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Variability of Biological Parameters in Blood Samples Between Two Consecutive Schedules of Hemodialysis

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1. Introduction

Epidemiologic studies have suggested that anemia may be associated with poorer outcomes in a variety of disorders. The WHO criteria define anemia by hemoglobin (HGB) concentration of < 130 g/L for adult men and<120 g/L for adult females. [1]
Nonetheless, recent evidence indicates that even mild anemia is independently associated with increased risk of recurrent falls, poorer physical function, hospitalization, and mortality in older adults. [2, 3]
A number of studies have reported differential distributions of anemia by age and sex, but less attention has been devoted to disparities in anemia by race. According to NHANES III estimates, older non-Hispanic blacks were 3 times more likely to have anemia compared to older non-Hispanic whites (27.8% vs 9.0%). [1]
Similar disparities in anemia prevalence have been observed in other population-based studies of older blacks and whites [4, 5]. These observations have led some to consider race-specific criteria for defining anemia. [6]
A recent study in Iceland defined mild anemia as a hemoglobin concentration between 10.0 and 11.9 g/dL in women and between 10.0 and 12.9 g/dL in men [7]. This cross sectional analysis provides evidence of anemia in 36.7% of hospitalized patients, and shows an association among anemia, poor nutritional status, and inflammation [8].
Future research on anemia in the elderly should focus on the age-related physiologic changes underlying this condition and whether anemia correction can reduce anemia-associated risks, and improve quality of life [9, 10].
Erythrocytes indices, derivatives from value of HGB and numbers of erythrocytes was used in correlation with serum iron to establish grades and types of anemia and was pathological results of these indices was noted as first signals of latent anemia in hematological diseases. Mean corpuscular volume (MCV) measures the mean or average size of individual red blood cells. To obtain the MCV, the hematocrit is divided by the total RBC count.

$$MCV = \frac{Hematocrit\ 1/1 x 1000}{Number\ of\ erythrocytes\ (x10^{12}/L)}$$

The MCV is an indicator of the size of red blood cells. MCV is measured in cubic micrometers or fento-liters (Reference values: adult men: 80-94 fl, women: 81-99 fl).
Mean corpuscular hemoglobin (MCH) measures the amount, or the mass, of hemoglobin present in one RBC. The weight of hemoglobin in an average cell is obtained by dividing the hemoglobin by the total RBC count.

$$MCH = \frac{Hemoglobin\ (g/L)}{Number\ of\ erythrocytes\ (x10^{12}/L)}$$

MCH is expressed in picograms of hemoglobin per cell (pg/L, 1 pg = 10-12 g).
(Reference values: adult men; MCH = 27 - 31 pg, women = 27-30 pg). Mean corpuscular hemoglobin concentration (MCHC) measures the proportion of each cell taken up by hemoglobin.

$$MCHC = \frac{Hemoglobin\ (g/L)}{Hematocrit\ 1/1}$$

The results are reported in percentages, reflecting the proportion of hemoglobin in the RBC. The hemoglobin is divided by the hematocrit and multiplied by 100 to obtain the MCHC. (Reference values: adults: MCHC = 32- 36 %}
RDW (red cell distribution width) reflects the size distribution of the erythrocyte population. The hematological instrument calculates it as a coefficient of variation (CV),

$$RDW = \frac{Standard\ deviation\ of\ red\ blood\ cell\ size\ distribution}{MCV}$$

(Reference values: adults RDW = 11.5 - 15.5)
The aim of this study was to identify the values and changes of hematological and biochemical parameters in blood samples between two consecutive schedules of hemodialysis and assesses the effect of plasma osmolality on errors of platelets count, to the hospitalized patients admitted in hospital with diagnosis chronic renal diseases complicated with chronic renal failure.

2. Method

The prospective study of laboratory was performed on 90 known patients with chronic kidney diseases(CKD) complicated with chronic renal failure(CRF), admitted in hospital, prior to undergoing schedules of dialysis, (55 men and 35 women), in average ages 35-65 years (mean, age 50, SD= +_2).
The patients were analyzed once a month, all at the same day, to connection and after connection of hemodialysis schedules, in medical internal department.
For diagnosis of specific anemia of chronic renal diseases, laboratory tests included hemoglobin (HGB), hematocrit (HCT), white blood cells and platelets count, differential count and red cell indices (mean cell volume (MCV), mean cellular hemoglobin (MCH), mean corpuscular hemoglobin concentration (MCHC) red cell distribution width (RDW), being performed using an automated analyzer (Coulter HMX with 22 parameters) and for

specific biochemical parameters in chronic renal failure as serum iron, total iron binding capacity, and index saturation transferrin (IST), usually and specific biochemical tests:
Glucose, Urea nitrogen, Creatinine, Sodium, Potassium, E CO2, was used a dry chemistry analyzer Vitros 700(Ortho Diagnostics), Johnson $ Johnson.
Reticulocyte count (RET %) was calculated after microscopic analysis of brilliant cresyl blue stained slides, (normal ranges adult: 0.5 - 1.5%). To evaluate rate of erythropoiesis, the Reticulocyte Production Index (RPI) was calculated using the formula: [RPI = RET% x HCT patient /45 /reticulocyte time maturation], where maturation time (reticulocytes survival days in peripheral blood**)** was considered 1 day
for HCT 36-45%, 1.5 days for HCT 26-35%, 2 days for HCT 16- 25% and 2.5 days for HCT < 15%. Reference interval for RPI in healthy individuals is 1.0-2.0; and RPI < 2 in a person with anemia indicates ineffective erythropoiesis, while values > 2 indicate compensation for decreased red cell survival (bleeding, hemolysis) [11].
Three methods were used to assess platelet counts of hemodialysis patients: optical microscopy, peripheral blood smear and user of the cytometry principle with impedance principle (VIC) by Coulter HNX hematological analysis. For to avoid systematic errors during platelets count by optical microscopy, a method of direct counting in the Burker-Turk chamber(hemacytometer) has been recommended for use in parallel with determination of the number of platelets counted on peripheral blood smear, (by optical microscopy). Calculation of the platelets counted in the Burker-Turk chamber considers the height of the chamber and the surface of the middle square of the chamber to yield a value of 0.2mm^2. [12].
The calculation formula for hemacytometer cell counts determines the number of cells within 1µL (1 mm^3) of blood. To make this determination, the total number of cells counted must be corrected for the initial dilution of blood and the volume of diluted blood used. The standard dilution of blood for platelet counts is 1:100; therefore the dilution factor is 100. The volume of diluted blood used is based on the area and depth of the counting area. The area counted is 2 mm^2 and the depth is 0.1 mm; therefore the volume factor is 0.2 mm^3. Total number of cells counted • dilution factor • 1/volume factor = cells/mm3 (cells/mm^3= cells/µL or cells/µL • 10^3µL /L = cells x 109/L).
Direct microscopy of the blood smear yields the number of thrombocytes count by counting those found between 1000 erythrocytes (5 microscopic fields of 200 red cells) multiplied by the number of erythrocytes/mm.^{3} and then divided /1000) with the results expressed as platelets/ mm^3. The estimate of platelet count from slides uses a semiquantitative method, whereby 1 platelet / oil immersion field is equivalent with 20.000 plt/mm^3. Figure 1 In optical microscopy, one assesses a panoptic colored blood smear under the immersion objective(100 X). Most platelets have a dendritic aspect and fringe-like extension. Normal platelets have diameter of 2-4 microns on the blood smear with 70% alone, 20% in groups of 2 or 3 and 10% in larger groups or “big pools”. Correctly executed blood smear reveal microscopic fields on the oil-immersion objective with an average of 10 platelets;as either isolated or grouped. Visualization of <5 platelets on the microscopic field connotes thrombocytopenia while >40 indicates thrombocythemia. [13].
Platelets are typically disk-shaped with a more dense central (granular) area and a peripheral (crystalline) area with functional dendritic fringes [14]. If activated by toxic metabolic factors, platelets become more spherical, which can yield a decrease in the intensity of the image in the microscopic lenses, due to light transmission and diffusion through samples. When platelets are activated, they become spherical with a hypogranular

cytoplasm and release small particles. This may lead to the erroneous detection of platelets when using the microscopy owing to their deformed morphology.

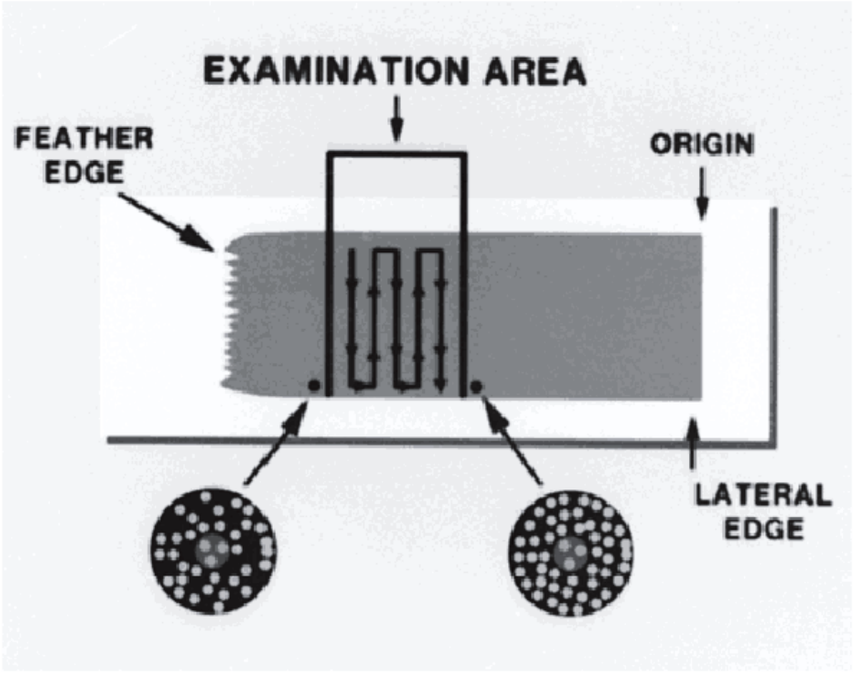


Fig. 1.

Recognizing erroneous results of platelet counts is especially critical for a consistent decision in the diagnosis of disseminated intravascular coagulation (DIC) and for clinical decision making regarding transfusion.

The platelet count is an indispensable parameter in the DIC scoring system proposed by the International Society on Thrombosis and Hemostasis Sub-Committee of the Scientific and Standardization Committee on DIC, in which platelet counts of less than 100 × 103/μL (100 × 109/L) and less than 50 × 103/μL (50 × 109/L) would score 1 and 2 points, respectively. [15, 16] The samples were assessed for platelet count by statistical parameters: [SD = (Xi- Xm)2 /n – 1; accuracy: (%Diff = X average – X target/ X mean x 100, with normal value until + − 25) and Z score(Z = X average-X target/SD, with normal value until +-2, R>0.95%), for average platelets 150-400 x10^3/μl, 95% CI.].

3. Results

From total patient in our study, a minority of patients, 36 patients (40%) had normal results for all hematological tests under monitoring treatment of specialty. In type of anemia from kidney chronic diseases, an additional 16 (18%) patients had normal HGB and HCT, but low MCV or MCH ((mean value 72 fL, SD= 2.1) or MCH (mean value 24.3pg, SD= 1.6). Other 28 patients (31%) had mild anemia (HGB decreased but > 106 g/L), while only 10 patients (11%) have had severe anemia. All individuals in the group with severe anemia had low RET (mean value 1.2%, range 0.5-1.5%), and RPI in mean value of <1.4, indicating a hypo-regenerative type of anemia.

To the 54 patients with anemia of chronic kidney diseases (ACKD) and chronic renal failure(CRF) were registered in 30.90% of cases normal TIBC values (mean value 282 microgram/ d L, SD=2.5), low RPI in mean value of 1.33, low IST in mean value of 7.62%, with middle ineffective erythropoiesis and moderate iron deficiency anemia (IDA) and to 19.10 % of patients with ACKD and CRF associated with renal inflammations, were calculated low RPI, in mean value of 1.21, high TIBC value (mean value 468 microgram/d L, SD =2.4) and low IST in mean value of 6.5%, with severe ineffective erythropoiesis and severe IDA.

In biochemical field, in this study on this cohort of hemodialysis patients, was obtained the variability of plasma osmolality past normal individual values (310 Osm/l), in the samples taken from the patients with chronic renal failure because of high values of Urea nitrogen (mean value 112 mg%; 40 mmol/L; SD = 2.40); Creatinine (mean value 5.5 mg/%; 4.85 mmol/L); SD=0.15); Sodium (mean value 170 mmol/L; SD=0.14); Potassium (mean value 14.5 mmol/l; SD=2.88); E CO2 (mean value 11 mmol/L; SD=0.26). Prevalence of anemia to patients admitted in hospital for undergoing schedules of hemodialysiss have been registered in percents: 60% of cases, with normochromic-normocytic anemia, 30% of cases with microcytic-hypochomic anemia and nutritional iron deficiency, 7% of cases with aplastic anemia and 3% with macrocytic and vitamin B12 deficiency.

In cases with microcytic-hypochomic anemia and nutritional iron deficiency were registered by this study that mean corpuscular volume (MCV) of red cells decreases below normal value before that the hemoglobin to be decreased under normal value. Iron deficiency anemia associated with ACKD was presented in three forms:

- Prevalent anemia with low serum ferritin (SF), when ferritin descends in early stages of iron deficiency, before changes of concentration of hemoglobin concentration, size of erythrocyte, level of iron serum value, with high TIBC (8%),
- Latent anemia with low SF and low circulating serum iron, TIBC is increased, urine iron is low and erythrocytes with low iron in content have aspect of hypochromic red blood cells (10%),
- Installed anemia with deficiency of erythropoiesis, low ferritin (< 50 microgram/L) in bone morrow, TSI < 16% in serum iron and hypochromic and microcytic erythrocytes (12%), [17,18]. When the aspect Iron/TIBC is less than 15%, we have had the certain diagnostic of ACKD associate with IDA. Low serum iron, serum ferritin increased and low TIBC means ACD. Low serum iron, low serum ferritin and TIBC increased means IDA [19].

In the two cases of study were registered suspect flags on Coulter HMX: neutropenia, lymphopenia and increased MCV erythrocyte index (109 f L). On blood smear from peripheral blood, in optic microscopy the reticulocyte count was decreased (0.4%), and neutrophil granulocytes showed multi-segmented nuclei, macrocytes (larger than normal RBCs) presence of ovalocytes (oval-shaped RBC) but Howell-Jolly bodies(chromosomal remnant) was absented. An elevated MCV should not be ignored because the patient is especially suspected of alcohol abuse. Blood chemistries will also showed: an increased lactic acid dehydrogenase (LDH) values of .increased of homocysteine, folic and vitamin B12 deficiency.

Bone marrow (checked in a patient suspected of megaloblastic anemia on hematological analyzer, in 3% from cases) showed megaloblastic hyperplasia~ 45%, ploycromathopil and acidophil erythroblasts with megaloblastic character, large metamielocytes and giant band forms. Biopsy results from gastric mucosa showed lesions of chronic gastritis, non-atrophic epithelium and the patient was receiving the recommendation from clinician doctor to assess B12 vitamin.

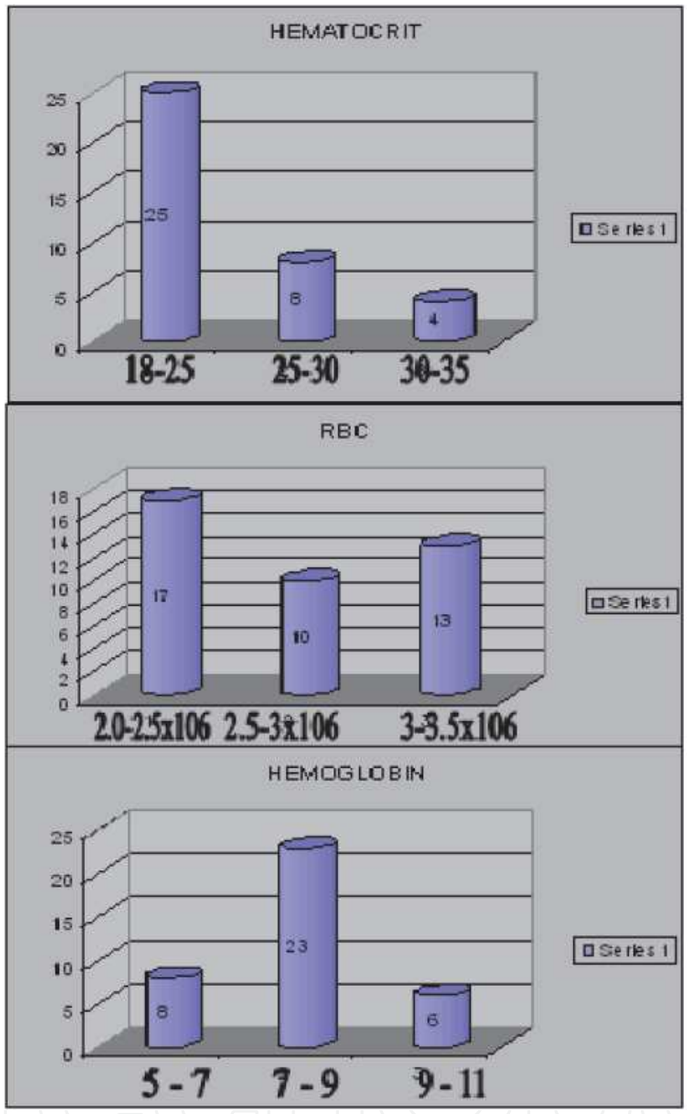
Diagnosis in all these patients has been established in collaboration with clinician doctors from department of hospitals in the system of evidence based medicine, on data encompassed in observation daily sheet of patients.
The suspect cases with hemolytic anemia were verified on biochemistry panel (unconjugated bilirubin, LDH) and in hematological field by Coombs test direct (DET) and indirect,reticulocytes presented in elevated number, haptoglobin levels decreased, also increased urobilinogen in urine analysis.
The bone marrow aspiration was performed by sternum bone puncture, to 7 patients with suspect chronic refractory anemia from myelodisplastic syndrome on evidence of aspect of peripheral smear with neutropenia, anemia and thrombocytopenia, (low cell counts of white and red blood cells, and platelets, respectively) with blast count <5% in the peripheral blood, beside macrocytosis and microcytosis. The morphological abnormality was observed in the granulocytes. These included bi-lobed or un-segmented nuclei (pseudo–Pelger-Huet abnormality) and granulation abnormalities in vary from.
After this aspect the clinician doctors recommended bone morrow puncture to National Institute of Reference Hematological Diseases, City Bucharest, (Romania). Was excluded the diagnosis of acute myeloid leukemia when < 20% blasts was observed on blood smear of bone morrow. In severe cases, red blood cells in eliptocytes forms accompanied microcytic and hypochromic cells on blood film. Low SI, IST%, and SF combined with elevated RDW, TIBC suggest IDA and this type of anemia must be differentiated from uncomplicated anemia from ACKD. An association between, HCT, HGB and RBC (Graphic 1) or HCT, TIBC, RPI and IST (Table 1) can be applied and in assessment of anemia from chronic diseases taken in this study.

HTC %	RPI	TIBC microgram/d L	IST %
35 - 30	1.52	225	29.1
29 - 25	1.33	282	7.62
24 - 18	1.21	468	6.5

Table 1. Correlation between Hematocrit (HTC), Reticulocytes Production Index (RPI) Total Iron Binding Capacity (TIBC) and Index Saturation Transferrin (IST) in Anemia of Chronic Renal Failure

The platelet count determined on the peripheral blood smear was used to complement data from the quantitative methods and provided morphological information.
The comparison between the platelet counts on the Coulter HMX (mean value $\bar{X}$ = 233 x 10^3µl; p=0.028; SD=2; % Diff=0.90; Z score = - 0.30) and by optical microscopy ($\bar{X}$ = 250 x 10^3µl; p=0.029; SD= 2.6; %Diff = -3.6; Z score =0.40) yielded similar values in a control group (120 male and female healthy subjects, ages 25-55 years(mean age 40).
For the dialysis patients, we found that results for platelet counts with the Coulter HMX, before and after hemodialysis were similar: (pre-dialysis mean $\bar{X}$ = 230 10^3 µl; p=0.024; SD=3.45; % Diff = -4.53; Z score =2.5; post dialysis mean $\bar{X}$ = 245 x 10^3µl; p=0.034; SD=2.1; %Diff = 6.34; Z score = 0.10) but differences appeared if counting was done using optical microscopy (pre-dialysis mean $\bar{X}$ =261 x 10^3µl; p = 0.020; SD=7.1; %Diff= 5.90; Z score=3.90); post-dialysis mean $\bar{X}$ = 167 x 10^3µl; p = 0.6; SD=4.2; %Diff= -7.10; Z score= -2.90). Table 2
The latter results may be attributable to the variability of plasma osmolality in the samples taken from the patients with chronic renal failure: Glucose (98mg%; 5.44mmol/L; SD=2.80); Urea nitrogen (112 mg%; 40 mmol/L; SD = 2.40); Creatinine (5.5 mg/%; 4.85 mmol/L);

SD=0.15); Sodium (170 mmol/L; SD=0.14); Potassium (14.5 mmol/l; SD=2.88); E CO2 (11 mmol/L; SD=0.26). (Table3. Graphic 2)



Graphic 1. Levels of HGB, RBC and HTC in chromic renal failure

The performance of devices used was assessed by **Z score = < 1 = optic performance; 1 < Z < 2 = good performance; 2 < Z < 3 = satisfactory performance and Z > 3 =unsatisfactory performance.** In parallel, we assessed platelet count using the peripheral blood smear and found that it provided information that was complentary to the other methods, especially with respect to morphological aspects of platelets.

Counting thrombocytes on slide from peripheral blood smear is necessary in quantitative platelet disorders, as isolated thrombocytopenia: immune versus nonimmune, thrombocytopenia associated with other hematological abnormalities or in differential diagnosis with platelet clump, thrombocytosis and qualitative disorders, as giant platelets (megathrombocytes), platelet inclusion or granule abnormalities, bizarre in shape and size.

The control group to 40 potential health persons (20 adult men and 20 adult females), on hematological analyzer Coulter HMX, was next results (mean value), form men: WBC=9700/dL, RBC=4500 000/dLHGB=13,9g/dL, MCV= 90 f L, RDW=13.5%, MCV = 29 f L, MCHC = 34%) and for women WBC=95/dL, RBC=4200 000/dL, HGB=12,5g/dL, MCV= 80 f L, RDW=14.5%, MCV = 27 f L, MCHC = 30%) [Sensitivity = (35/ 40) x 100 = 87.50%].

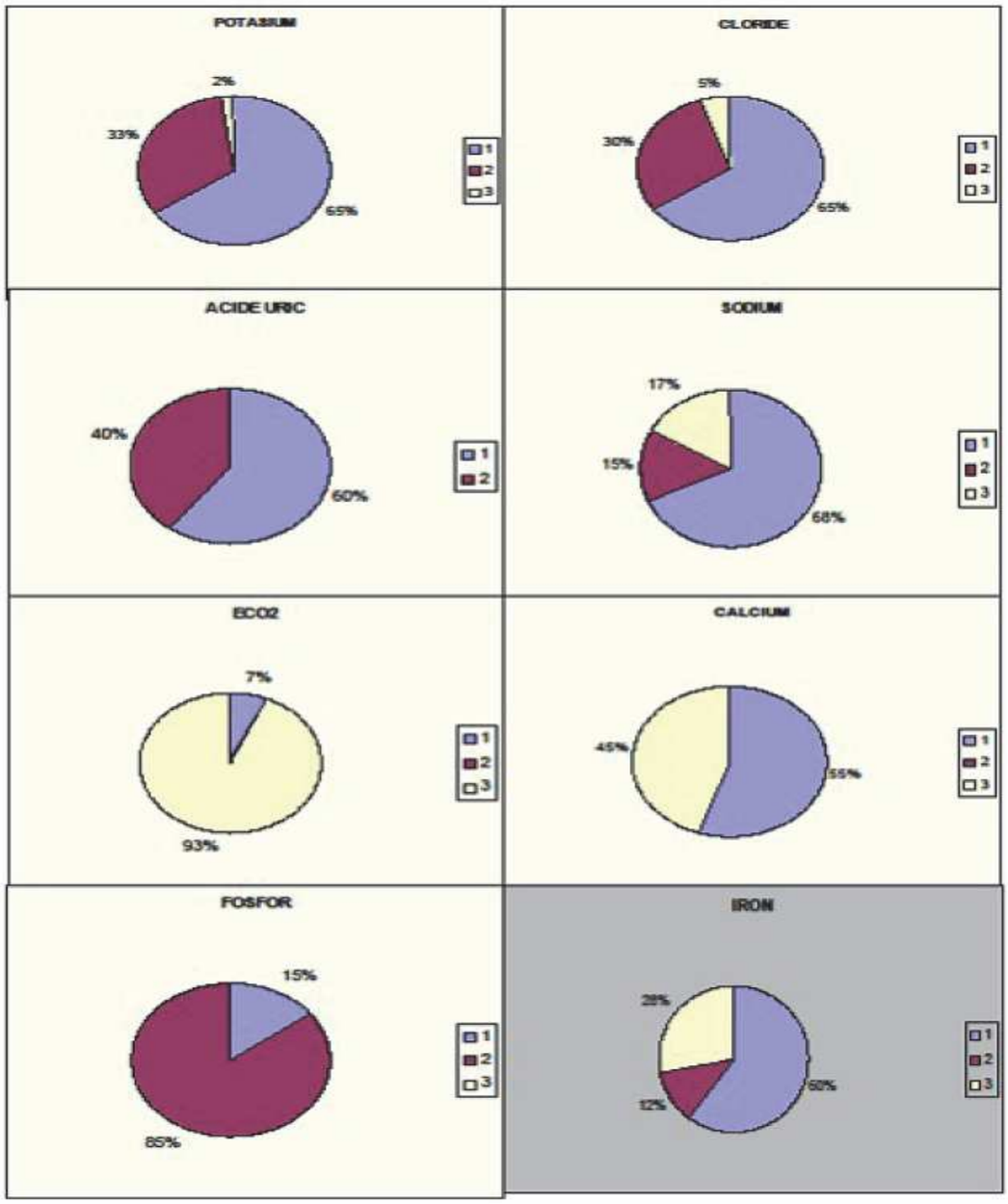
In biochemistry field, normal results of the same group control were registered next results: Creatinine, 1.2 mg/dl, with SD=0.15,CV%=29, accuracy [Z] =-1.36; Iron, 100 microgram/dl, SD=2.88, CV%=1.8, Z=-0.56; Phosphate, 27.mEq/dl, SD=0.14.CV%=2.2, Z=-0.8; Urea, 40mg/dl, SD=2.40, CV=2.2, Z=-0.13; Uric acid, 8mg/dl, SD=0.26; CV=3.2, Z=-0.79; [Normal Z = ±2 in Control of Levey Jennings Chart.].

The precision to our cohort in study was registered as next results: CV < 2% for RBC, CV < 1% for HGB and CV < 2% for HCT, (Accuracy: r > 0.95 for HGB and HCT, 95% CI), mean SD=2.2 and p=0.04 for HGB, mean SD = ± 2.5 and p < 0.05 for MCV in CBC, MCHC with CV =2%, MCH with CV=1.5%, RDW with CV = 3%. [Specificity = (124/140) x 100 = 88%]. Positive predictive value (107/124) = 86%.

Coulter HMX Normal Patients;	**Microscopy Normal Patients**	**Microscopy Normal slide blood**
$\bar{X}$ = 233 x 10^3µl; p=0.028; SD=2; %; Diff=0.90; Z score = - 0.30;	$\bar{X}$ = 250 x 10^3µl; p=0.029; SD= 2.6; %; Diff = -3.6; Z score =0.40.	$\bar{X}$ = 240 x 10^3 µl;CV=5.3%, SD= 12.7; %; Diff= 8.30; Z score= 3.33;
Coulter HMX Patients with CRF before connected to dialysis devices	**Optic Microscopy Patients with CRF before connected to dialysis devices**	**Microscopy slides Patients with CRF before connected to dialysis devices**
$\bar{X}$ =230 x10^3 µl; p=0.024; SD=3.45; % Diff = -4.53; Z score =2.5),	$\bar{X}$ =261 x 10^3µl; p = 0.020; SD=7.1; %; Diff= 5.90; Z score=3.90	$\bar{X}$ = 275 x 10^3 µl; CV=5%; SD= 13.75; %; Dif= 15.75; Z score = -3.46,
Coulter HMX Patients disconnected from dialysis devices	**Optic Microscopy Patients disconnected from dialysis devices**	**Microscopy slides Patients disconnected from dialysis devices**
$\bar{X}$ = 245 x 10^3 µl; p=0.034; SD=2.1; %; Diff = 6.34; Z score = 0.10),	$\bar{X}$ 167 x 10^3 µl; p = 0.6; SD=4.2; %; Diff= -7.10; Z score= -2.90	$\bar{X}$ =190 x10^3 µl;CV=4.6%; SD= 8.74; %Diff =18; Z score =7.60;

Table 2. Assessment of performances for methods used in platelets count to patients with Chronic Renal Failure, undergoing dialysis

Functional ID was closely related to the production of hypochromic red cells, and measurement of red cells hemoglobinization provides a sensitive method for determining the quantity of circulating iron incorporated into the red blood cells which, reflect recent changes in erythropoiesis.



Graphic 2. Biochemical parameters of chronic renal failure which are frequently increased in Chronic Kidney Diseases (CKD)

Parameters in Chronic Renal Failure before schedules of Dialysis (mean value)	Parameters in Chronic Renal Failure after undergoing the schedules of Dialysis (mean value)	Normal Range of Blood Tests used in Diagnosis of CRF (laboratory reference)
Glucose: 98mg%; (5.44mmol/L); SD=2.80;	Glucose: 105 mg%; (5.76 mmol/L) SD=1.04;	Glucose: 65- 115 mg%; (3.9-6.1 mmol/L)
Urea nitrogen: 112 mg%; (40 mmol/L); SD = 2.40;	Urea nitrogen: 65 mg%; (23.2 mmol/L); SD = 1.60;	Urea: 17-45 mg%; (1.7-8.3 mmol/ L)
Creatinine; 5.5 mg/%; (4.85 mmol/L); SD=0.15;	Creatinine; 1.8 mg/%; (1.58mmol/)L; (SD=0.20;	Creatinine: 0.2-1.25; (0.07-0.12 mmol/L)
Sodium: 170 mmol/L;SD=0.14	Sodium: 145 mmol/L;SD=0.70	Sodium: 137-145 mmol/L;
Potassium14.5 mmol/l;SD=2.88	Potassium7.1 mmol/l;SD=2.90	Potassium: 3,6—5mmol/L
E CO2: 11 mmol/L;SD=0.26;	E CO2: 19 mmol/L;SD=2.29;	E CO2: 22-30mmol/L
Hb= 8.5g/- 9.2mg/dl; SD=2.20	Hb= 10.5- 11.2mg/dl SD= 2.45;	Hb=11.4-13.6mg/dl; SD=2.7

Table 3.Values of biochemical and hematological parameters in blood samples from patients with Chronic Renal Failure, undergoing the schedules of dialysis

4. Discussions

Diagnosis in all these patients has been established in collaboration with clinician doctors from department of hospitals in the system of evidence based medicine, on data encompassed in observation daily sheet of patients.

Anemia of chronic kidney (ACKD) diseases associated with the iron deficiency (IDA) was microcytic and hypochromic, especially once the HGB level fall below 100g/L and HCT are somewhat lower that seen in normochromic, normocytic anemia from chronic diseases(ACKD).

Proportion of hypochromic red cells is a time average marker, was similar in anemic patients like glucose, HbA1c in diabetes patients. The marker for IDA, hypochromic erythrocytes, has been investigated for every patient, on blood film slide, May Gunwald stain. The hypochromic cells >10% were considered functional ID, in correlation low with iron. Various cut off values for functional ID is reported in literature ranging from 2% to 10% of hypocromic cells [17]

Measuring of TIBC was made as an indirect method of assessing transferrin and provided comparable information [18]. TSI indicates the percent of iron binding sites on transferring that is carrying iron. TSI is derived from a calculation using the formula: [(SI/TIBC) x100] and 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 cut off level of TSI >55% identifying iron overload and TSI < 15% meaning IDA. Red distribution width (RDW) is a mathematical expression of size variation used to quantify anisocytosis. The higher the RDW means the greater the anisocytosis. Increased RDW may be an early indication of iron deficiency, where it may precede the onset of microcytosis.
These measurements, known as erythrocyte or red blood cell indices, provide an important information about various types of anemia. If the MCV is low, the cells are microcytic or smaller than normal. Microcytic red blood cells have been seen in iron deficiency anemia and thalassemia minor. If the MCV is high, the cells are macrocytic, or larger than normal. Macrocytic red blood cells were associated with pernicious anemia or folic acid deficiencies. If the MCV is within the normal range, the cells are referred to as normocytic and nomocytic anemia was met with more frequency in chronic diseases/inflammation, small MCH under 27% show hypocromic erythrocytes, frequently encountered in IDA. In the same correlation with MCHC less than 32% indicates that the red blood cells are deficient in hemoglobin concentration.
This situation is most often seen with iron deficiency anemia. RDW is a measurement of anisocytosis. IDA and thalassemia are both microcytic-hypochromic anemia. As screening tests for discovery of anemia to elderly we used, beside additional tests, erythrocytes indexes such as MCV, MCH, and RBC number to distinguish this anemia types. MCH is just the equivalent of Retyculocites –Hemoglobin (Ret-He) that indicates the long term of life span of erythrocytes.
Both serum transferrin receptor and erythrocyte zinc protoporphyrin have been demonstrated to be useful in a variety of clinical situations. Serum transferrin receptor can be best used in diagnosing iron disorders, especially for patients with pathologies that may affect iron metabolism. Erythrocyte zinc protoporphyrin can be best used as a primary screening test for assessing iron status, especially in patients likely to have uncomplicated iron deficiency hemoglobin status and life span of erythrocytes [18].
Other anemia, most notably thalassemia, are also characterized by low MCV, MCH, MCHC and additional tests are needed for confirmation of thalassemia Patient with a ratio target cells/normal cells > 1% in low power field and with >20% microcytic red cells on blood film (magnification x 400), were suspicious for beta-thalssemia. RBC count result 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 hematocrit can be normally but only MCV and MCH decreased in thalassemia silent carrier. (Graphic 3)
The bone morrow hemosiderrin and microscopic bone marrow examination have been recommended in clinical management in most elderly patients with anemia in Mielodysplastic Syndrome (MDS) The problems in diagnostic anemia occurs when the iron reserves are depleted and not.
The peptide hormone Hepcidin appears to play a central role in the pathogenesis of the anemia of chronic disease, but is extremely difficult to measure in the serum. Thus the "anemia of chronic disease" may include patients with a variety of patho-physiological mechanisms. The peptide hormone Hepcidin, secreted by the liver, controls plasma iron concentration by inhibiting iron export from macrophages cells(cut off, 15 ng/d L, Elisa

method). The effect of Hepcidin is to increase intracellular iron stores in ACD, decreased dietary iron absorption and decrease circulating iron concentration in chronic anemia from inflammations and infections [19].

Laboratory Test Results

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

Graphic 3. The RBC count is increased for the amout of hemoglobin present. The concentration of hemoglobin in the RBCs is slighlty decreased (hypochromic) and the cells are small (microcytic). The varioation in RBC size (RDW) is whitin normal limits.

In chronic renal failure (CRF), the peripheral blood smear can reveal activated thrombocytes with fingers(burr cells) as isolated cells or organized in groups. By contrast, with diabetic ketoacidosis, one can see the reverse phenomenon, thrombocytes that are isolated, with round shape form and without activated fringes. Figure 2 It is interesting that platelet activation markers were associated with the severity of DIC and erroneous platelet counts, suggesting that platelet activation is a potential source for the inter-method variation in platelet counts. More attention needs to be given to improve the accuracy of platelet counts, especially in clinical conditions with high levels of platelet activation.

It is well known that white light is comprised of luminous waves with different wave lengths of 750-250 nm. Optical microscopy uses light diffraction but can have light reflection, refraction, diffusion and dispersion phenomena, especially through media with non-homogenous densities.

Thus, it has been recommended that one conduct platelet counts using phase-contrast microscopy, which helps eliminate such light interference phenomena because it the image is formed by a diffraction process in two stages: incident light diffraction and diffraction of the light refracted in the objective.

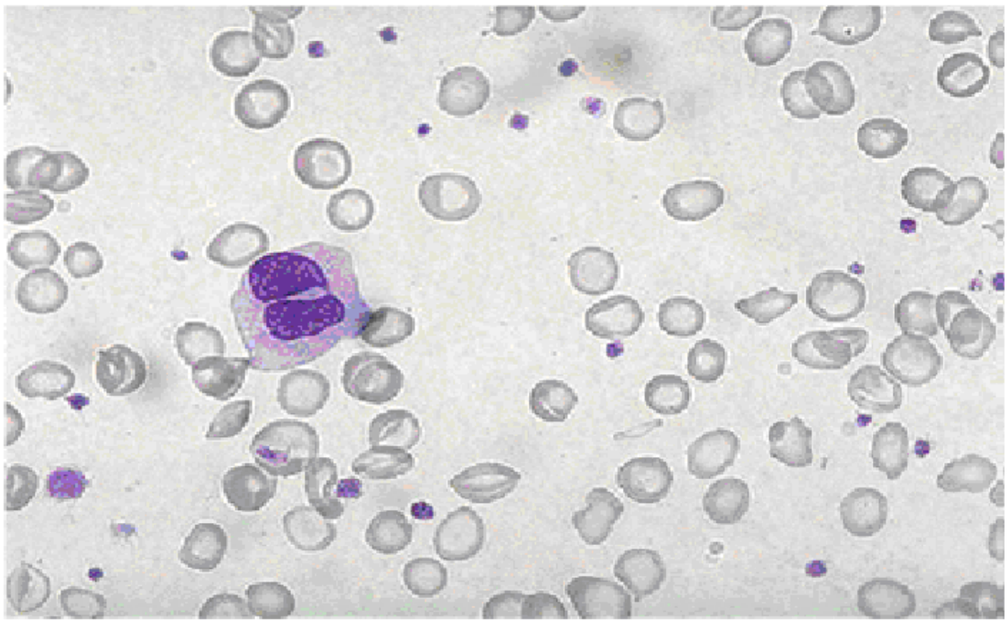
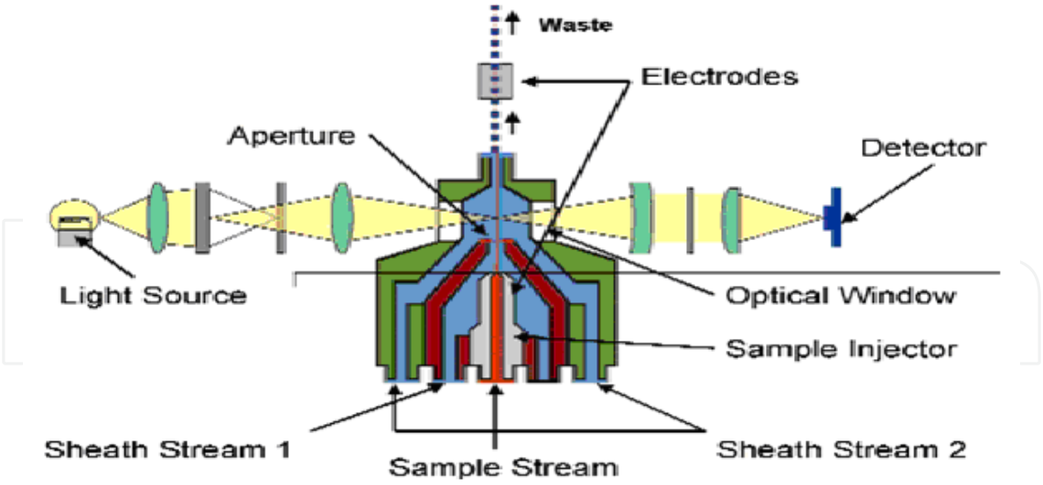
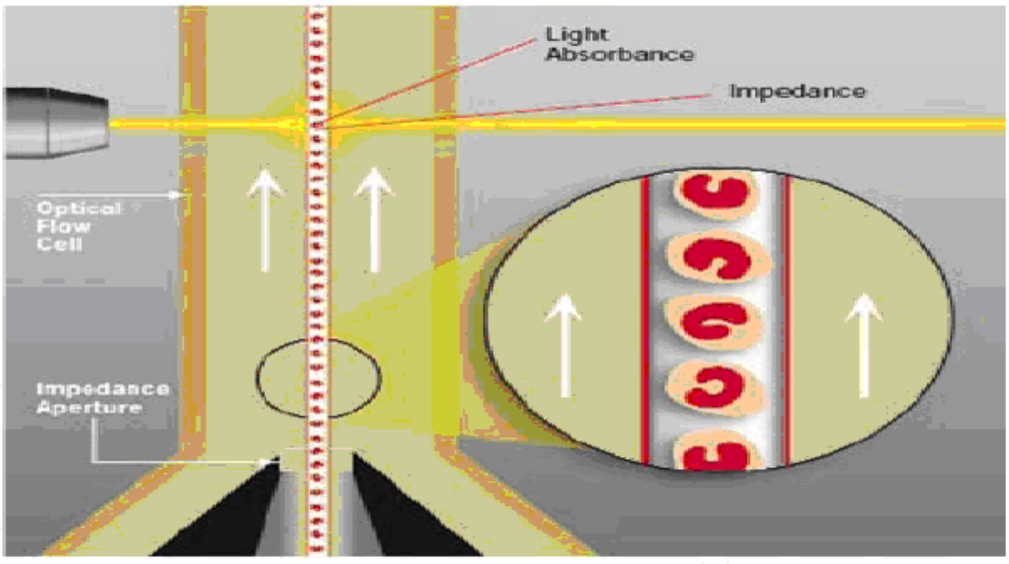


Fig. 2. Qualitative platelets disorders: platelets with abnormality bizarre in shape and size

The optical conventional techniques used for platelet counting have limits that are influenced by the human eye, especially for detection of objects <5 microns. Thus, the modern trend is to replace optical systems and introduce some electronic optical systems.
Electronic microscopy with Beta rays and wave lengths thousands of times smaller than the white light gives a higher power of resolution and thus, analyzers well-suited for platelet count in biological fluids are ones that use either of two methods: WCS technology of impedance (Volume, Conductivity and Scatter Light), {Graphic 4} and WOC analysis by laser ray (White Cell Optical Count) [20], Graphic 5. In WCS, the fat within the cell membrane behaves as an object that facilitates generation of an electronic impulse with an amplitude proportionate as the cell volume and helps create a potential difference next to the count cleft Since VCS technology includes a highly accurate measure of cell volume, we can use this information to correct the conductivity and scatter signals. The result of this volumetric compensation is a pair of measurements that are very powerful, and unique to Beckman Coulter. The HMX Coulter Analyzer utilizes the Coulter principle to provide cellular information for the complete WBC differential. The system measures the amount of light "lost" due to diffraction and absorbance as compared to full transmission when no cell is present.
The signals collected are converted into voltage pulses and are processed. The size and shape of the voltage pulses are equivalent to the unique nuclear and morphologic structure of the cells being analyzed conductivity offers information about opacity, which is directly proportional to cell density [21].
In WOC technology the laser light measures cellular elements in 4 specific angles and every angle of light scatter from 0° through 90° is influenced by cellular size. The low angles are the most affected, and are often used as an indirect estimation of cellular size. The zero angle measures the dimension of cells and impedance is used to count RBC corpuscles > 36 fL and platelets, corpuscles with the dimensions between 2-20 fL [22].



Graphic 4. VCS Technology includes a highly accurate measure of cell volume and this information is added to the Conductivity and Scatter signals. Every angle of light scatter from 0° through 90° is influenced by cellular size. The low angles are most affected, and are often used as an indirect estimation of cellular size.



Graphic 5. WOC channel (White Cell Optical Count) is used for counting blood cells and differential count by laser technokigy. The result of this volumetric compensation is a pair od measurements that are very powerful, and unique to Beckman Coulter.

The main elements that maintain the plasma osmolality in normal values (310 Osm/l) are; Na, K, urea and glucose. Serum osmolality is normal whenever the osmotic pressure set by urea and glucose is negligible and the Na+ concentration can largely define osmolality [Osm = 2.1 x conc Na mEq/L).Whenever the level of plasma urea or glucose is high, the osmolality becomes: 2.1 (Na + K) mmol/L + urea mg% / 2.8 + glucose mg% /18.02), result expressed in Osm /L[23]. In metabolic states with high osmolality (e.g. from chronic renal

failure), errors in platelet counts occur in optical microscopy due to the double refraction phenomenon.
This phenomenon occurs because particles <5µ create reflection, refraction, diffusion and diffraction of light through environments with different properties (ε) and in solutions with higher osmolality. The diffraction of rays by objects < 5µ are not sufficiently dispersed and only a part of the issued light falls on the object from the objective of microscope.
The angle comprised between the rays which delimit the light cone represents the numerical aperture(A) and the resolution power or the spectral separation power, dependent of light diffraction (D), light wave length (L) and numerical aperture (A) , (D = L / A), [24].
Optical instruments contain light separation media that are non-homogenous, including glass (ocular, objectives, prisms, air) and thus yield losses in the intensity of the incidence, reflection, refraction and diffraction rays through the media crossed by them. After the expression: $S = [n1 - n2/n1 \times n2]^2$, where "n" represent the refraction index from the environment, the losses of the incidental ray, because of interference, is 4% from the intensity of incidental fascicle [25].
Platelets with dimensions <2µ and are met by light rays, with a very high speed of propagation through liquid environments may not be seen in optical microscopy if increased osmolarity concentrations are present. In accordance with Huygens interference principle, clefts S1 and S2 become secondary oscillation sources. The sources of secondary vibration of the light generated waves can overlap between the interference areas and fringes, thus yielding what is termed the interference domain.
Thus, the average of the intensity values of the object light image in the ocular may has the range between 0 value and 4 'e" ($e= {}^1/4nS$) in the minimal, respectively, maximum interference phase.
The minimal intensity state of the light reflected on the object in order to create its reversed image in the ocular leads image loss for the human eye.
The normal thrombocytes having the diameter of 2-4 microns, create reflection, refraction, diffusion and diffraction of light through microscopy and become more less visible to manual counting.
There has been some debate over which counting principle, between the impedance and optical methods, measures platelet counts more accurately. Some studies suggested that the accuracy of the optical methods was superior for thrombocytopenic specimens, while recent studies demonstrated the impedance method to be more accurate for samples from patients undergoing cytotoxic chemotherapy [26].

5. Conclusions

The anemia of hospitalized patients with chronic or acute renal diseases undergoing hemodialysis exists in our study in 60% from studied cases and must be managed of laboratory medicine in collaborative with the clinician. A routine anemia screening should be recommended using HGB, HCT and erythrocytes indexes MCV, MCH, MCHC and must be redefined the anemia by these common parameters.
An iron panel (serum iron, TIBC, IST% and RPI) is useful in differentiating anemia of chronic disease from iron deficiency. By this study the anemia can be defined as a decrease of HGB and or hematological indexes with 10%from initial normal values, with cut of 117g/L HGB for men and 108g/L HGB for women.
The methods used to assess platelet counts of hemodialysis patients, optical microscopy, peripheral blood smear and use of the cytometry principle with impedance principle (VIC),

yielded similar results with samples from normal subjects but the accuracy of the automatic method ensures a high quality count of hemodialysis patients.
The all three methods yielded similar results with samples from normal subjects and that the accuracy of the automatic method ensures a high quality count but apparently not so, for patients post-dialysis.
Examination of the peripheral blood smear appears to offer important advantages, in particular for dialysis patients, so as to assess for qualitative as well as quantitative changes in platelets in such patients We concluded that should be a clinical guideline for the management of anemia in the elderly with chronic renal diseases.

6. Abbreviations

ACD - Anemia of Chronic Disease;
CFR- Chronic Renal Failure;
CBC-complete blood count;
CHr -reticulocyte hemoglobin;
EPO -erythropoietin;
HGB-hemoglobin;
HCT - hematocrit;
IDA - iron deficiency anemia;
IST - index saturation transferrin;
MA -megaloblastic anemia;
MCV -mean cell volume;
MCH - mean cellular hemoglobin;
MCHC -mean cell hemoglobin concentration;
RPI-Reticulocyte Production Index;
TS - transferrin saturation;
RDW 0 red cell distribution width;
RET - reticulocyte count;
SI - serum iron;
sTR - soluble transferrin receptor;
TIBC - total iron binding capacity

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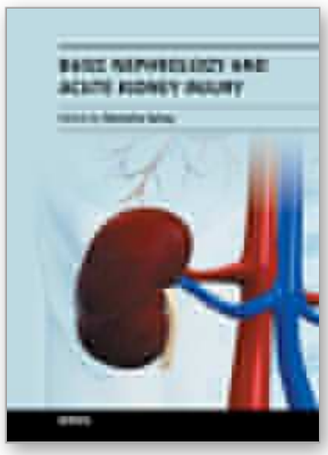

Basic Nephrology and Acute Kidney Injury

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The first section of the book covers the basics of nephrology and second section focuses on acute kidney injury. This easy to reference text examines the physiological and biochemical aspects of renal diseases - all in one convenient resource. Experts in the field discuss topics of increasing concern in nephrology including newer methods of assessing renal function. The field of acute kidney injury in nephrology is a rapidly evolving one with research translating into clinical guidelines and standards. This text brings together experts to provide an authoritative reference for management of AKI in various clinical settings. Pregnancy related AKI is an important entity which has also been discussed in detail. The recent advances in the field of critical care AKI have been incorporated as well and help the reader to update their knowledge.

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