

First Hematological Signals of Latent Anemia to Aging Population

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- Demographic data combined with the results of recent studies indicate that anemia is a significant health concern for our aging population. Diagnosis of anemia and determination of its causes are highly dependent on the results of clinical laboratory assays.
- Defining Anemia in the Elderly
- Traditionally, anemia in general population has been defined by criteria developed by the World Health Organization (WHO) in 1968. Anemia is defined for adult males and adult females as hemoglobin (HGB) concentration of < 130 g/L and < 120 g/L, respectively.
- There is some evidence that blood HGB may decline slightly with age. For this reason the anemia can be defined as a decrease of HGB with 10% from initial normal value to persons with age > 66 years.

- The aim of this study was to identify the first hematological signals of latent anemia to aging population using as screening the hematological aspects of cells from peripheral blood and additional tests, establishing the etiology of anemia in the elderly.
- Method
- Erythrocytes indices (MCV, MCH and MCHC), are useful for classifying anemia based on red cells size and HGB content, thus providing a first step in determining a causes of anemia.
- Additional to level of HGB and hematocrit (HCT) have been included Hematological Indexes, red cell distribution width, (RDW), reticulocyte count (RET), serum iron (SI), total iron binding capacity, (TIBC), transferrin saturation index, (TSI), serum ferritin (SF), on an study of 140 adult hospitalized patients with ages between 65-90 years, males and females from wars of chronic diseases.

- It is very important to apply the criteria with very carefully, to individuals, to ensure that reference interval for hematological indices and biochemical markers of iron metabolism are correct. The controls for this study were selected by the following procedure:
- -was established reference interval for normochromic-normocytic red cells with criteria for Hb <140g/l for men and <123g./l for women, RDW <15%, a corrected reticulocyte index <2%, a leukocyte count <10 000 /micro L.
- -was established good specific interval for the biochemical markers of iron metabolism with no functional ID.
- *Plasma specimen was used to determine the standard biochemical marker of iron metabolism. Because of TIBC is considered a sensitive analyte, for detection of ID, the reliability of this marker was tested using Vitros Fe slide and sample TIBC Calibrators Kit 4.*
- *Diagnosing testing has been done on principle of the procedure with reagents: Vitros TIBC column, iron saturating reagent, Fe slides.*

- Blood counts were measured by assaying one specimen from every patient for Hb, red blood cells,
- Hematocrit, mean cellular volume (MCV), RDW, and white blood cells on automated hematology analyzer,
- Coulter HMX, Reticulocyte measurement included percentage of reticulocytes (% ret) and absolute reticulocyte count(# ret = no. red blood cells* % ret).
- Card with expected values to normal control group, in average mean, to 40 health patients, at HMX –
- Coulter Hematological analyzer, were: WBC=9700/dl; RBC=4500 000/dl; HGB=13,9g/dl; HCT=42.2%;
- MCV=93fl; MCH=30,6pg; MCHC=33g/dl; RDW=15.1 units; PLT=270 000/dl; MPV=8,2fl; LIMPH=4.,4%;
- MONO=6.3%; GRAN=479%

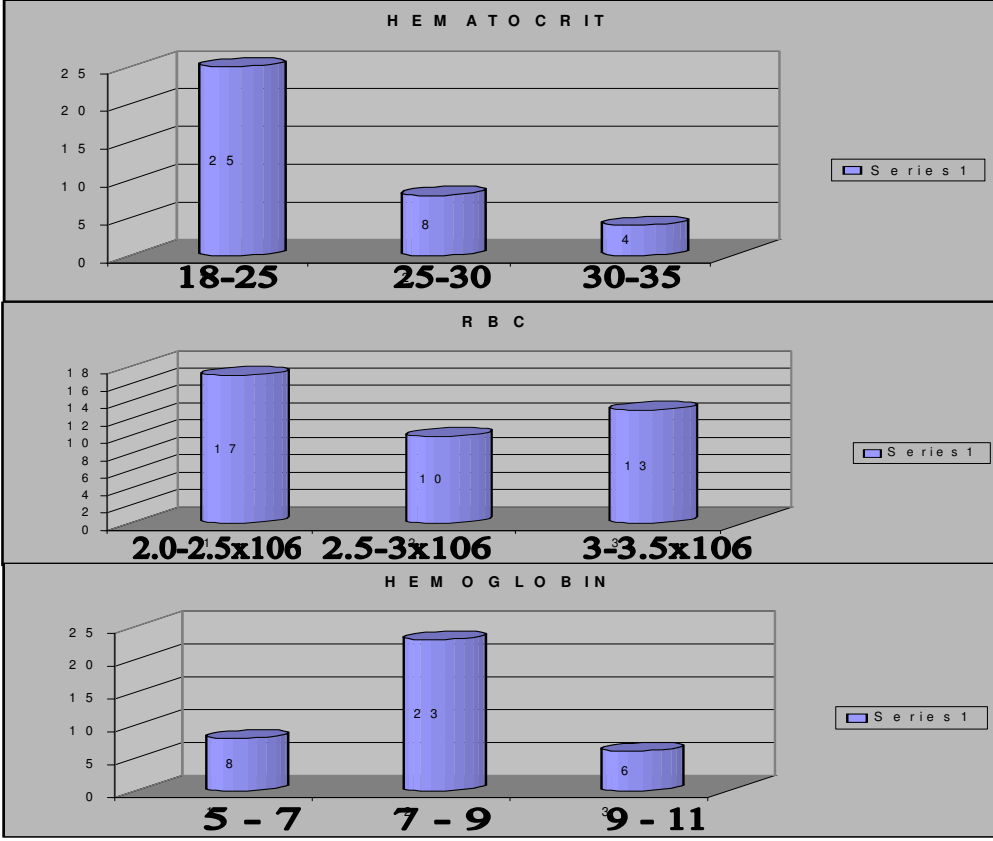
- A new measurement, the reticulocyte hemoglobin (CHr), is reported by some hematology analyzers when RET count are ordered. CHr has been shown to be an early indicator of developing iron deficiency, before HCB levels decline.
- Anemia of chronic disease (ACD), also referred to as anemia of inflammation, was clearly responsible for many cases of anemia observed in the elderly past 66 percent.
- Results:
- Anemia of chronic diseases (ACD) is more common in the elderly than other group
- In ACD correlation of hematological testes was following: 22 % with normal hemoglobin and hematocrit, 31.5 % with only MCV or HCV decreased, 22.5 % patients with Hemoglobin (Hb) in value 10.8 g/dl for women and 11.08 g/dl form maen , 15 % patients with Hb 9.8-10.6g/d L and 9% patients with Hb 7.8-9.6, g/d L (mean SD=2,2); average number of Reticulocytes =1.2%; Corrected Index Ret = 0.6%; Production Index Reticulocytes, (PIR) = 1.4, meaning the hypo-regenerative anemia with globular value, GV =1.2.

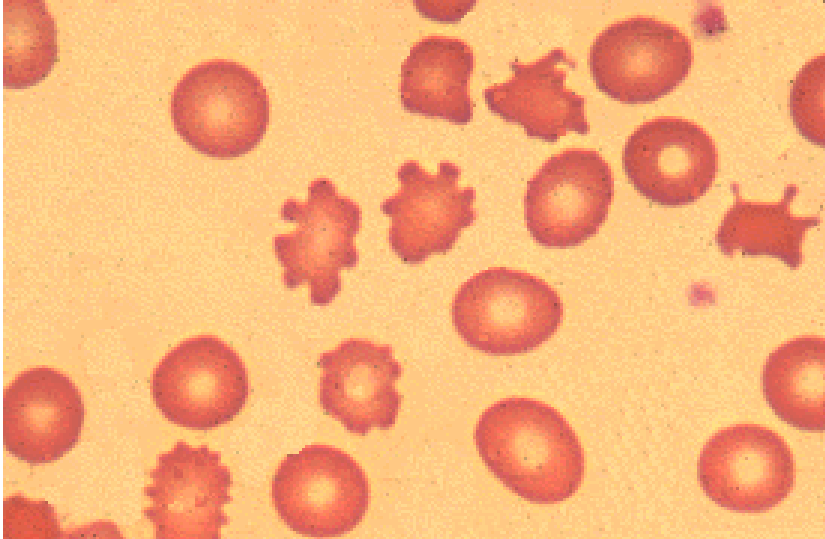
- Anemia of renal disease is considered to be a distinct form of anemia and is typical due to decreased EPO production in 20 percent
- *As indicator of erythropoiesis was used Ret. % and PIR [Ret% * Ht patient /45 /reticulocyte time maturation] when PIR <2 means ineffective erithropyesis, and hypoproliferative anemia. Table 1.*
- To Emergency County Hospital Targu Jiu, we have made in last study the differentiation of ACD from renal disease which is considered a category of anemia distinct of another type of anemia.
- In anemia of renal disease HGB concentration begins to decrease when the level of blood nitrogen rises with 30 mg/d L/ past normal values. The anemia is typical normochromic and normocytic and HGB levels are between 5-8g.dl. Graphic 1
- Poikilocytosis is moderate to severe with burr cells, acanthocytes and schizocytes present. RET count are decreased. Figure 1.
- Other useful assays include tests of renal function such creatinine, urea and urine analysis. Graphic 2

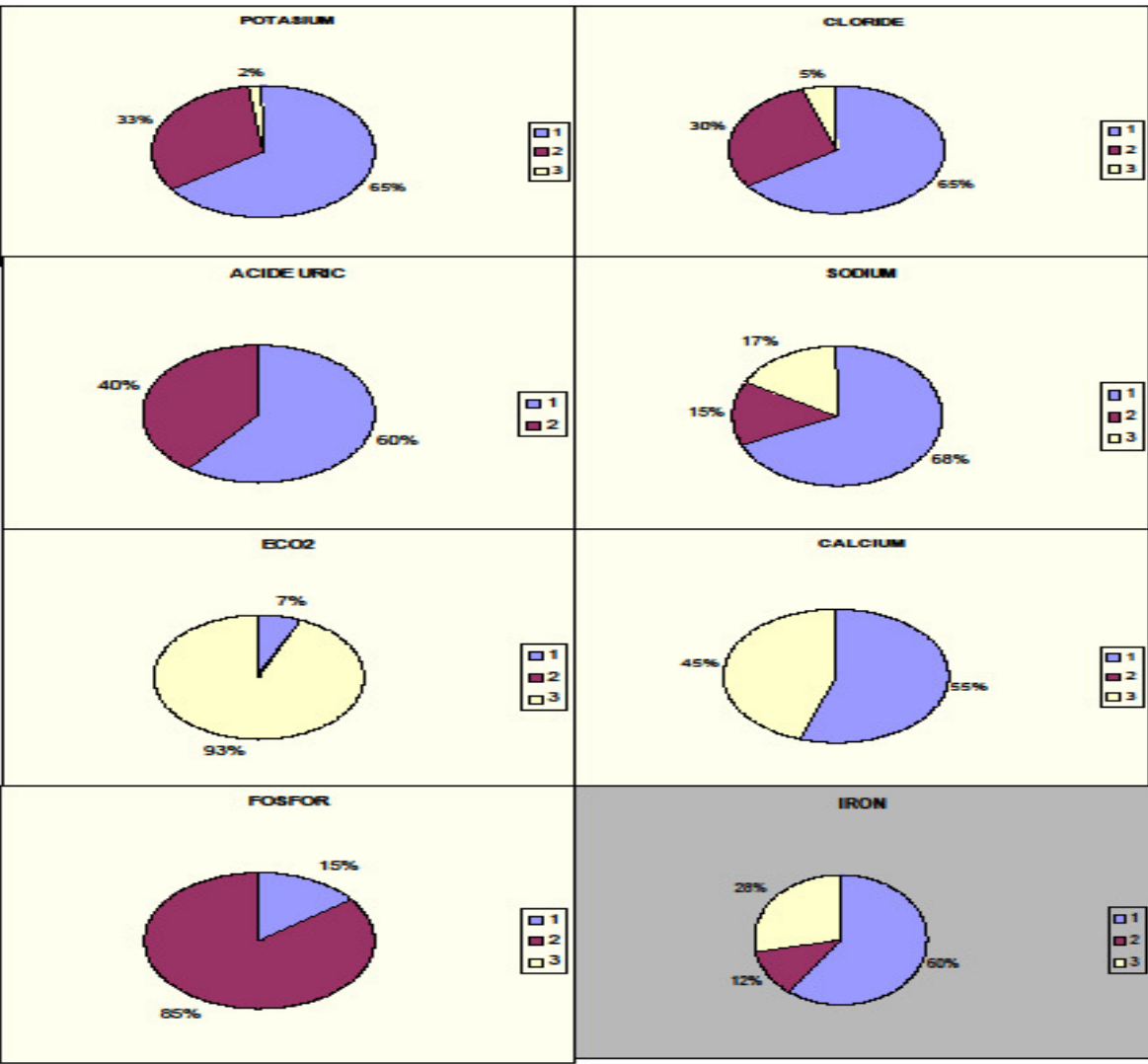
Ht%	PIR	TIBC microgram/d L	TSI %
35 - 30	1.52	225	29.1
30 - 25	1.33	282	76.2
25 - 18	1.21	368	6.5

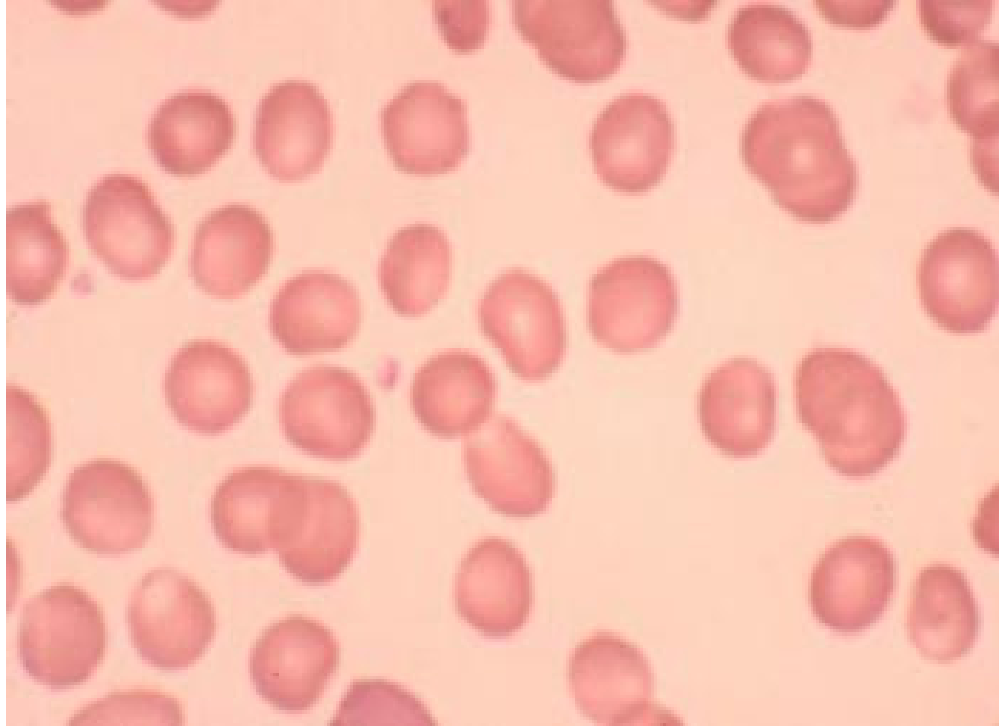
Table 1

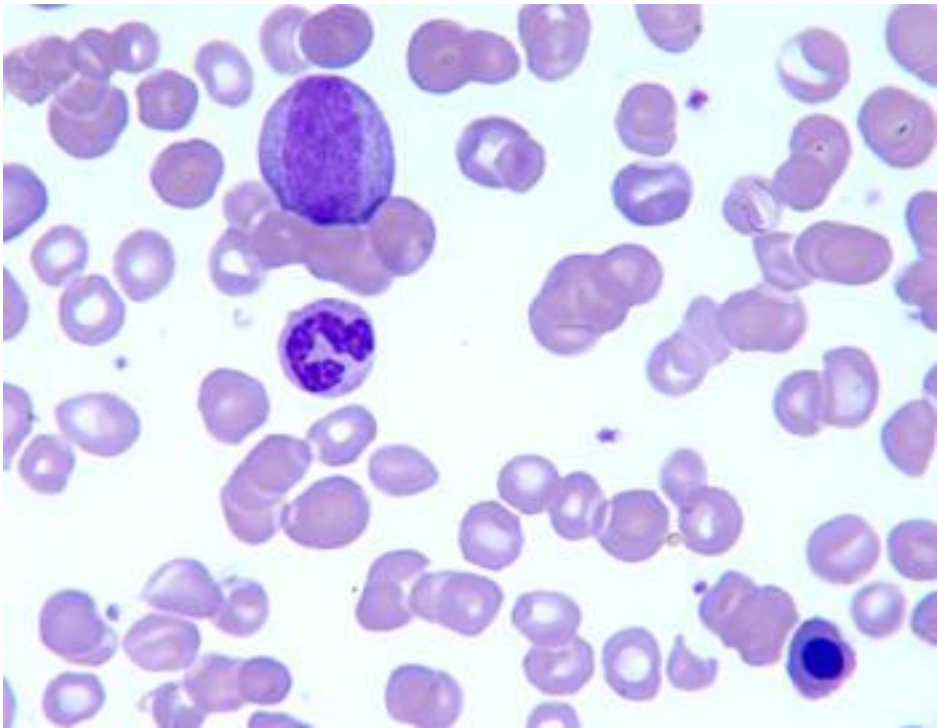
Correlation between Ht, PIR, TIBC and IST in chronic renal failure

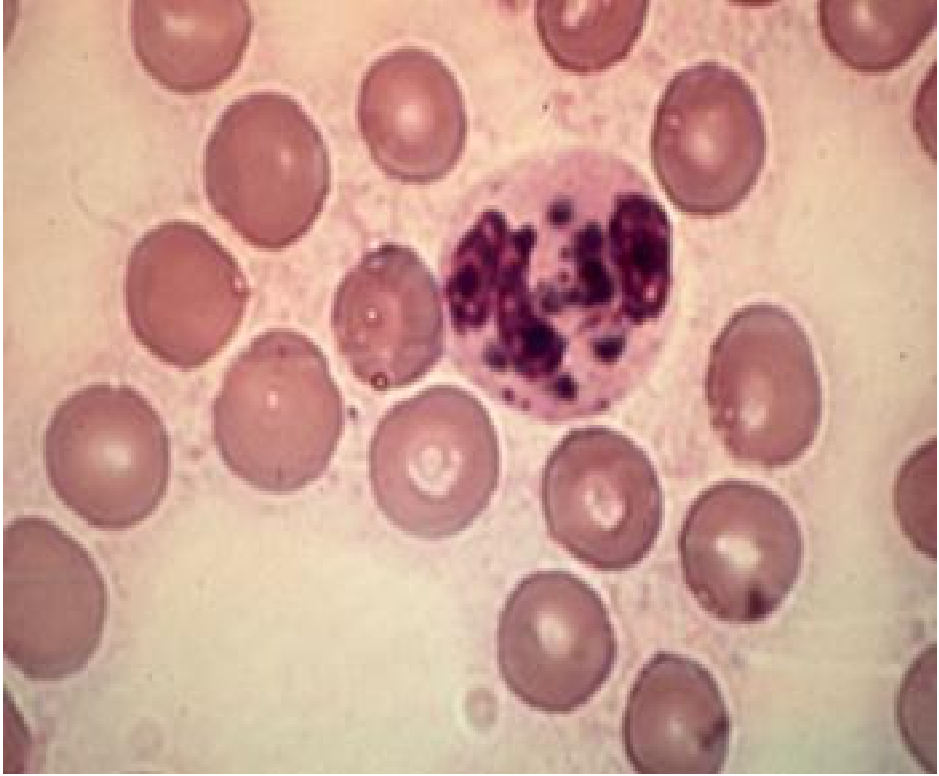




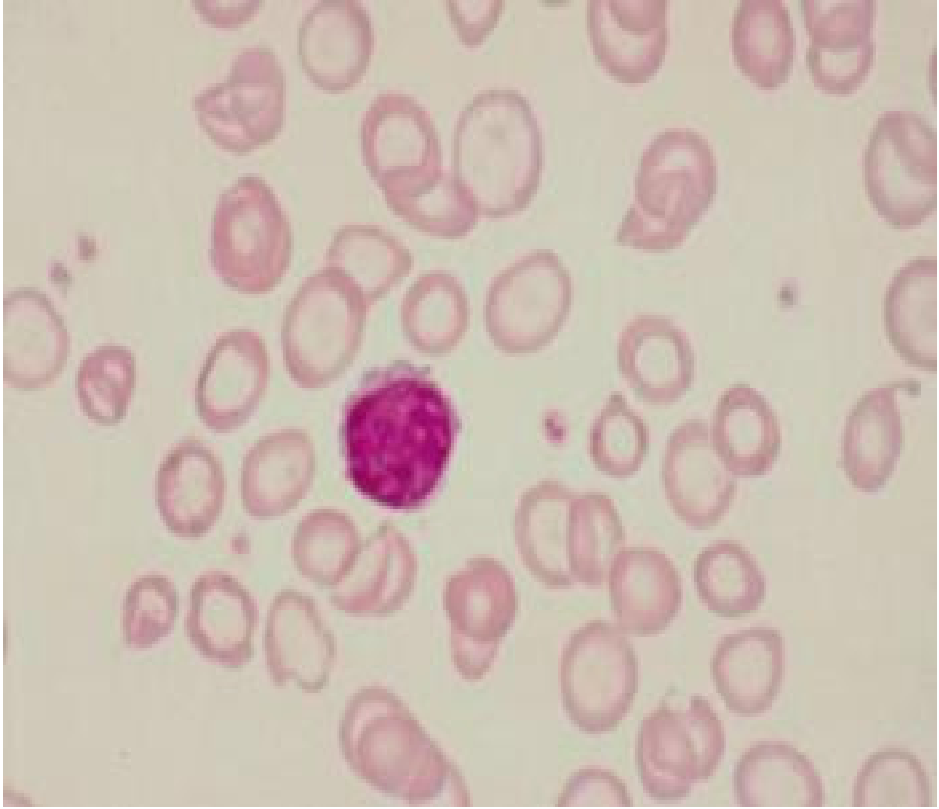


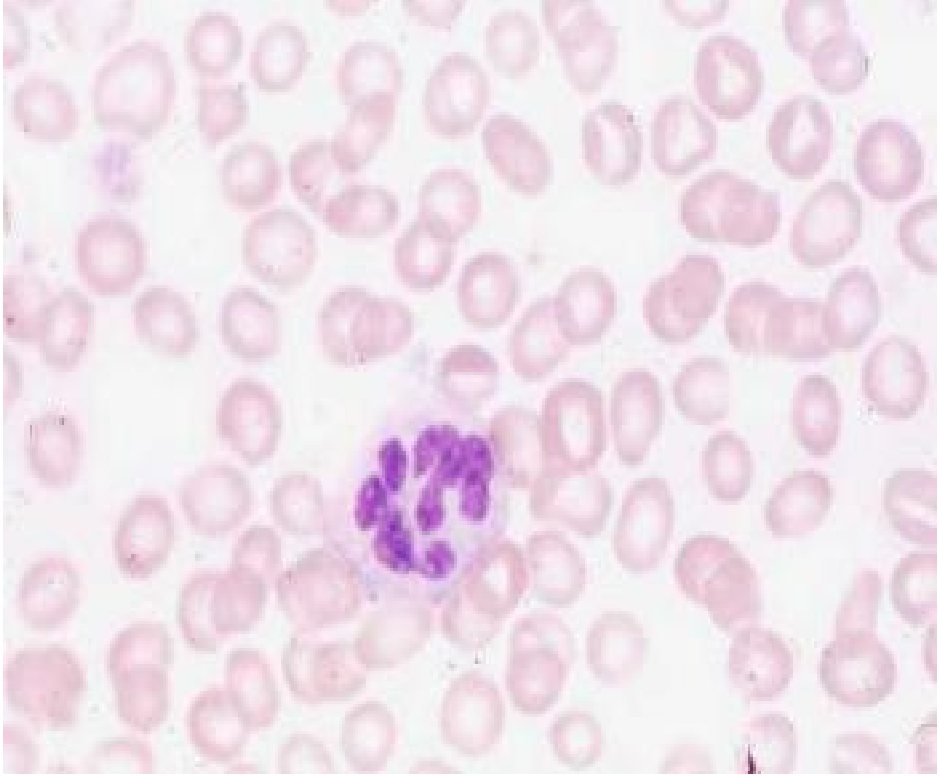






- ACD and IDA may be found in combination and in these situations interpretation of confirmatory tests is difficult.
- When patients are treated with hemodialysis, bleeding and loss of iron or folate results in anemia due to other causes.
- Laboratory of Emergency County Hospital Targu Jiu made a presentation of case of laboratory with the atypical megaloblastic anemia intrigued with iron deficiency anemia to an elderly patient which was admitted in neurological and psychiatry department without specific diagnostic of hematological disease. Figure 5
- Megaloblastic anemia (MA) is macrocytic and normocytic with numerous morphologic abnormalities evident in erythrocytes, including macro-ovalocytes, Howell-Jolly bodies, and basophilic stippling. MCV is usually >110 fL, RDW is elevated and RET is normal or decreased.
- Hypersegmentation of neutrophils is an early and common indicator of MA, and pancytopenia is typical. Figure 6

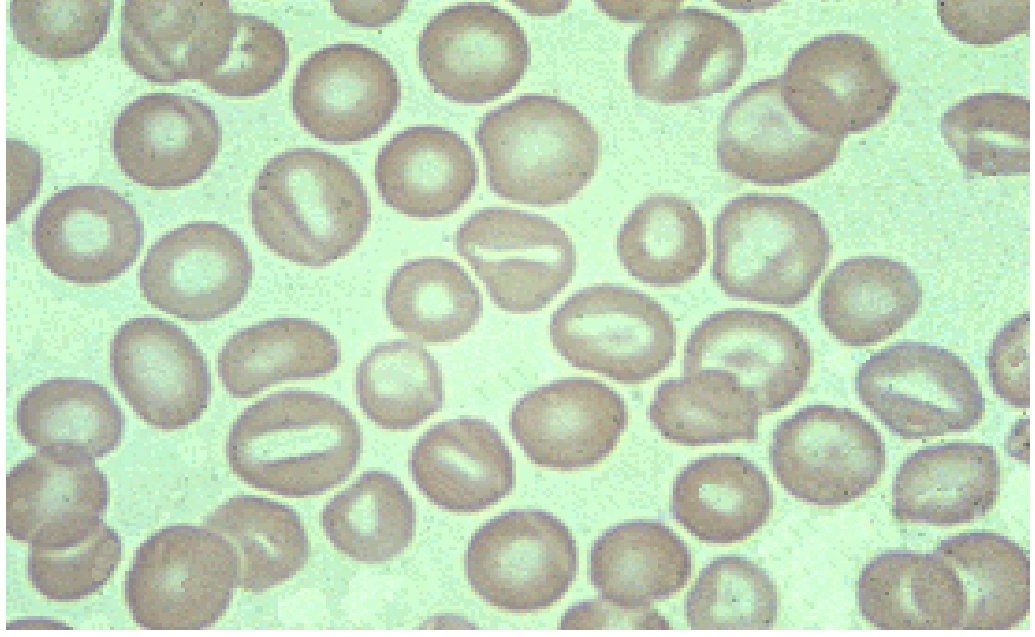


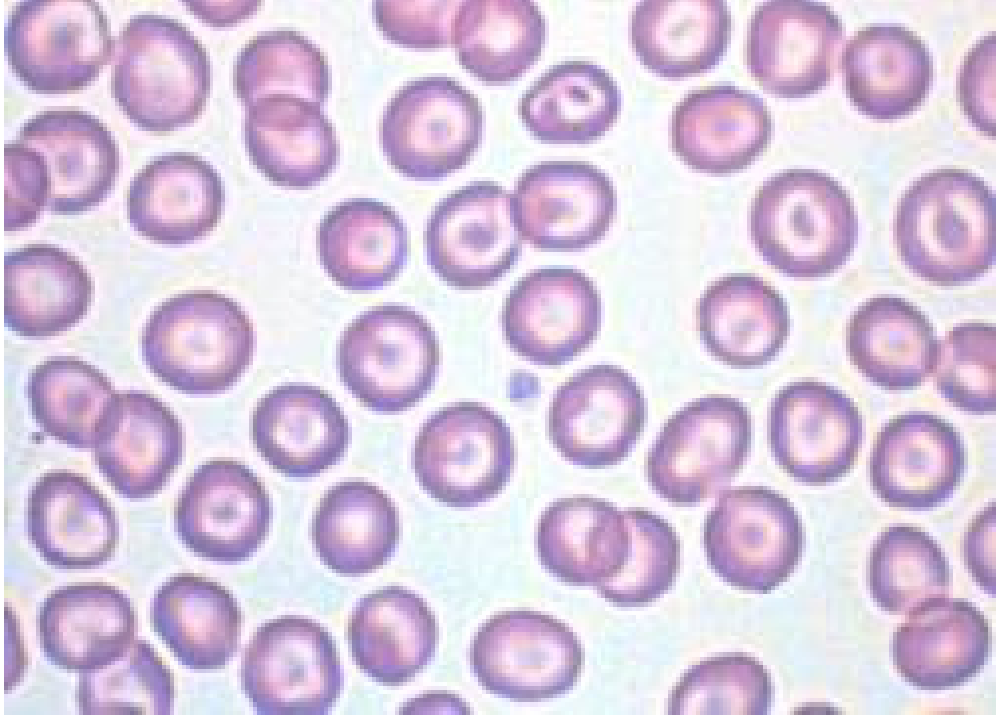


- Assay for serum cobalamin and serum folate are needed to document deficiencies of one or both as cause of MA. Other relevant laboratory test include assay for methylmalonic acid, antibodies for parietal and gastric cells, bilirubin, lactic dehydrogenase and homocysteine.
- Other causes of macrocytosis include alcoholism, liver diseases and reticulocytosis. In generally, MCV is less elevated in these condition than in MA, and elevated RET are a key feature of hemolytic anemia and severe blood loss.
- An elevated MCV should not be ignored because the patient is especially suspected of alcohol abuse
- Discussions and Differential Diagnosis
- The problems in diagnostic anemia occurs when the iron reserves are depleted and no clinical symptoms found. This is actually iron deficiency without any clinical symptoms, also known as iron deficiency without anemia. Second problem is chronic inflammation infections and neoplasm. Quantitative analysis of iron in serum without TIBC and transferin in the serum is of little importance.
- Anemia due to iron deficiency (IDA) is microcytic and hypochromic, especially once the HGB level fall below 10g/L and this was emphasized in 15% from studied cases
- Typically, HGB and HCT are somewhat lower that seen in ACD Figure 7



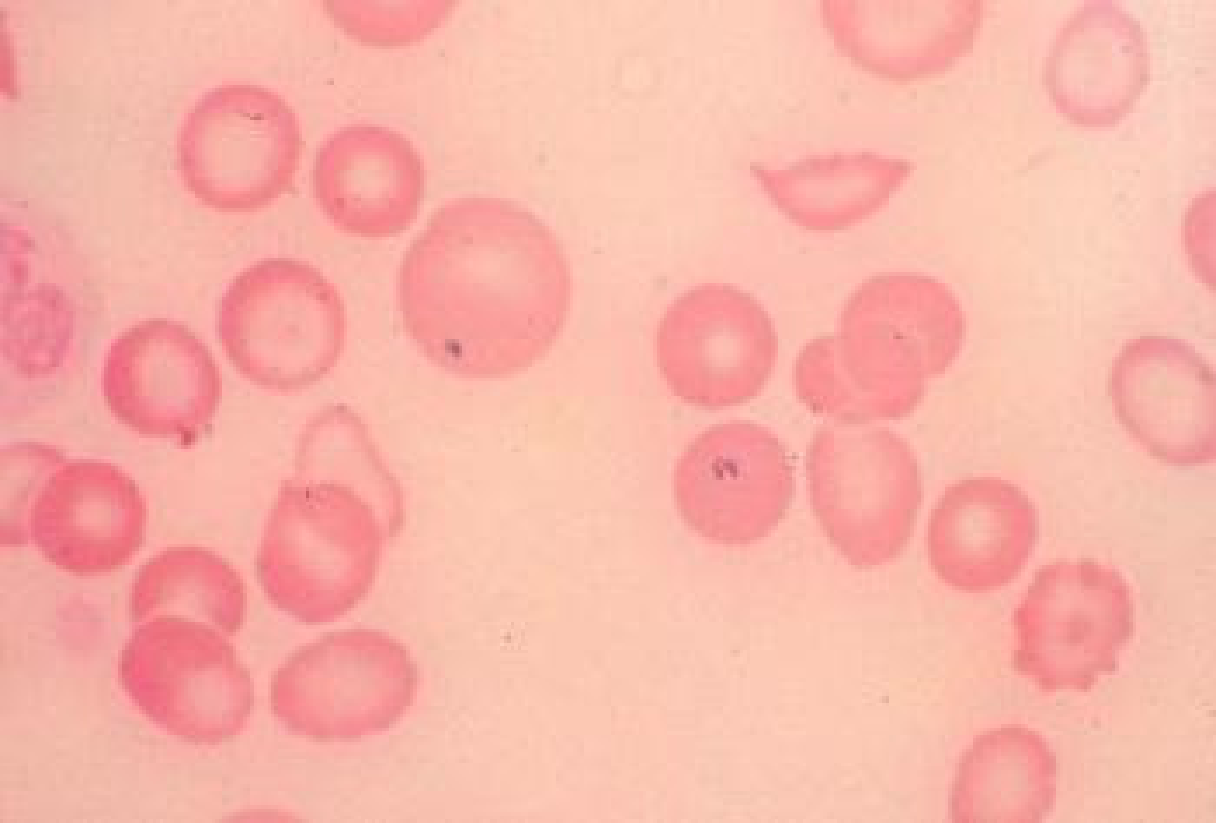
- In severe cases, elliptocytes may accompany microcyte and hypochromic cells on blood film. Stomatocytes have a slit like appearance in the area of central pallor - many chemical agents may cause this abnormality. Figure 8
- Low SI, ITS, and SF combined with elevated RDW, TIBC, and sTR are typical for IDA and must be differentiated from ACD and thalasemia.. Figure 9
- Measuring of TIBC is an indirect method of assessing transferrin and provides comparable information. The TIBC (or transferrin) are typically performed along with the STI. Taken together, these determinations are useful in the differential diagnosis of many disorders affecting iron metabolism, including hereditary hemochromatosis (HH) and iron deficiency anemia. Transferina (Tf) and TIBC are typically low-normal or decreased in HH and are increased in iron deficiency. .
Serum is the specimen of choice for Tf and TIBC. TIBC is less subject than SI to day-to-day variation and other causes of variability





- (TSI) is usually reported along with the SI and TIBC. TSI indicates the percent of iron binding sites on transferrin that are carrying iron. TSI is derived from a calculation using the formula:
$$\text{TSI} = (\text{SI}/\text{TIBC}) \times 100$$
TSI results are reported as percentages. Typical reference intervals for TSI are 20% to 55% for males and 15% to 50% for females.
- TSI is generally considered to be the most sensitive laboratory test for detecting altered iron metabolism in hereditary hemochromatosis (HH). It may be elevated prior to significant deposition of tissue iron. TS levels increase as additional iron is accumulated.
- A drawback to using the TS is that it is dependent on performing both the SI and TIBC.
- Current guidelines from the American College of Physicians include a normal level of TSI encompassed between 20-40%, a cutoff level of TSI >55% identifying iron overload and TSI < 16% meaning IDA.

- Serum [ferritin](#) (SF) level reflects the amount of storage iron in tissues. This parameter can not be used in acute or chronic inflammations, infectious diseases and malignant diseases, being increased as a protein of acute phase.
 - RDW is a mathematical expression of size variation used to quantify anisocytosis. The higher the RDW means the greater the anisocytosis. RDW is increased in iron deficiency, and tends to be normal in thalassemia minor and intermedia. Increased RDW may be an early indication of iron deficiency, where it may precede the onset of microcytosis.
 - We registered by ambulatory study that MCV of red cells decreases below normal before of hemoglobin in IDA.
 - Because IDA in elderly adults is most commonly a result of iron loss due to chronic gastrointestinal bleeding, treatment includes stopping loss and replacing iron with an oral supplement. Ferritin levels may be periodically re-ordered to asses the efficacy of treatment.
- Figure 10



- IDA can be presented in three forms:
- 1) -Prevalent anemia with low SF, when ferritin descends in early stages of iron deficiency, before changed in concentration of hemoglobin, size of erythrocyte or amount of iron or high TIBC.
- 2) -Latent anemia with low SF and low circulating serum iron, TIBC is increased, urine iron is low and erithrocytes with low iron in content as in hypochromic anemia.
- 3) -Installed anemia with deficiency of erithropyesis, low ferritin (< 50 micro.gram/L) in bone morrow, TSI < 16% in serum iron and hypochromic and microcytic erythrocytes.
- When the report Iron/TIBC is less than 15%, we have certain diagnostic of IDA.
- Low serum iron, serum ferritin increased and normal or low TIBC means ACD. Low serum iron, low serum ferritin and TIBC increased means IDA.
- IDA and thalasemia are both microcytic-hypochromic anemia. We used erythrocyte indicators such as MCV, MCH, and RBC number to distinguish this anemia types.

Test	Patient Result	Reference Intervals (Adult female)
White blood cell (WBC) count	3.7 x 10 ⁹ /L	4.4 - 11.3 x 10 ⁹ /L
Red blood cell (RBC) count	5.6 x 10 ¹² /L	4.1 - 5.1 x 10 ¹² /L
Hemoglobin (Hb)	10.5 g/dL	12.3 - 15.3 g/dL
Hematocrit (HCT)	36.6%	35.9 - 44.6%
MCV	65.8 fL	80.0 - 96.0 fL
MCH	19.9 pg	27.5 - 33.2 pg
MCHC	26.7%	33.4 - 35.5%
RDW	14.0	<14.5
Platelets	249.0 x 10 ⁹ /L	100.0 - 450.0 x 10 ⁹ /L
Total serum iron	165 µg/dL	60 - 150 µg/dL
Iron-binding capacity	230 µg/dL	250 - 400 µg/dL

The RBC count is increased for the amount of hemoglobin present. The concentration of hemoglobin in the RBCs is slightly decreased (hypochromic) and the cells are small (microcytic). The variation in RBC size (RDW) is within normal limits.

Other anemias, most notably thalassemia, are also characterized by low MCV, MCH MCHC and additional tests are need for conformation of IDA.

- Patient with a ratio target cells/normal cells > 0.9 in low power field and with $>20\%$ microcytic red cells on blood film, were suspicious for
 - beta-thalsemia and was discovered in 1% from studied cases.
 - RBC count result is higher in thalassemia minor group in comparison with IDA. Microcytic, hypochromic and polyglobulia are more evident in thalassemia minor compared with IDA and hemoglobin and hematocit can be normally but only MCV and HCV decreased in thalassemia silent carrier.
- Graphic 3
- Conclusions;
 - The results of these studies suggest that HB concentration may decline with age and therefore reference interval specific to geriatric are needed.
 - Most anemic adults are asymptomatic until HGB fall below normal values. For this reason a routine anemia screening should be recommended by HB, HCT and MCV, HCV, MCHC to persons with age past $>_{65}$ year.
 - The anemia of aging exists and must be managed of laboratory medicine in collaborative with the clinician.