Standardisation of erythropoietin (epo) measurements

Background

In polycythemia vera (PV), there is a an autonomous bone marrow hyperactivity of clonal origin. The overproduction of erythrocytes is the hallmark of the disease, but leucocytosis and thrombocytosis will in most cases sooner or later develop. The erythrocytosis is not driven by the ordinary feedback system but by the clonal activity, which means that epo, the normal regulator of eryhtrocyte production, is not a mediator in the disease development.

On the contrary, epo production is reduced, and plasma levels of epo are suppressed in PV. Erythroblasts from PV patients differ from normal in that they grow in culture media without addition of epo, and he erythroblasts have an increased sensitivity to epo.

In secondary polycythemia, on the other hand, the increased production of erythrocytes is *caused* by an increase in epo production, either as a response to hypoxia – as in pulmonary disease, at high altitude etc – or because of an autonomous overproduction of epo caused by tumours, cystic kidneys etc.

Hb and hematocrit levels may also be increased because of a reduced plasma volume that gives an apparent but not real erythrocytosis. This may be caused by dehydration, long term stress etc. Since this situation does not alter the oxygenation status, there is no change in epo production or epo plasma levels.

The diagnostic problems connected to high haemoglobin (Hb) levels most commonly occurs when a high Hb is detected in patient either without symptoms or with diffuse symptoms not obviously connected to the Hb level. The immediate need is to differ between the three major causes of elevated Hb levels: PV, secondary erythrocytosis and relative or pseudo-erythrocytosis. In MPD research, there is a need for solid diagnostic criteria for PV.

Epo measurements in MPD.

It could be expected from the pathophysiology of PV that plasma levels of epo are low. For a long time only biological epo assays were available, notably the polycythemic mouse assay(1), and these did not have enough sensitivity to measure low levels of epo. An international reference standard was developed early that has been very important for the standardisation of epo assays (2). When radioimmunological assay methods were developed, the sensitivity was improved, but there were still problems to measure subnormal levels (3). The problems mainly arose from the presence of an unspecific immunological cross-reactant in plasma, that gave inappropriately high values (4,5). This could be eliminated by heat treatment (6), but later more specific antibodies reduced or eliminated the problem (7). However, commercially available assays have not until lately become available that avoided this problem (8), which probably is the reason why there has been controversy over the role of plasma epo measurement in the diagnosis of PV (9).

A number of studies have shown that measurement of epo in serum or plasma differentiates between PV, secondary erythrocytosis and pseudo-erythrocytosis with a high degree of certainty, provided that the sensitivity and specificity of the assay in the low range is sufficient (7,10-16).

A plasma epo value below the reference range (<3.3IU/L) has a specificity of 97% with a positive predictive value of 97.8% (16). This was further improved by applying an even lower threshold of 1.4 IU/L, which gave 100% specificity and predictive value in a cohort of 186 patients with erythrocytosis. Plasma epo levels remain low in PV patients during phlebotomy, even if some reach the reference range. (7,11) For the diagnosis of PV the ability to measure subnormal Epo levels is the most important.

Low serum epo levels have also been detected in a minority of ET patients (11,13).
For the diagnosis of secondary erythrocytosis a good precision is also needed around the upper reference range limit, since even a slight increase in epo may produce erythrocytosis if it is longstanding.
A number of commercially available Elisa assay kits have the quality necessary, and lately even automated analysis systems fill these criteria.

Demands on Epo assays:

- **A sensitivity and specificity in the low range that makes it possible to differentiate between Epo levels below the reference range, down to 0.5 IU/L.**
- **A well defined reference range making it possible to detect slightly elevated Epo levels**

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