The clinical utility of RET-He in diagnosing and treating anaemia
Erythropoiesis
What is anaemia?

• A reduction in haemoglobin concentration of blood with reference to healthy individuals of the same age group, sex, physiological state and environment (altitude).
How to diagnose anaemia?

• After thorough history and physical examination of the patient, FBP including the blood counts and morphology is the most important step in diagnosis.
Red cell indices

- Rbc
- Hb
- HCT/PCV
- MCV
- MCH
- MCHC
- RDW
Iron deficiency anaemia (IDA)

• Iron deficiency anemia is one of the most common diseases worldwide.

• In the majority of cases, the presence of hypochromic microcytic anemia and biochemical evidence for depletion of body iron stores makes the diagnosis relatively straightforward.
However, in several clinical conditions, classic biochemical indices such as serum iron, transferrin saturation, and ferritin may not be informative or may not change rapidly enough to reflect transient iron-deficient states (functional iron deficiency), such as the ones that develop during recombinant human erythropoietin (r-HuEPO) therapy.
Functional iron deficiency (FID) is a state in which there is insufficient iron incorporation into erythroid precursors in the presence of apparently adequate body iron stores, as defined by the presence of stainable iron in the bone marrow together with a serum ferritin value within normal limits.
• Iron is essential for the production of haemoglobin, and the most common reason for anaemia is classical iron deficiency.

• In hospitalised patients, however, the most common type of anaemia is anaemia of chronic disease (ACD).
Anaemia of chronic disease (ACD) is often present in chronic inflammatory diseases, such as rheumatoid arthritis, end stage renal disease and tumours.
Pathophysiology of ACD

- ACD is mainly caused by a disturbance of iron metabolism due to the **reactive state of the immune system**: the uptake of iron from the gut is impaired, and the iron inside the body cannot be appropriately used to produce haemoglobin. The iron is being locked away in certain inflammatory cells (macrophages).
- This phenomenon is also called **functional iron deficiency** since the iron is there but not available.
- Another characteristic feature of ACD is that due to the inflammatory process the development of red blood cells is impaired, and many of them die at early stages of their development.
Questions to be raised

• How do you decide whether anaemic patients have classical iron deficiency or functional iron deficiency, or a combination of both,

• How do you schedule them for the appropriate therapy, and how do you monitor whether the treatment is effective or not?
• The traditional serum markers to assess the iron status are ferritin and transferrin saturation.

• Both of these measure the degree to which the body’s iron stores are complete.
• Under normal circumstances, classical iron deficiency can easily be diagnosed by performing one of these two tests in blood serum.

• If these parameters have low values (i.e., below ~15μg/l ferritin or below ~15% transferrin saturation), classical iron deficiency is proven.
• The opposite, however, is often not true, especially in patients with ACD, since inflammation itself has an influence on the results of these two lab tests.
• Ferritin production is increased in inflammation, and in this condition even very high serum ferritin values cannot rule out functional iron deficiency.
• Ferritin is further elevated in different forms of liver disease and is produced by various kinds of cancer.
• On the other hand, the production of transferrin is to some degree suppressed in inflammation, which influences the result of the transferrin saturation test.
• In summary, these tests (ferritin and transferrin saturation) are of very limited value in ACD.
The ferritin index

• A better laboratory test is the so-called soluble transferrin receptor (sTfR), as it is more robust against the influence of inflammation. It can be demonstrated, however, that a combination of the two laboratory tests serum ferritin and sTfR is superior to the measurement of sTfR alone.

• From the results of these two laboratory tests an index can be calculated: ferritin index = sTfR/log ferritin.

• To date, the ferritin index is the best marker to assess the iron status: the higher the index, the more depleted the iron stores.

• But this index alone does not answer the question of whether this anaemia is functional iron deficiency as in ACD full iron stores do not mean that iron is available for the production of haemoglobin.
A new approach

- Many patients with chronic disease suffer from anaemia, but a new approach allows the
  1. identification of the cause of anaemia,
  2. scheduling the patient for appropriate therapy and
  3. monitoring the effectiveness of the treatment
• In hospitalized patients the commonest type of anaemia is anaemia of chronic disease
• The new diagnostic concept comprises clinical chemical serum tests and a haematological parameter (reticulocyte haemoglobin content)
Fortunately, this blood test that tells us whether the iron in the stores is actually being used for the production of haemoglobin or not.

This test is termed reticulocyte haemoglobin content (abbreviated RET-He)
Reticulocytes are early stages in the development of red blood cells. They are produced in the bone marrow and expelled into the blood, where they develop into mature red blood cells within a day or two.
Reticulocytes

• Reticulocytes actively produce haemoglobin, which the mature red blood cells do not.
• Looking at reticulocytes, we get a picture of the current red blood cell production of the bone marrow.
• With some modern haematological analysers it is possible to routinely specifically measure the haemoglobin content of the reticulocytes.
• This test tells us whether the reticulocytes are able to produce sufficient haemoglobin, and indicates the appropriate utilisation of the body’s iron stores.
• RET-He can be determined on Sysmex’s haematology system XE 2100.
If the RET-He is low and the iron stores are depleted, as determined with the help of the ferritin index, we can diagnose classical iron deficiency and treat the patient with iron tablets alone.
However, if RET-He is low despite full iron stores, we can diagnose functional iron deficiency caused by ACD where the reticulocytes cannot appropriately use iron.

In this case EPO, together with intravenously administered iron, is required to treat the anaemia.
Reticulocyte haemoglobin content (RET-He)

- RET-He is measured on the basis of automated fluorescent flow cytometry which in the reticulocyte channel, using a polymethine dye, also measures the mean value of the forward light scatter intensity of mature red blood cells and reticulocytes.
- RET-He is a direct index of iron availability and reflects the cellular availability of iron.
- These values equate with reticulocyte hemoglobin content.
Reticulocyte haemoglobin content (RET-He)

Diagnostic/therapeutic plot generated by Thomas and Thomas
Detection of iron deficiency by Ret-He in haemodialysis patients on rHuEPO.
(Dr Ehram Jamian, Dr. Marini Ramli, et al)
In the process of being published.
This research showed that iron management based on Ret-He is simple and practical to perform. The test can be performed during routine blood testing for FBC, by the same haematology analyzer, at a little increment in the cost.

It also showed more value of Ret-He in anaemia diagnosis in comparison to ferritin and sTfR.
This research suggests using Ret-He as a diagnostic tool for the detection of IDX and FID as well as monitoring of response to IV iron therapy in haemodialysed patients.
Thank you