

# MEAN CORPUSCULAR VOLUME (MCV) – its value in clinical medicine

## OVERVIEW

The Mean Corpuscular Volume (MCV) is the most important of all the red cell indices and has been available as a reliable, directly measured value for more than 40 years. It is measured either by electrical impedance or light deflection. Measurements are proportional to cell size.

In any newly diagnosed anemia the first question to ask is “what is the MCV?”. The answer will have a profound influence on the diagnosis and subsequent investigation. The MCV can be a useful indicator of clinical state without anemia but, due to changes in reference ranges and confusion over appropriate follow up testing, it is felt that some clarification of the utility of the MCV is needed.

## REFERENCE INTERVALS

Traditionally the normal reference range has been given as 80-100 fl and clinical action plans have been based on these ranges. Now that it is necessary for laboratories to scientifically establish a ‘reference range’, MDS has modified the range to be 80-97 fl, which is also consistent with several hematology texts.<sup>12</sup> However, clinical action plans continue to use only the old range. There is no literature guidance as to what action to take when the MCV is between 97 and 105 fl and it would seem best to follow traditional practice and do nothing.

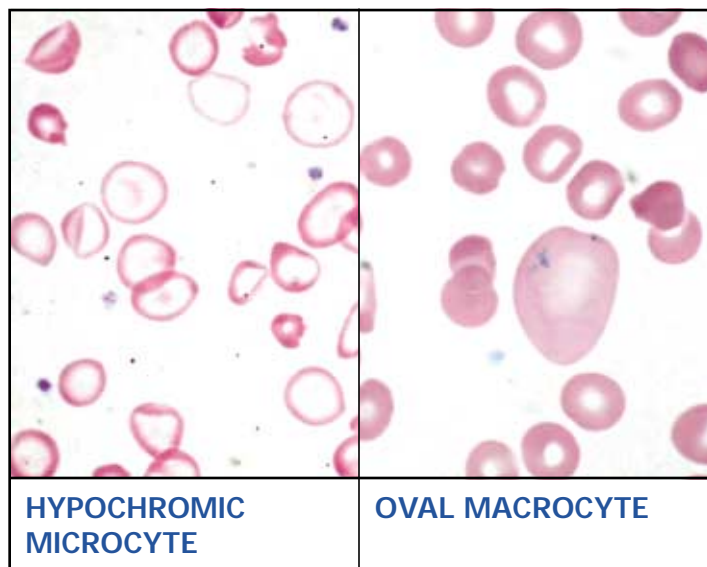
## CLINICAL IMPLICATIONS OF THE MCV:

### 1. MCV is low < 80 fl

Reduced production of hemoglobin whether due to low heme synthesis, as in iron deficiency, or low globin synthesis, as in thalassemia, results in the production of small red cells hence **MICROCYTOSIS**. Appropriate action on identifying microcytosis is to look for iron deficiency (the commonest deficiency disorder in the world) or thalassemia (the commonest genetic disorder in the world). Appropriate investigations have been described in previous MDS newsletters.

The anemia of chronic disease, such as occurs in rheumatoid arthritis, may also result in microcytosis, although usually to only a minor degree, and differentiation from iron deficiency may be difficult. (See article in this issue on Serum Transferrin Receptor)

*Caution: Sub-clinical iron deficiency may be present with a normal MCV since this is a late change in deficiency and the MCV will fail to detect many hemoglobin genetic disorders that are unassociated with red cell size changes.*



### 2. MCV is elevated > 100 fl

A recent hospital based study has demonstrated varying clinical implications based on the degree of the presenting elevation of the MCV.<sup>3</sup>

#### Level of the MCV

MCV 100 – 110 fl

#### Clinical Association

Alcohol, Liver Disease (with and without Alcoholism), Drug Therapy (HIV, Oncology and Epilepsy), Reticulocytosis due to hemolytic anemia

MCV > 110 fl

Megaloblastic Anemia due to B12 or Folate Deficiency, Myelodysplastic Syndrome

These associations are by no means clear-cut but, do indicate a direction for investigation. The hospital nature of this study indicates a late stage in the disease process. In the outpatient or community setting there is likely to be considerable overlap in categories. A patient becoming Vitamin B12 deficient will clearly have a progressively increasing MCV over time.

The degree of any associated anemia and examination of the blood film are of limited utility in defining the cause in most cases. ■

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## LABORATORY INVESTIGATION OF AN ABNORMAL MCV

MCV	Reading	Action
MCV	< 80 fl	Clinical History Ferritin Hb investigation HbA2 HbH Possibly Serum Transferrin Receptor
MCV	97 – 100 fl	No Action
MCV	100 – 105 fl	Possibly Investigate
MCV	> 105 fl	Clinical History Vitamin B12 Serum & Red Cell Folate Liver function tests Reticulocyte Count Possibly Bone Marrow Aspiration Methylmalonic Acid Homocysteine

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## SOLUBLE TRANSFERRIN RECEPTOR (sTfR) - a new test for iron deficiency

### OVERVIEW

Iron deficiency is the most common nutritional deficiency worldwide affecting a quarter of the world's population.<sup>1</sup> Several tests are available for the detection of deficiency including the serum ferritin and the serum iron and iron binding capacity. However, both of these analytes are acute phase reactants and serum levels are rendered diagnostically unreliable in inflammatory conditions such as rheumatoid arthritis. Even iron staining of bone marrow particles can be unreliable in such circumstances.

The soluble transferrin receptor (sTfR) in the plasma however, has been found to be increased in iron deficiency and is not affected by tissue inflammation.

### WHAT IS THE SOLUBLE TRANSFERRIN RECEPTOR?

Transferrin receptors are membrane glycoproteins that serve as the gateway for circulating transferrin-bound iron to enter the interior of

all body cells.<sup>2</sup> The largest proportion of transferrin receptors is on erythroblasts in the bone marrow but receptors are also present on other rapidly dividing cells and the placenta. Synthesis of transferrin receptors and ferritin are carefully regulated by the body's need for iron.

The soluble form of the receptor is present in plasma and has been cleaved from the membrane bound receptor.<sup>3</sup> The plasma (or serum) level accurately reflects the tissue mass of erythroid cells or the iron status of the body. Hence sTfR will be increased in hemolytic anemia, where there is an increase of erythroid cells, and in iron deficiency. A reduced level of sTfR will be seen in aplastic anemia and renal failure when erythroid cell mass is reduced.

### CLINICAL VALUE OF sTfR

This new assay appears to be of clinical value in two distinct clinical situations.

#### 1. Possible iron deficiency complicated by inflammation

In the anemia of chronic disease there is suppression of red cell production due to release of inflammatory cytokines and a reduction of the normal production of erythropoietin. The anemia may be microcytic and, particularly in conditions such as rheumatoid arthritis, there could be a suspicion of bleeding due to the therapeutic use of aspirin and non-steroidal antiinflammatories. Ferritin will likely be elevated and the serum iron and % saturation may be reduced due the cytokine effects. In this situation sTfR will be elevated only if there is iron deficiency or an increased red cell production as in hemolytic anemia.<sup>4</sup>

#### 2. Monitoring the therapeutic response to erythropoietin (EPO) in chronic renal disease and malignancy

Low levels of sTfR at the start of EPO therapy, along with >20% increment after two weeks of therapy, strongly predict a good clinical response to EPO. In this situation the elevated sTfR reflects the increased red cell mass rather than impending iron deficiency. Ferritin and careful monitoring would still be necessary to identify iron deficiency, which is the commonest cause of failure to respond to exogenous EPO.

### FUTURE CLINICAL USE OF sTfR

Recognizing that sTfR levels are dependant on both 1) iron status and 2) red cell production, there have been various attempts to improve the sensitivity and specificity by use of calculations, the most promising of which is log sTfR:Ferritin ratio.<sup>5</sup> However these formulae are not yet widely used and will not be calculated by MDS at this time.

### AVAILABILITY OF sTfR

The sTfR assay is available from MDS through our affiliation with the esoteric testing capabilities of the Toronto Medical Laboratories (TML), a joint venture between the University Health Network and MDS. As this test is not funded by most Provincial Health Insurance plans, there may be a charge for the test. ■

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# MEASUREMENT OF ERYTHROPOIETIN (EPO)

## OVERVIEW

Erythropoietin (EPO), a glycoprotein produced primarily in the kidney, is the growth factor responsible for the maintenance of the red cell mass. EPO binding to a receptor on red blood cells triggers a series of enzymatic reactions that result in increased synthesis of globin chains, transferrin receptors and structural proteins. EPO also increases red cell mass by inhibiting apoptosis.<sup>1</sup>

EPO synthesis is controlled by an elegant system that detects venous oxygen saturation in the renal tubular area, which is independent of the rate of blood flow. Production is increased in conditions that cause hypoxia, and is suppressed by a variety of inflammatory cytokines, which also interfere with its action. EPO levels are low in most patients with chronic renal disease.

## EPO LEVELS IN DISEASE STATES

**A) Increased EPO:** As EPO production is stimulated by low oxygen saturation, levels are increased in conditions associated with hypoxia. These include anemia caused by acute blood loss, iron deficiency or hypoplasia; or erythrocytosis associated high altitude, cyanotic heart disease, lung disease, sleep apnea, smoking and high-oxygen affinity hemoglobins. In iron deficiency anemia there is an inverse correlation between the hematocrit and the EPO level.

Abnormal production of EPO has been shown to be the cause of erythrocytosis in a number of neoplastic conditions including renal carcinoma, hepatocellular carcinoma, cerebellar hemangioblastoma and uterine leiomyoma. Abnormal levels are seen in use of EPO to enhance athletic performance.

**B) Decreased EPO:** Levels are inappropriately low in the anemia of chronic renal disease, although the oxygen sensing system is intact.<sup>1</sup> An exception is polycystic renal disease, in which hemoglobin and EPO levels are maintained at better levels than for other dialysis patients. EPO levels are variable in patients with chronic infections, inflammatory conditions and malignancies, but often are low relative to the degree of anemia, presumably due to the presence of inflammatory cytokines.<sup>2</sup>

## DIAGNOSTIC TESTING

The ability to measure EPO reliably with the introduction of immunoassays has increased the diagnostic uses of the test. The reference range should be obtained from the laboratory performing the test. Some diurnal variation has been noted and antibodies to EPO have been detected in systemic lupus erythematosus (SLE), which may interfere with the assay.<sup>3</sup>

### 1. Polycythemia

Measurement of EPO is used frequently by hematologists in the investigation of polycythemia.<sup>4</sup> The level of EPO is normal in Polycythemia Rubra Vera, as red cell production is EPO-independent. In contrast, the level of EPO is increased in secondary polycythemia, either as a physiological response to hypoxia or resulting from inappropriate EPO production.

### 2. Anemia

The role of EPO measurement in the investigation of anemia is less clear-cut. Measurement of EPO, along with serum transferrin receptor and the reticulocyte count – markers of red cell production – may be helpful in complex cases to understand the mechanism of the anemia.<sup>5</sup> In straightforward cases, measurement is not justified.

## PREDICTING RESPONSE TO EPO TREATMENT

Recombinant EPO (rHuEPO) is standard treatment for the anemia of chronic renal disease and is increasingly recommended to ameliorate moderate to severe anemia associated with a number of chronic conditions, such as malignancy, HIV infection and rheumatoid arthritis. It may be worthwhile measuring EPO prior to starting treatment to determine those most likely to respond.<sup>6</sup>

### EPO abuse

Urine EPO measurement has been used to detect the presence of recombinant EPO as the recombinant drug has a different carbohydrate content than native protein. However, its value is limited in that it is expensive, time consuming and will detect only recent use (within 3-4 days). Recently, a study has shown that detection can be improved by analysis of serum EPO along with markers of red cell production, including hemoglobin, reticulocyte count and soluble transferrin receptor.<sup>7</sup>

## AVAILABILITY

As each province has specific arrangements, please contact your local laboratory for instructions. ■

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## ONTARIO NOTES

# PATIENT SAFETY AND THE LABORATORY QUALITY SYSTEM

In recent years there has been a recognition that measurable outcomes in healthcare depend on the integrity and integration of the entire system rather than on a particular point in the system. Quality control processes improved quality within the analytical laboratory but, quality improvement must be directed at the whole system and in fact, most errors that impact patient safety occur in the pre-analytical or post-analytical phases.

The MDS Quality System aims to deliver an accurate, timely, clinically useful analytical result, or interpretation, on an appropriately handled specimen unequivocally attributable to an individual patient. A failure of the Quality System may compromise patient safety. Clinicians and their staff, MDS employees and contractors all contribute to the integrity of the Quality System.

Proper procurement and handling by clinicians and their staff are essential to maintain the integrity of patient samples so that an accurate analysis is possible. Specimen handling instructions are in the MDS Directory of Laboratory Services.

Clear identification of samples using at least two unique identifiers, including the patient's full name plus, for example, date of birth or health card number, is needed to ensure an analytical result is attributed to the correct person. Patient samples should be procured and labeled one patient at a time to avoid misidentification. When clear identification of specimens is deficient the MDS Specimen Rejection protocols are invoked to protect patient safety.

Transcription presents a point of potential error in a quality system and one which should be eliminated whenever possible. Completion of requisitions is best and most accurately undertaken by individuals responsible for requesting a test because they can gather correct patient demographic information and are also able to obtain patient consent when this is required.

Certain laboratories, including Public Health Laboratories (PHL) and those in Genetic Centres, rely on MDS to transport samples and frequently to collect specimens for tests they perform. Often samples and/or patients arrive at MDS facilities without the appropriate requisition and/or informed consent. In these cases, an OHIP requisition is not acceptable. In the past, MDS has completed the appropriate requisition on behalf of the requesting clinician. To ensure patient safety and informed consent, the PHL or Genetic Centre requisitions must be completed by those originally requesting PHL or genetic tests. MDS will no longer perform transcription of these requisitions.

Please ensure you have a supply of requisitions available for completion in your office and understand which tests must be performed at PHL. ■

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## TRANSPORTATION OF DANGEROUS GOODS REGULATIONS - Updated

Clinical specimens that require transportation must comply with the Federal Transportation of Dangerous Goods (TDG) Act. For diagnostic specimens that are collected in the clinician's office the clinician (not the courier) is considered the "consignor", and under the TDG Act, the clinician is responsible for ensuring the specimen transport meets TDG regulations.

Diagnostic specimens for screening, or where low risk organisms are expected (e.g. throat culture for Group A Streptococcus, routine urine culture), are not considered dangerous goods.

Diagnostic specimens are considered dangerous goods if the specimen is **reasonably expected** to contain a high-risk organism. The following table lists common examples of high-risk organisms that require special handling.

Test/Suspect Organism	TDG Requirements
HIV viral load, CD4/CD8 for HIV monitoring, SARS West Nile Virus, Avian Influenza, Creutzfeldt-Jacob disease, Tuberculosis	<ul style="list-style-type: none"> <li>▪ 1A Container</li> <li>▪ Infectious Substance Label</li> <li>▪ Shipping document</li> </ul>
Hepatitis B monitoring (eg/ Hep BeAg, Hep B PCR), Stool for <i>Vibrio cholera</i> , Stool or Blood for <i>Salmonella typhi</i> , Throat culture for <i>C. diphtheriae</i> , Culture for Mycoplasma	<ul style="list-style-type: none"> <li>▪ 1B container</li> <li>▪ Infectious Substance Label</li> <li>▪ Shipping document</li> </ul>

*Clarification: in the previous issue of Inside Diagnostics, Enterovirus was included in the 1A-packaging group. The one Enterovirus that must be packaged in this manner is swine vesicular disease virus. In addition, the reference to Smallpox referred to serology for vaccinia (small pox vaccine) strain. Obviously for a suspected case of Smallpox, Public Health must be contacted immediately.*

For complete and up to date TDG information, see <http://www.tc.gc.ca/>

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