AMPATHCHAT no. 88

ERYTHROPOIETIN

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TEST CHARACTERISTICS

- Siemens IMMULITE 2000 Erythropoietin Assay
- Mnemonic: **ERYTHRO**
- Specimen type: Serum on ice
- Container: SST on ice
- Minimum volume: 250 µl
- Turnaround time: 120 hours
- Collection instruction: Morning samples taken between 07:30 and 12:00 preferred due to diurnal variation. Serum sample kept cool, not frozen.

INTRODUCTION

Erythropoietin (EPO) is a glycoprotein hormone that is responsible for erythropoiesis. This process usually happens at a low rate, at baseline, where aged erythrocytes are slowly replaced by new ones.[1,2]

The EPO is secreted by the peritubular renal capillary endothelial cells in adults and the liver in foetuses. It stimulates the erythroid progenitor cells in the bone marrow that results in erythropoiesis. Reduced oxygen delivery to the kidneys will cause the hypoxia-inducible factor-2 (HIF-2) to be released. This signals the transcription of EPO.[1,3]

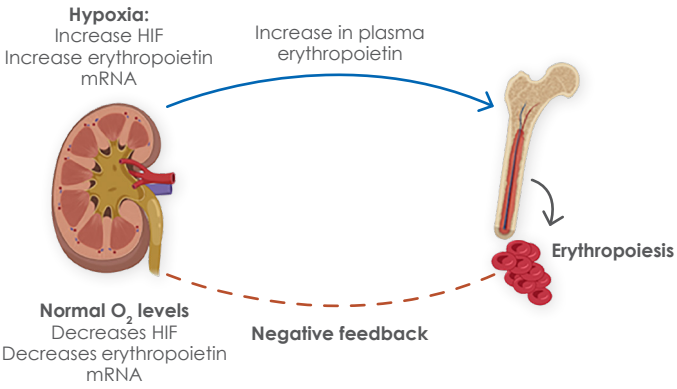

FIGURE 1: DEMONSTRATION OF THE NEGATIVE FEEDBACK MECHANISM BETWEEN EPO RELEASE AND ERYTHROPOIESIS AS ADAPTED FROM COLD SPRING HARBOR PERSPECTIVES IN MEDICINE 2013;3:A011619[3,4]

Erythropoiesis can increase up to eight times the baseline production rate during haemorrhage, haemolysis or any situation that results in decreased oxygen delivery to the kidneys.[5]

When the stimulus of the hypoxia is removed, i.e. sufficient oxygen delivery to the kidney receptors is re-established, the release of EPO is terminated.[2] This feedback mechanism resembles a negative feedback loop (see Figure 1).[3]

CAUSES OF LOW LEVELS OF EPO

- **Renal failure:** Patients with renal failure commonly develop severe anaemia with a multifactorial etiology; mainly the suppression of erythropoiesis, due to the accumulation of metabolic waste, and the reduction of the erythrocyte longevity. The most important contributor to anaemia associated with the uraemia of renal failure is the insufficient production of erythropoietin. The degree of anaemia correlates roughly with the extent of renal failure.[6]
- **Inflammation:** The cause of the insufficient EPO production is varied: it can be either due to direct damage to the EPO-producing cells or because of suppression by inflammatory cytokines. Inflammatory disorders like rheumatoid arthritis, cancer and AIDS may all result in EPO gene expression being suppressed due to the release of inflammatory mediators – although the suppression is less pronounced than in renal failure.[7,8] Increased plasma viscosity, as seen in patients with monoclonal gammopathies, has been associated with EPO production suppression.[10]
- **Heavy metal exposure:** This may lead to structural and functional disruption of the proximal renal tubule, resulting in suppression of EPO production. This mechanism of anaemia has been described in cis-platinum treated cancer patients. Cadmium toxicity is another condition that is associated with the suppression of EPO production.[9]
- **Polycythaemia vera (PV):** This is a myeloproliferative neoplasm that is associated with increased erythrocyte production. PV is associated with a mutation in the Janus kinase-2 (JAK2) gene in the majority of patients. The low plasma EPO is due to a negative feedback mechanism.[11] In PV, an Hb of >16.5 g/dL or an Hct of >49% is observed in men and an Hb of >16 g/dL or an Hct of >48% is observed in women.[15]

CAUSES OF HIGH LEVELS OF EPO

- **Hypoxaemia:** When an individual has chronically low arterial oxygen saturation – due, for example, to a right-to-left cardiac shunt, chronic obstructive pulmonary disease or living at a high altitude, the resultant erythrocytosis would be due to an increase in EPO production.

- **Neoplasms:** Erythrocytosis due to the inappropriate overproduction of EPO has been associated with various neoplasms, e.g. renal, hepatic, cerebellum tumours and other organs where erythropoietin expression occurs normally. Secondary erythrocytosis is commonly seen as part of a paraneoplastic syndrome in patients with renal carcinomas, Wilms tumour, hepatomas and cerebellar hemangioblastomas due to ectopic EPO secretion by the tumour cells.[12]
- **Genetics:** This is usually seen in autonomous EPO secretion and chronically increased levels – in the absence of any discernible cause. This may be due to a mutation in the genes associated with oxygen sensing and regulation of HIF-1.

Congenital erythrocytosis follows an autosomal recessive inheritance pattern. Affected individuals have high EPO levels, and an increased incidence of thrombotic and haemorrhagic-associated complications.

CLINICAL INDICATIONS FOR EPO TESTING

1. Useful in differentiating between primary and secondary polycythaemias
2. Assessment of CKD patients for EPO replacement therapy
3. Evaluation of a patient with an elevated haemoglobin and haematocrit

ANALYTICAL CONSIDERATIONS

Synthetic EPO is used in clinical practice to treat anaemias. It is available in many recombinant forms. Some of these have amino acid sequences that differ from the natural human form. This results in reduced reactivity with the assay. Some recombinant EPO forms may be additionally glycosylated to delay renal clearance and prolong bioavailability. The glycosylation of the synthetic EPO forms may further alter the molecular structure compared to natural human EPO.

A study that investigated the cross-reactivity of synthetic EPO with the IMMULITE 2000 assay demonstrated slight cross-reactivity in their small cohort. Clients should be aware of this potential interference.[13,14] EPO demonstrates diurnal variation. Higher levels are expected in the mornings.[11]

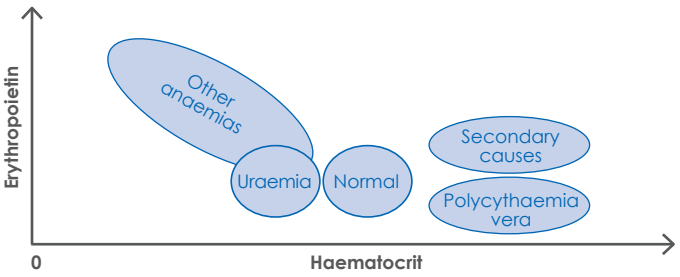

FIGURE 2: THE RELATIONSHIP BETWEEN HAEMATOCRIT AND ERYTHROPOIETIN AND SELECTED CONDITIONS AS ADAPTED FROM COLD SPRING HARBOR PERSPECTIVES IN MEDICINE 2013;3:A011619[3,4]

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