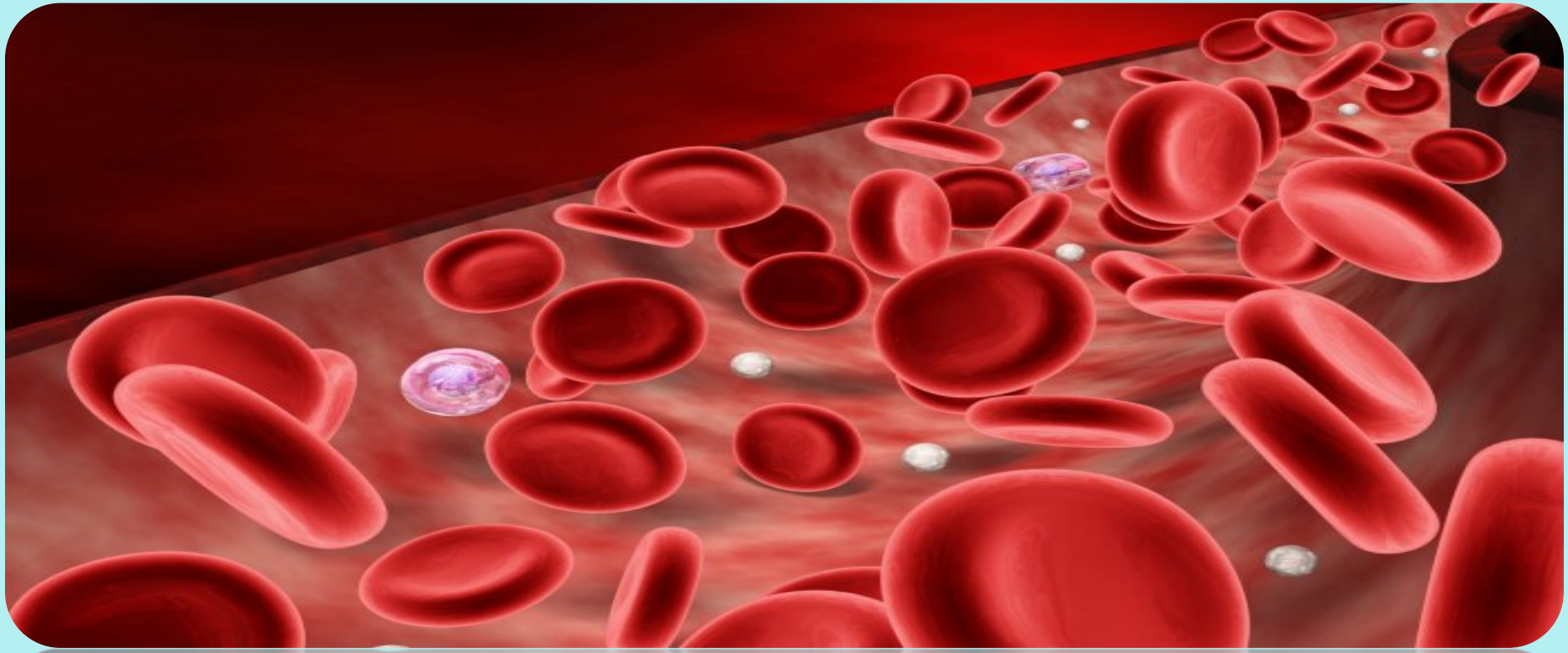


Quantitative Determination of Serum Iron, Unsaturated Iron Binding Capacity (UIBC), and Total Iron Binding Capacity (TIBC)

Objectives

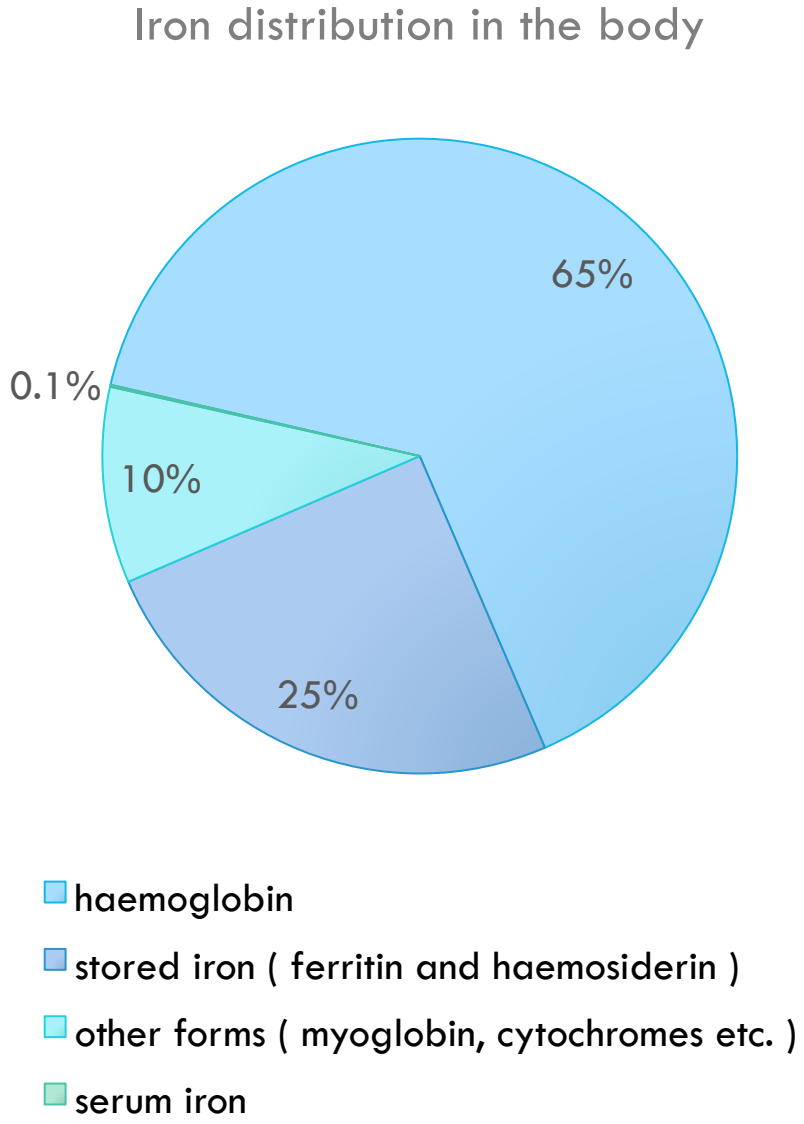
- To determine the normal level of serum iron.

- To determine the use of this test in diagnosis of anemia (iron deficiency).

Iron in The Body

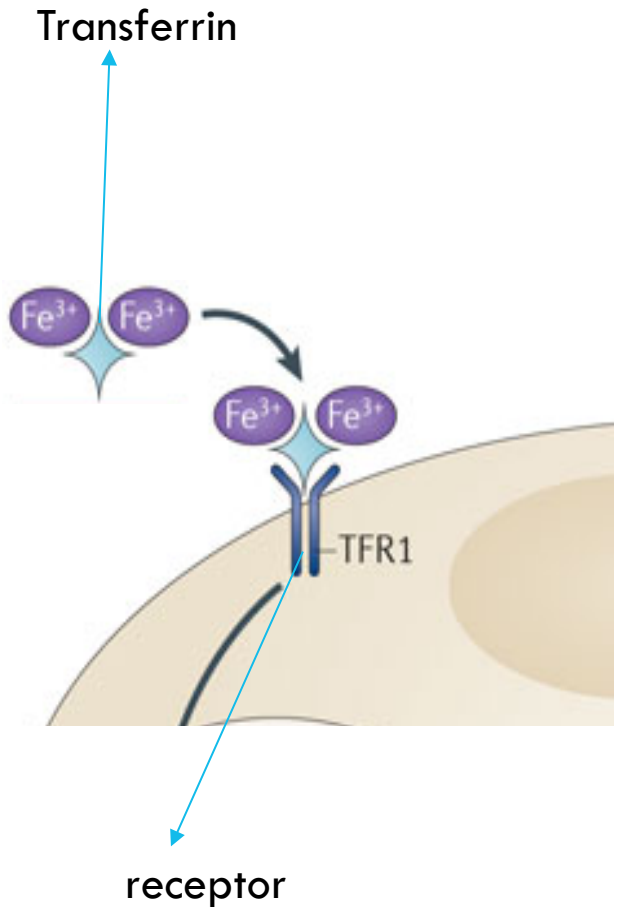
- Iron is the metal component of haemoglobin, myoglobin, cytochromes and some proteins of the electron transport chain.

- The total iron of an adult male is 4-5g and of a female is 3-4g.



Iron Transportation

- Iron is carried in Fe3+ state bound to a specific iron transport protein known as transferrin.
- transferrin are iron-binding blood plasma glycoproteins that control the level of free iron in biological fluids
- It contains **two** specific high-affinity Fe(III) binding sites.
- largely synthesized by the liver
- Transferrin distributes iron to those tissues which have a demand for its utilization.
- The transferrin–iron complex enters the cell through specific receptors and the iron ions are released for metabolic functions



Iron Transportation

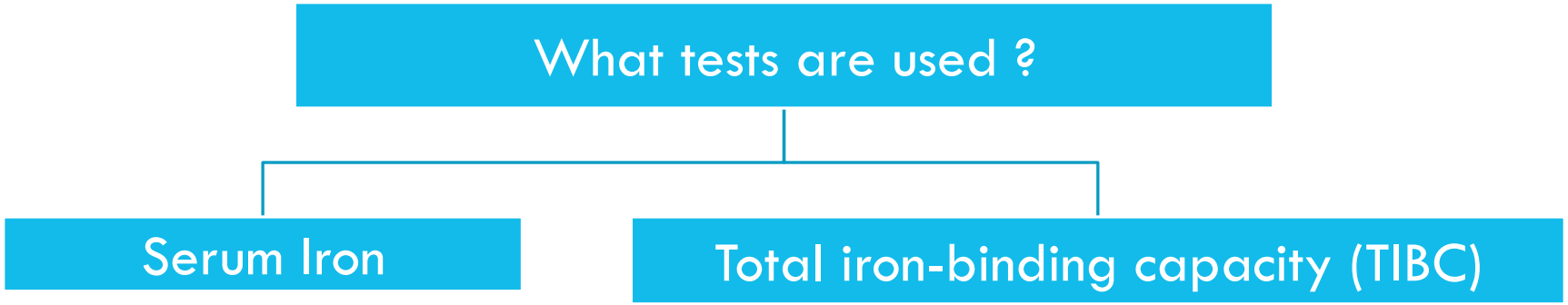
- When iron stores become low, transferrin levels will **increase**. When there is too much iron, transferrin levels are **low**

- Individuals who lack transferrin show severe hypochromic anemia and are also susceptible to bacterial and viral infections

Iron Level in Blood

- It is important to measure **iron** and **iron-binding capacity** to detect iron deficiency or overload.

Serum iron on its own provides no complete information on iron level

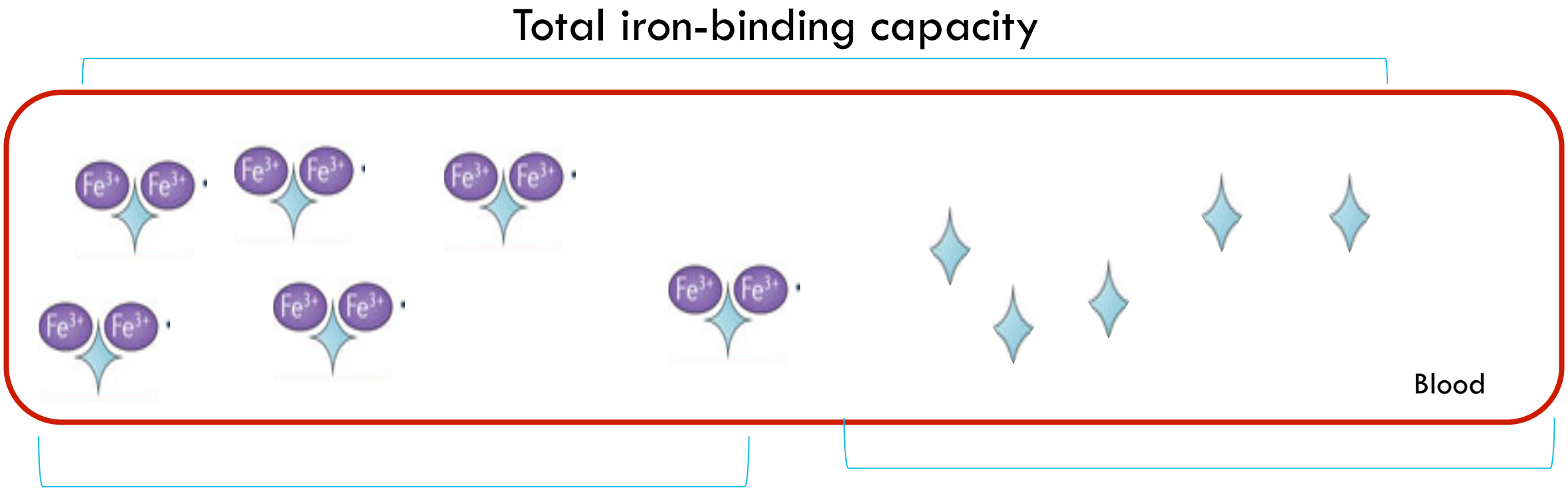


TIBC or UIBC may be ordered along with serum iron when it appears that a person has iron deficiency or overload. These tests may be ordered when there are signs of anemia, especially when a CBC is performed and shows red blood cells that are microcytic and hypochromic and the hemoglobin and hematocrit levels are low

Total Iron-binding Capacity (TIBC)

- It is a medical laboratory test that measures the blood's capacity to bind iron with transferrin.
- It is measuring the maximum amount of iron that it can carry, which **indirectly** measures transferrin
- It is calculated by adding serum iron and unsaturated iron binding capacity (**UIBC**)
- It is most frequently used along with a serum iron test to evaluate people suspected of having either iron deficiency anemia or iron overload (**hemochromatosis**)

SERUM IRON, TIBC, UIBC



Principle

- **Serum iron**: The iron is dissociated from its Fe-III-transferrin complex by addition of acidic buffer containing hydroxylamine which **reduces the Fe(III) to Fe(II)** .
- Then the **chromogenic agent** (**PDTS**) form a highly **colored Fe(II) complex** that is measured spectrophotometrically at **565nm** .
- **UIBC**: Determined by adding **Fe(II)** to serum so that it binds to unsaturated iron binding site on transferrin . The excess Fe(II) react with **PDTS** to form color complex which is measured spectrophotometrically at 565nm. The difference between the amount of Fe(II) added and the amount of Fe(II) measured represent the UIBC
- **TIBC**: is determined by adding serum iron to UIBC value.

Principle

- **Serum Iron:**
- **Fe-III-transferrin complex** —acidic buffer→ **Fe(II)** —**PDTS**→ **Colored Fe(II) complex**

- **UIBC:**
- **Unsaturated iron binding site on transferrin**

Adding excess **Fe(II)** to serum →

Fe-III-transferrin complex
+
The excess Fe(II)

↓ **PDTS**

Colored Fe(II) complex → **UIBC = Amount of excess Fe(II) added - amount of Fe(II) measured**

- **TIBC: is serum iron + UIBC value.**

Method

	Serum Iron		
	Blank	Standard	Test
Iron buffer (pH 4.5)	2.5 ml	2.5 ml	2.5 ml
Iron Standard	-------	0.2 ml	-------
Sample	--------	--------	0.2 ml
Water	0.2 ml	--------	------

	UIBC		
	Blank	Standard	Test
UIBC buffer	2 ml	2 ml	2 ml
Iron Standard	-------	0.2 ml	0.2 ml
Sample	--------	--------	0.2 ml
Water	0.4 ml	0.2 ml	------

Method

Mix. Read the Abs. of Std. and test (IRON) at 565 nm against their blank, this is (A^0) Also read the Abs. of Std. and test (UIBC) at 565 nm against their blank, this is (A') Then add:						
Iron color reagent	0.05 ml	0.05 ml	0.05 ml	0.05 ml	0.05 ml	0.05 ml
Mix and incubate at 37 ^{0}C for 10 min. Read the Abs. of Std. and test (IRON) at 565 nm against their blank, this is (A^1). Also read the Abs. of Std. and test (UIBC) at 565 nm against their blank, this is (A'').						

Calculations

Serum iron conc. in test (μ g/dl) =

$[(A^1 - A^0)$ test$/(A^1 - A^0)$ std$]$ x Std. iron conc.

Serum UIBC in test (μ g/dl) =

Std. iron conc. – { [(A" – A') test/(A" – A') std] x Std. iron conc.}

Serum TIBC in test (μ g/dl) = Serum iron + Serum UIBC

Transferrin saturation (%) = [Serum iron concentration/ TIBC] x 100

***** The std iron conc. = 500 μ g/dl***

Normal Ranges

- Serum iron (50 -160 µg/dl)
- TIBC (250 - 450 µg/dl)
- Transferrin saturation (20 – 55 %)

Defect in Serum iron

-**Serum iron is low** in iron deficiency anemia whether due to:

- insufficient intake, malabsorbtion, blood loss or inability to retrieve storage iron.

-**Serum iron concentration is high** when:

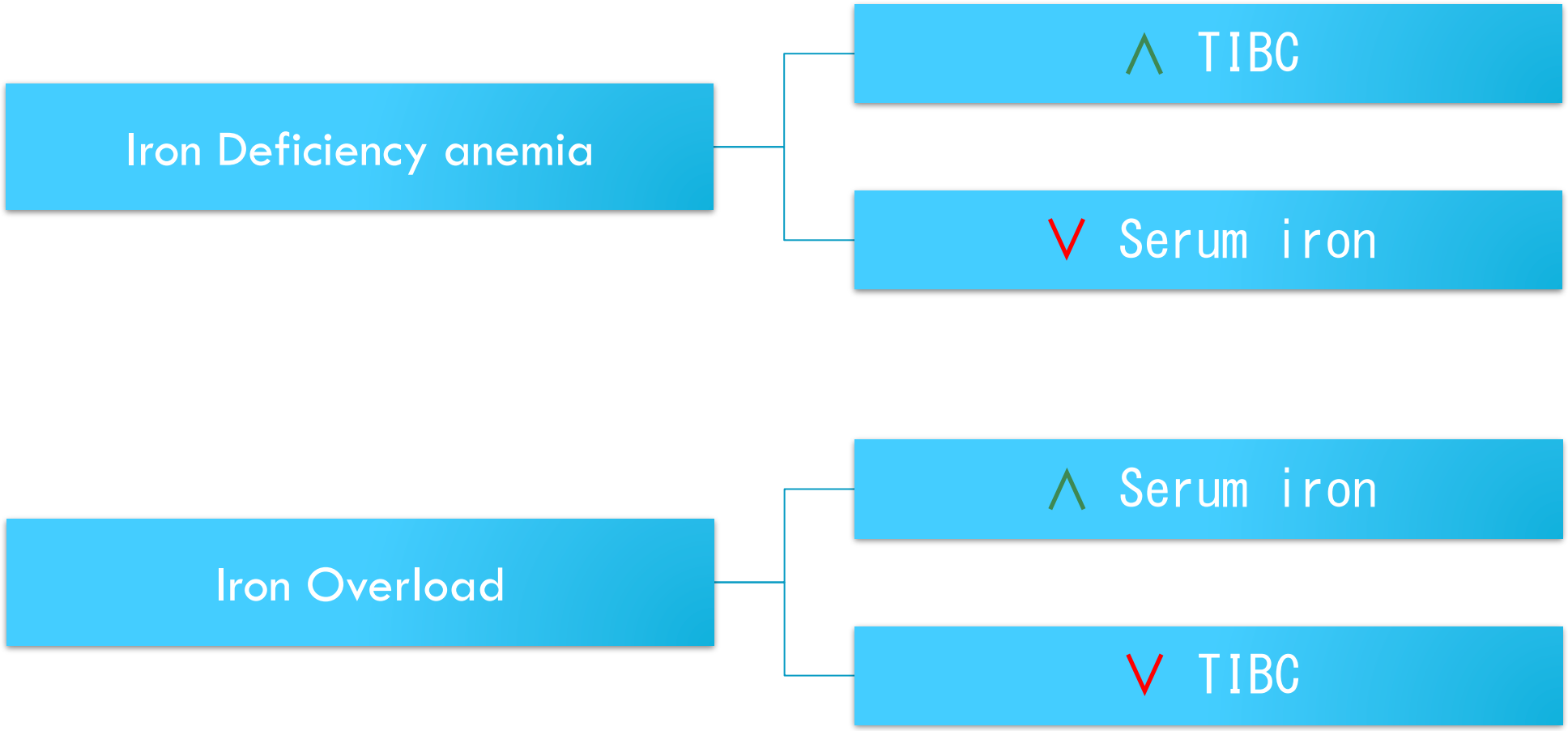
- marrow cannot utilize iron, hemolysis, increased absorption or defects in storage capabilities.

-High values are also found in severe hepatitis due to release from liver cells.

Defect in Total iron binding capacity (TIBC)

Increase in iron deficiency anemia

Decrease in hemochromatosis, malignant or rheumatic fever.


Iron Deficiency anemia
∧ TIBC
∨ Serum iron
Iron Overload
∧ Serum iron
∨ TIBC