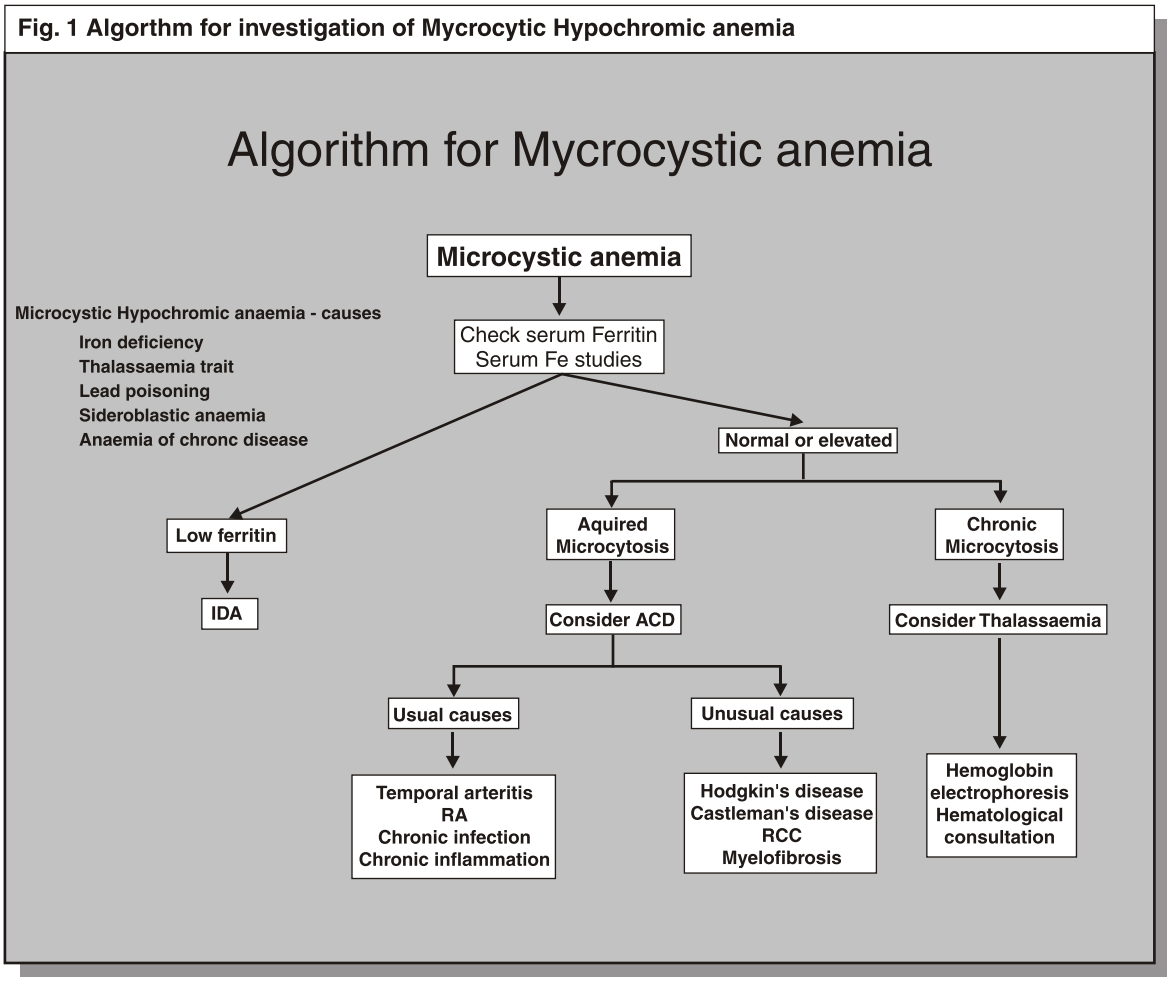
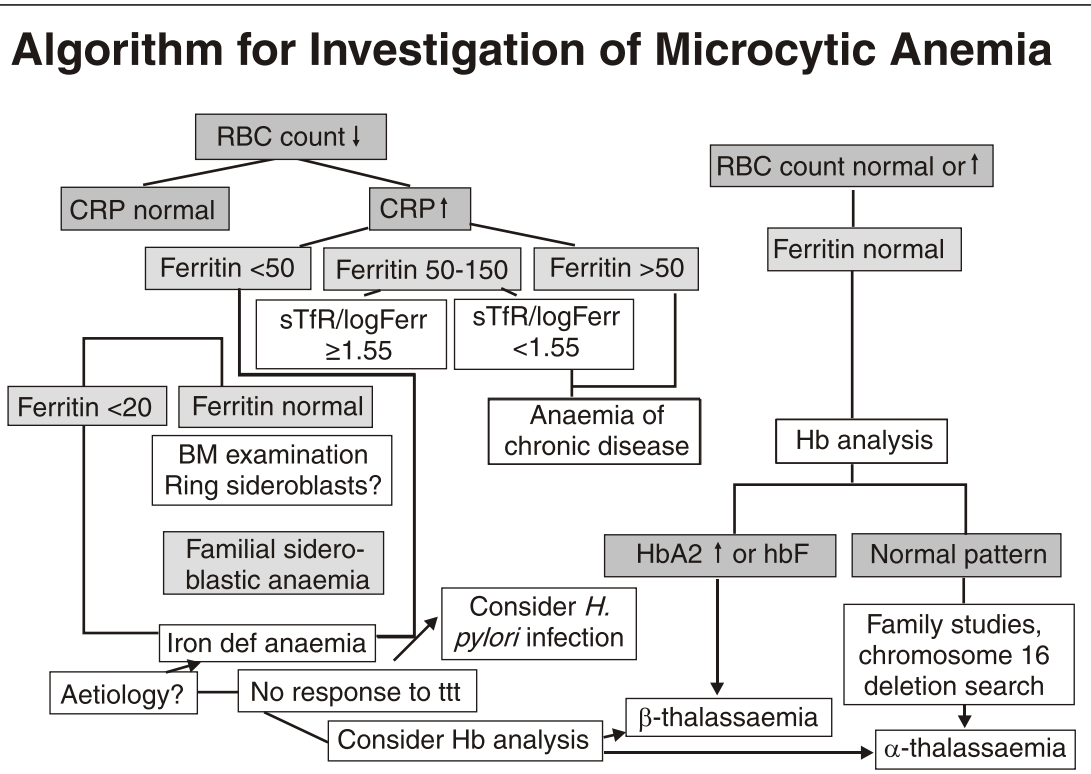


Table 2. Differential diagnosis of Microcytic hypochromic anemia

Category of anemia	Differential diagnosis	CBC clues	PBS clues
Microcytic	IDA	↑RDW	Anisocytosis
		Thrombocytosis	Poikilocytosis
			Elliptocytosis
	Thalassemia	N or ↑RBC count	Polychromasia
		N or ↑RDW	Target cells
			Basophilic stippling
	ACD	N RDW	Unremarkable (typically)
			Rouleaux formation (CD)

We know that serum ferritin is an acute phase reactant and hence will be affected by inflammation as well as infection. Hence CRP & estimation of ratio of sTfR/log Ferritin is used in newer algorithm for evaluation of microcytic hypochromic anemia as shown in Fig. 2.

Fig. 2. Current algorithm for evaluation of Microcytic hypochromic anemia using newer parameters like CRP & sTfR/log Serum ferritin



Reprinted from Lambert JF, dt al. In C Beaumont, P Beris, Y Beuzard, C Brugnara, eds. Disorders of iron homeostasis, erythrocytes, erythropoiesis. Forum service editore, Genoa, Italy, 2006 page 73 figure 1.

Macrocytic Anemia

Causes of Macrocytosis:

- B_{12} and folate deficiency
- Macrocytosis secondary to reticulocytosis
- Drug induced
- Constitutional Hypoplastic anemia
- Myelodysplastic syndrome
- Liver disease
- Hypothyroidism
- Hemolysis

B_{12} deficiency is common in strict vegetarians and is often associated with severe periungual pigmentation and knuckle pigmentation

B_{12} deficiency is common in Indian children especially strict vegetarian and they are very commonly associated with severe periungual pigmentation and knuckle pigmentation.

It is also important to remember that megaloblastic anemia is very often associated with pancytopenia and their LDH levels are markedly elevated.

Bone Marrow evaluation is very important in Macrocytic anemia, as this would help rule out Myelodysplastic syndrome.

Other investigations that may help are thyroid function test, liver function test and depending on clinical condition work up for hemolysis may be necessary.

Fig. 3: Algorithm for investigation of Macrocytic anemia

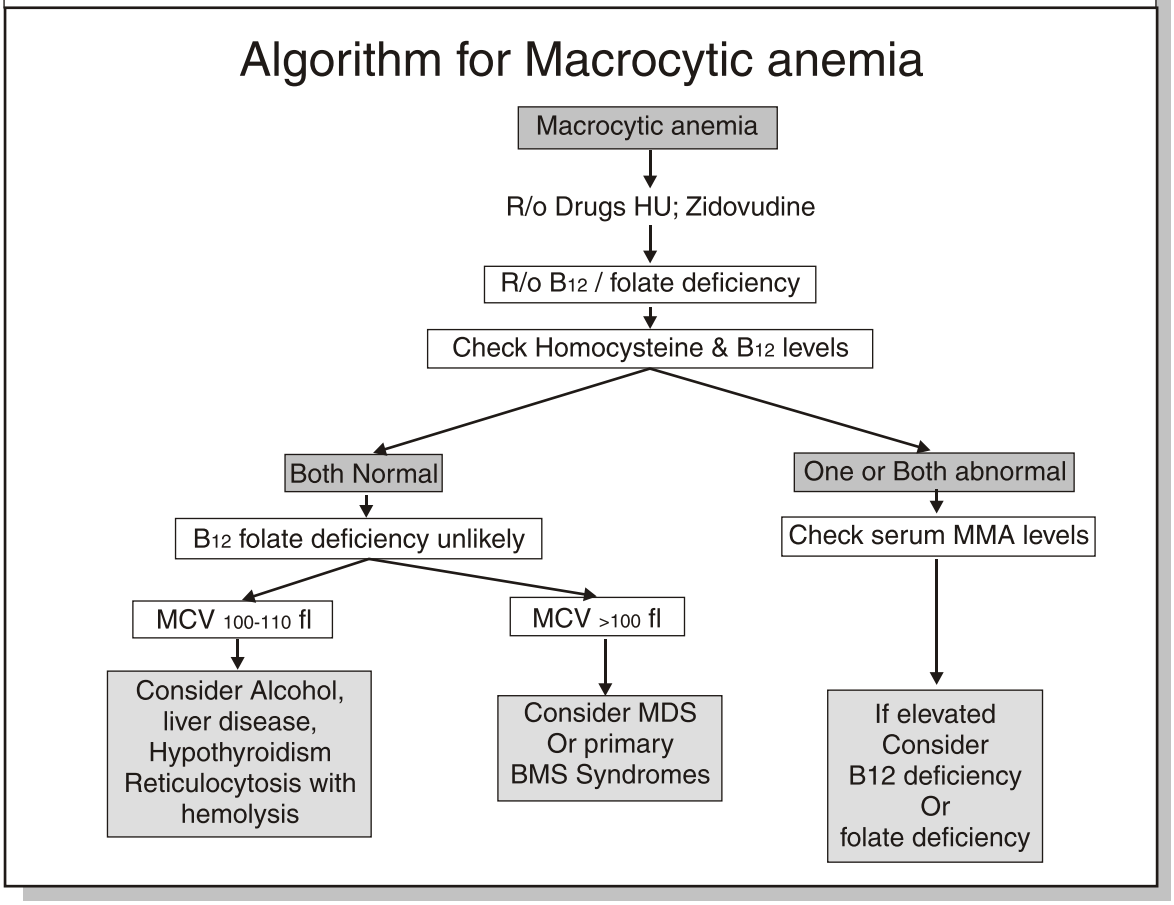


Table 3. Differential diagnosis of Macrocytic anemia

Category of anemia	Differential diagnosis	CBC clues	PBS clues
Macrocytic	Drug-induced	↑RDW	Oval macrocytes
		Marked or mild macrocytosis	
	Nutritional	↑RDW	Oval macrocytes
		Marked or mild macrocytosis	Hypersegmented neutrophils
	MDS or other bone marrow disorder	Increased RDW	Dimorphic RBCs
			Pseudo Pelger-Huet anomaly cells
			Oval macrocytes
	Liver disease, alcohol use	N. RDW	Round macrocytes
		Thrombocytopenia	Target cells
	Hypothyroidism	N. RDW	Round macrocytes
	Hemolysis	N or↑RDW	Polychromasia

Normocytic anemia

Causes of normocytic anemia:

- Dimorphic anemia
- Anemia of chronic disease (ACD)
- Hemolysis
- Bleeding
- Anemia of renal insufficiency

In a patient with normocytic anemia exclude potentially treatable causes like nutritional anemia combined iron and B12 deficiency, bleeding, anemia of renal insufficiency, and hemolysis.

Retic count high may provide clue for hemolysis and presence of hemolysis

Elevated LDH may help detect hemolysis

Elevated creatinine will confirm anemia of renal failure.

Peripheral blood smear may help diagnose sickle cell, dimorphic anemia.

The differential diagnosis of a normocytic anemia that is not linked to bleeding, nutrition, renal insufficiency or hemolysis is either normocytic ACD or primary bone marrow disorder.

Fig. 4. Algorithm for evaluation of normocytic anemia

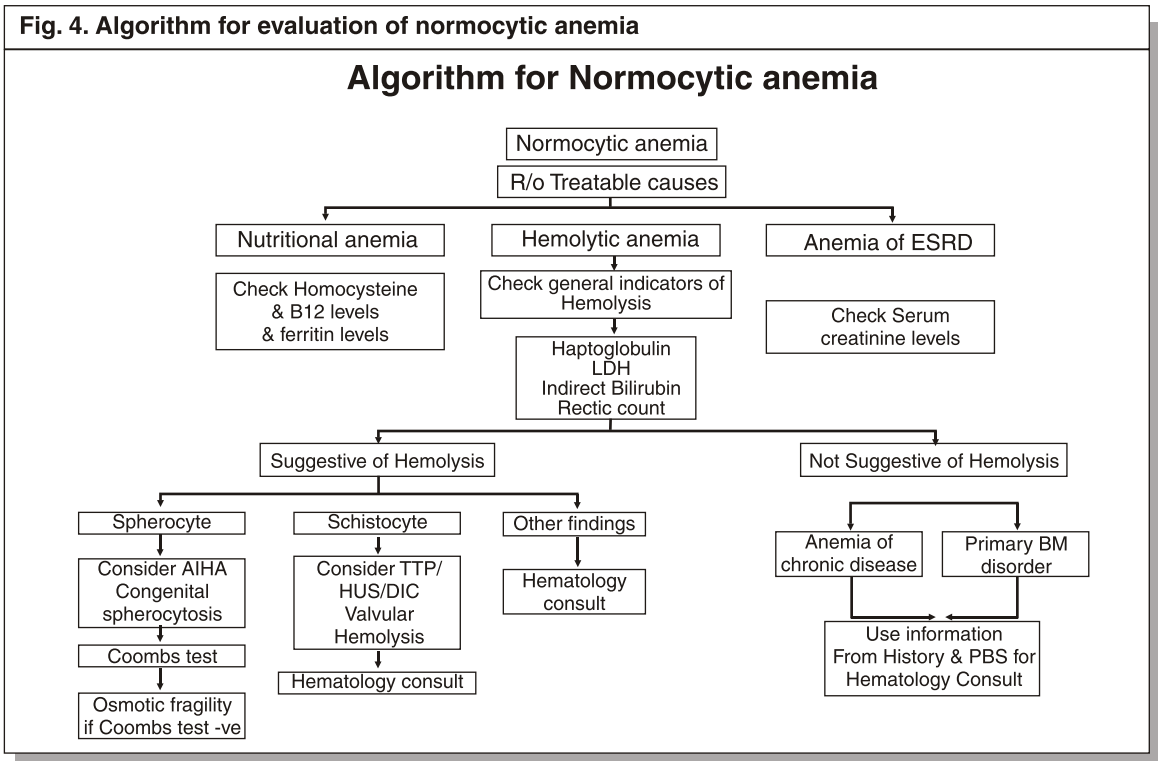


Table 4. Differential diagnosis of Normocytic anemia

Category of anemia	Differential diagnosis	CBC clues	PBS clues
Normocytic	Bleeding	Usually unremarkable	Polychromasia
	Nutritional anemia	↑ RDW	Anisocytosis
			Dimorphic RBCs
	Anemia of renal insufficiency	N RDW	Usually unremarkable
	Hemolysis	N or ↑RDW	Polychromasia
		Thrombocytosis	Spherocytes
			Schistocytes
			Bite cells
	Anemia of chronic disease	N RDW	Unremarkable
	A primary bone marrow disorder	↑ RDW	Dimorphic RBCs (MDS)
		Other cytopenias	Pseudo Pelger-Huet anomaly (MDS)
		Monocytosis	Oval macrocytes (MDS)
		Leukocytosis	Myelophthisis (MMM) †
		Thrombocytosis	Rouleaux (myeloma)
		Abnormal differential	Blasts (acute leukemia)
			Presence of abnormal cells

Thrombocytopenia

As mentioned earlier the first step in evaluation of thrombocytopenia is to exclude the possibility of spurious thrombocytopenia caused by EDTA-induced platelet clumping.

Exclude the possibility of spurious thrombocytopenia caused by EDTA-induced platelet clumping

Examining the Peripheral blood smear will show adequate platelet count or repeating the CBC using sodium citrate as an anticoagulant.

Macroplatelets suggests that thrombocytopenia is peripheral, more likely to be immune mediated

Look at the size of platelet on Peripheral blood smear; if there are macroplatelets than it is likely that the cause of thrombocytopenia is peripheral and more likely to be immune mediated.

Always look for Schistocytes or fragmented RBC's on PNS to rule out the possibility of thrombotic thrombocytopenic purpura/hemolytic uremic syndrome (TTP/HUS) because of the urgency for specific therapy for these diagnoses (ie, plasma apheresis). It is essentially a peripheral blood smear diagnosis and an expert hematomorphologist with background of history of patient is necessary to make this life threatening diagnosis.

Drug-related thrombocytopenia should be considered but there is no specific diagnostic indicator on CBC. History of drug ingestion and its temporal association is extremely important in these cases.

Presence of atypical lymphocytes on PBS suggests a viral aetiology.

Look for abnormal blast cells to rule out leukaemia

Idiopathic thrombocytopenic purpura (ITP) is the major contender in the differential diagnosis of isolated thrombocytopenia. However, ITP is a diagnosis of exclusion that requires consideration of other causes of immune-mediated thrombocytopenia including connective tissue disease, lymphoproliferative disorders, and human immunodeficiency virus (HIV) infection.

ITP is a diagnosis of exclusion requiring consideration of other causes of immune-mediated thrombocytopenia

Leucocyte Abnormalities

Principles of interpretation of WBC abnormalities:

Is the WBC count abnormal for age is the first consideration?

Which WBC is affected, Neutrophil, Lymphocyte, Eosinophils, Monocytes or Basophils?

What is the absolute value of these cells? E.g absolute neutrophil count. Always interpret in terms of absolute values and not in term of percentage of cells.

Are these WBC normal in morphology or have abnormal morphology; look at the peripheral smear and rule out leukaemia and classify whether this is granulocytosis, monocytosis, and lymphocytosis.

What is their trend in the disease process?

LEUCOCYTOSIS:

Neutrophilic leucocytosis:

Pyogenic infections

Drug induced e.g. steroids

In case of brisk bleeding

Systemic onset Juvenile Rheumatoid Arthritis (JRA)

Periodic fever syndrome

Stress

Myeloid leukaemia

Growth factor use like Granulocyte Colony Stimulating Factor (GCSF)

Myeloproliferative disorders

Peripheral smear evaluation will help rule out leukaemia and may pick up clues, which may suggest infiltration of bone marrow like leukoerythroblastic response.

Important point to note is every leucocytosis does not mean pyogenic infection and there are noninfectious causes of neutrophilic leucocytosis.

Every leucocytosis does not mean pyogenic infection

Steroids, Myeloproliferative Disorders, periodic fever syndromes, Systemic onset JRA are important causes of noninfectious Neutrophilic leucocytosis.

Lymphocytosis:

Viral infections

Infectious mononucleosis

CMV infection

Lymphoproliferative disorder

Whooping cough

Koch

Addison's disease.

Peripheral blood smear should be seen to rule out Acute Lymphoblastic leukaemia.

Presence of Atypical Reactive T-cell lymphocytosis suggests viral infection.

Presence of Atypical Reactive T-cell lymphocytosis suggests viral infection. Repeat CBC and PBS after the patient recovers, to confirm that abnormality has resolved

If the clinical scenario is consistent with viral infection; after the patient recovers, the CBC and PBS should be repeated to see whether the abnormality has resolved.

Extreme Lymphocytosis can be seen with whooping cough and the patient may be often referred to you as a case of acute leukaemia. However; peripheral smear show morphologically mature lymphocytes and not abnormal lymphoblast.

Monocytosis (Absolute Monocyte count > 400):

Enteric fever

Koch

Recovery from neutropenia

Viral infections

Primary hemopoietic disorders like Juvenile myelomonocytic leukemia

Absolute monocytosis that is persistent should be considered a marker of a myeloproliferative disorder (eg, chronic myelomonocytic leukemia) until proved otherwise by bone marrow examination and cytogenetic studies.

A hematology consultation is advisable for further evaluation.

Eosinophilia:

Worms
Allergic conditions
Tropical eosinophilia
Loffler pneumonia
Drug induced hypersensitivity syndrome e.g. Carbamezapine
Hypereosinophilic syndromes
Addisonian crisis
Hodgkin's disease
Churg Strauss syndrome
Poly arteritis nodosa
Myeloproliferative disorders
Acute myeloid leukemia M4 with eosinophilia
Eosinophilic fascittis

A trial of deworming and a course of diethyl carbamazine should be given to all patients with eosinophilia

Firstly exclude causes of "secondary" eosinophilia like parasite infestation, drugs, comorbid conditions such as asthma and other allergic conditions, vasculitides, lymphoma, and metastatic cancer.

Stool test for ova and parasites.

In suspected case of primary eosinophilia Bone Marrow is required along with Cytogenetics

A trial of deworming and a course of diethyl carbamazine should be given to all and response followed by measuring the absolute eosinophil count and not by eosinophil percentage as emphasized.

Basophilia:

Allergic conditions
Myeloproliferative disorders
Ulcerative colitis
RA
Influenza, Chicken Pox
Koch
Hypersensitivity reactions

Is extremely rare to see basophilic leukaemia.

This is just a short list and a detailed list can be found in textbook.

LEUKOPENIA

Neutropenia:

Neutropenia is a sinister problem and requires immediate evaluation

Neutropenia is severe if ANC, $<0.5 \times 10^9$/L because of the associated high risk of infection.

Causes of severe neutropenia:

Drug induced

Post viral

Hemophagocytic Syndromes

Congenital Neutropenia Syndrome

Cyclic neutropenia

Leukaemia

Aplastic Hypoplastic anemia

Autoimmune neutropenia

Felty's Syndrome

Any drug should be assumed to be a potential offender until proved otherwise

Table 5. Drugs commonly associated with Neutropenia

Drug category	Drugs
Anticonvulsants	**Carbamazepine, valproic acid, diphenylhydamtoin**
Thyroid inhibitors	**Carbimazole, methimazole, propylthiouracil**
Antibiotics	**Penicillins, cephalosporins, sulfonamides, chloramphenicol, vancomycin, trimethoprim-sulfamethoxazole**
Antipsychotis	**Clozapine**
Antiarrhythmics	**Procainamide**
Antirheumatics	**Gold salts, hydroxychloroquine, penicillamine**
Aminosalicylates	
Nonsteroidal anti-inflammatory drugs	

Any drug should be assumed to be a potential offender until proved otherwise.

Infection with viruses and sepsis is another common cause of neutropenia.

Discontinue the presumed offending agent.

Close monitoring of daily CBC advised.

Use G CSF if clinical condition demands it.

Lymphopenia:

Causes of lymphopenia:

Drugs

Corticosteroids

Immunosuppressive drugs

Anti Thymocyte globulin

Infections

HIV

Measles

Tuberculosis

Collagen vascular disorders

Lupus

Rheumatoid Arthritis

Sarcoidosis

Thymoma

Critical illness in ICU

Congenital primary Immunodeficiency Syndrome

- Severe Combined Immunodeficiency (SCID)

In infancy if a child has lymphopenia suspect severe combined immunodeficiency and it is the easiest diagnosis to make if pediatricians pay attention to Absolute Lymphocyte Count in CBC report.

Like Neutropenia Lymphopenia should be paid attention to and should be routinely seen for when one looks at the CBC parameters.

Thrombocytosis:

Because of wide availability of automated CBC count we now increasingly see Thrombocytosis where platelets come as a part of the evaluation. Practitioners have started paying attention to this parameters and it is worth knowing more about it.

Causes of thrombocytosis:

Infections

- ❑ Viral
- ❑ Tuberculosis

Iron deficiency anemia

Bleeding

Surgery

Asplenia

Chronic inflammation

Inflammatory syndromes

Myeloproliferative disorders

- ❑ CML
- ❑ Essential thrombocytosis, PRV, AMM

Malignancies

- ❑ Neuroblastoma
- ❑ GI malignancies

Reactive

- ❑ Juvenile Rheumatoid Arthritis, collagen vascular disorders

An MT to rule out Koch should be done in all cases of thrombocytosis

The distinction between Primary essential Thrombocytosis and Reactive Thrombocytosis is clinically relevant because the former but not the latter is associated with increased risk of thrombohemorrhagic complications.

Presence of splenomegaly leucocytosis favour primary essential thrombocytosis.

It is extremely rare in children.

In our context an MT to rule out Koch should be done in all cases of thrombocytosis.

Associated microcytosis suggest the possibility of IDA.

Stool Occult blood must be done to rule out presence of blood loss and GI

Malignancy in adults definitely.

If ESR is markedly elevated think of associated collagen vascular disorder

Review old medical records if there is chronic thrombocytosis look for asplenia

If asplenia is ruled out than think of primary thrombocytosis, which is more a problem in the adults.

Some times a Bone Marrow examination is necessary to r/o marrow infiltration irritating the marrow and causing thrombocytosis.

C-reactive protein should be done if elevated the possibility of an occult inflammatory or malignant process, as a cause of reactive thrombocytosis should be considered.

Conclusion

A practitioner should be able to address some but not all CBC abnormalities. We hope this provides some guidance in this regard.

Remember following basic points from a hematologist perspective.

Always do CBC on automated Cell Counter and pay attention to all parameters provided by the counter report and it is cost effective to do CBC on coulter counter.

Refer your patients to centres that standardize their machines regularly.

Always provide history to the pathologist for a meaningful interpretation of CBC.

Your initial CBC should include a retic count & ESR if evaluation is for a child with anemia.

Classify anemia as per ndices. Look for indicators on CBC indices suggesting possibility of thalassaemia minor in every CBC report. This is our obligation to society

Peripheral blood smear is extremely important in children to complement your diagnosis so have an expert see them.

When interpreting WBC always think in terms of absolute values of cells

Besides paying attention to neutrophil also pay attention to absolute lymphocyte counts in clinical practice.

Always review with old records and compare

In case of treatment of anemia follow up CBC must be done to evaluate response

and you must always ensure that not only Hb increases to normal but also abnormal indices have returned to normal.

In case of therapeutic trial in patient of anaemia it is important to follow up CBC to judge response.

It is a good idea to ask for Retic count along with CBC in case of evaluation for patient of anemia.

A prompt hematology consultation is encouraged in patients with severe cytopenia, pancytopenia, or extreme cytosis of any type or when a PBS report suggests TTP or acute leukemia.