CBC data for diagnosing classical megaloblastic anemia. Other features such as pancytopenia, elevated RDW, serum LDH and indirect bilirubin, as well as LDH₁>LDH₂ are also helpful. If a patient has a normal or low MCV, megaloblastic anemia is not usually the first consideration, which might lead to a missed diagnosis or delay in giving treatment. The present study shows that the combined disease is not difficult to diagnose when all features of megaloblastic anemia except a normal MCV are present. Other features including the RDW, WBC and platelet count, total and direct bilirubin, elevated LDH with LDH₁>LDH₂, decreased vitamin levels were similar to those in the patients with uncomplicated megaloblastic anemia. When we compared the parameters between groups, we found that group A was more similar to group B than to group C. Thus, a patient with combined megaloblastic anemia and thalassemia may tend to manifest megaloblastic anemia rather than thalassemia. As only MCV was different between the uncomplicated and the complicated megaloblastic anemia combined with thalassemia is still an easily diagnosed disease provided the clinician is alert to the possibility of this kind of disease.

References


Novel erythropoiesis stimulating protein exerts an effect on platelet function in uremia equivalent to that exerted by recombinant human erythropoietin

Recombinant human erythropoietin (rHuEPO) improves platelet function and signaling through tyrosine phosphorylation in uremic platelets in response to thrombin. Novel erythropoiesis stimulating protein (NESP) has been recently introduced for the treatment of anemia in uremic patients with the advantage of requiring less frequent dosing. We analyzed the effects of NESP on intraplatelet signaling to thrombin, and compared these with the effects of rHuEPO. Results indicate that NESP is equivalent to rHuEPO with respect to its effects on platelet function.

Since its introduction into clinical practice rHuEPO has been widely used for the treatment of renal anemia. Although it has improved the quality of life of uremic patients, most patients require 2-3 doses per week. Novel erythropoiesis stimulating protein (NESP), a hyperglycosylated form of rHuEPO, is a new recombinant erythropoietic protein with the same mechanism of action as the native hormone, but developed to reduce the frequency of dosing. Uremic platelets have functional and biochemical alterations, with a defective association of the contractile proteins that constitute the cytoskeleton in response to activation. We demonstrated that treatment with rHuEPO enhances the response of uremic platelets to thrombin, by improving the assembly of contractile proteins and the signaling through phosphotyrosine proteins. In this study we compared the effects of NESP with those of rHuEPO on uremic platelets, using the same experimental design previously applied. We included 8 patients with end-stage renal disease (ESRD) on hemodialysis. There were 4 men and 4 women, their mean age was 65±3.85 years, and the mean time they had been on hemodialysis was 45.1±13.1 months. The cause of ESRD was: nephrosclerosis (2), polycystic kidney disease (2), unknown (1), analgesic nephropathy (1), diabetic nephropathy (1) and bilateral nephrectomy (1). Studies were performed while patients were under rHuEPO treatment (7500±1225 IU/week, iv, three times a week) and after a month of shifting treatment to NESP (36.7±6.2 µg/week, iv, once a week). These patients were included in an multicenter, open label, prospective protocol to assess the efficacy and safety of NESP in hemodialyzed patients already treated with rHuEPO.

The clinical parameters evaluated and platelet aggregation responses to thrombin did not differ between treatments (Table 1). Activation of control platelets with 0.1 U/mL of thrombin
Table 1. Clinical parameters and aggregation studies.

<table>
<thead>
<tr>
<th>Treatments</th>
<th>Clinical parameters</th>
<th>Platelet aggregation to T(0.1U/mL) (%)</th>
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</thead>
<tbody>
<tr>
<td></td>
<td>Hematocrit (%)</td>
<td>Hemoglobin (g/L)</td>
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<tr>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>HUePO</td>
<td>33.5±0.9</td>
<td>113.9±3.5</td>
</tr>
<tr>
<td>NESP</td>
<td>36.3±2</td>
<td>119.5±2.52</td>
</tr>
</tbody>
</table>

HUePO: recombinant human erythropoietin; NESP: novel erythropoiesis stimulating protein; T: thrombin.

Figure 1. Effect of NESP on tyrosine phosphorylation of proteins induced by thrombin activation. Platelets were obtained from healthy donors (CONTROL) and from uremic patients while under rHuEPO treatment and after a month of initiating treatment with NESP, as indicated. Aliquots of resting platelets (+) or platelets activated with 0.1 U/mL thrombin (+) were treated to obtain whole platelet lysates (A) or to extract the polymerized cytoskeletal fraction (B). Proteins were resolved by 8% SDS-polyacrylamide gel and transferred to nitrocellulose membranes. Phosphotyrosine proteins were detected by a specific antibody and developed by the chemiluminescence technique. Profiles are representative of 8 different experiments.

In our previous work,6 the effect of rHuEPO was significantly more marked when compared with the effect of NESP.7-9 These findings, together with the advantage of a less frequent dosing, provide an additional biological value to this new recombinant erythropoietin and allow NESP to be considered as an alternative treatment for anemia in uremic patients.

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References

Low affinity and unstable hemoglobin variant caused by AAC→ATC (Asn→Ile) mutation at codon 108 of the β-globin gene

We describe the clinical presentation and DNA analysis of a patient who harbors the AAC→ATC (Asn→Ile) mutation at codon 108 (G10) of the β-globin gene. Our case represents the second report of this hemoglobin (Hb) variant that shows characteristics of both low oxygen affinity and unstable Hb.

β108 (G10) Asn is located at the εββ sub-unit interface at the central cavity of the Hb molecule. At this site, the asparagine residue is uncharged but through its amide group forms hydrogen bonds with other residues of the ε- and β- globin chains. Four Hb variants have been described at this site: Hb Yoshizuka A→Asp (negative charge), Hb Presbyterian A→Asp (positive charge), Hb Shizuku Ile→His (positive charge), and Hb Schlierbach A→Ile (hydrophobic). Both Hb Yoshizuka and Hb Presbyterian show low oxygen affinity and high co-operativity, suggesting that any charge, positive or negative, at this position β108 disrupts the contact and alters the electrostatic properties of the central cavity. It results in destabilization of the Hb molecule, favoring the deoxy (T) over the oxy (R) conformation. Other aspects of the pathophysiology of these Hb variants are different. Hb Presbyterian shows an increased Bohr effect while Hb Yoshizuka shows a decreased Bohr effect. Moreover, the oxygen affinity of Hb Yoshizuka is insensitive to changes at low chloride concentrations. We describe the clinical presentation and DNA analysis of Hb Schlierbach (β108(G10) Asn→Ile; AAC→ATC) in a Chinese female.

A 53-year-old housewife with long-standing anemia underwent a cholecystectomy for gallstones at the age of 40. Her blood counts showed: Hb 10.5 g/dl, mean corpuscular volume 101.1 fl, reticulocytes 3.3%, white blood cells 9.1×10^9/L, and platelets 295×10^9/L. Although not obviously cyanotic, pulse oximetry showed a low oxygen saturation 83% that was increased to 95% with oxygen given at a flow rate of 2 L/min. The cardiac and respiratory systems were normal. Arterial blood gas analysis showed a normal pH and partial pressures of oxygen and carbon dioxide. Arterial blood gas co-oximetry revealed: oxyHb 83.1% (normal range: 94-97%), carboxyHb 0.2%, MethylHb 0.2% and deoxyHb 16.5% (normal range: 0-5%). The low SaO2 coupled with low oxyHb and increased deoxyHb suggested the presence of a low oxygen affinity Hb variant. Hb analysis by high performance liquid chromatography (Variant Hb Testing System, Bio-Rad, Hercules, CA, USA) showed a Hb variant (29.7%) that was eluted at the HbA2 window. Hba and Hbf levels were 68.7% and 1.5%, respectively. The variant was not separated from HbA on electrophoresis at alkaline and acidic pH. Red cell inclusion bodies were demonstrated on two-hour incubation with supravital dye. Tests for unstable Hb using heat and isopropanol precipitation both showed positive results. Other investigations including vitamin B12 and folic acid, total bilirubin, lactate dehydrogenase and haptoglobin were within normal limits. Her ferritin level was increased slightly at 336 pmol/L (normal range: 10-291 pmol/L). The patient had three children and two were found to carry the same Hb variant. Owing to the proportion of Hb variant among total Hb, a β-chain variant was anticipated. Direct sequencing of the β-globin gene based on a protocol previously described showed that the patient was heterozygous for AAC→ATC (Asn→Ile) mutation at codon 108 (Figure 1). While the α-globin genes were not directly sequenced, they showed normal configuration on Southern blot analysis with α- and ε-globin gene probes.

Ours is the second report of AAC→ATC (Asn→Ile) mutation at codon 108 (G10) of the β-globin gene, the first case being described in a Swiss family and termed Hb Schlierbach. It is interesting that the same Hb variant originates from two separate geographic areas, especially that the nucleotide substitution involves replacement of asparagine by a hydrophobic amino acid isoleucine that is not used at all in the production of normal ε- and β- globin chains. The εββ contact is expected to be perturbed by mutations at β108 and may contribute to instability of the Hb molecule. This is evident by positive Hb instability tests reported in Hb Presbyterian and Hb Schlierbach. In our case, the presence of gallstones, slight reticulocytosis and positive Hb instability tests are in accordance with the unstable nature of AAC→ATC (Asn→Ile) mutation at codon 108 (G10) of the β-globin gene, although the hemolysis may be episodic in nature. The arterial blood gas results in the present case are similar to those previously reported for Hb Schlierbach and the proportion of Hb variant is also consistent in the two cases (29.7% in our case versus 31% in the previous case). While oxygen dissociation studies in the previous report of Hb Schlierbach clearly demonstrated reduced oxygen affinity, this has not been repeated in our case.

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Figure 1. Direct nucleotide sequencing of the β-globin gene, showing AAC→ATC mutation at codon 108.

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