Anemia in Systemic lupus erythematosus: from pathophysiology to clinical assessment

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**Abstract**

Hematological abnormalities are very common in systemic lupus erythematosus. Anemia is found in approximately 50% of patients, with anemia of chronic disease being the most common form. Impaired erythropoietin response and presence of antibodies against erythropoietin may contribute to the pathogenesis of this type of anemia. Patients with autoimmune hemolytic anemia usually belong to a distinct category, which is associated with anticardiolipin antibodies, thrombosis, thrombocytopenia and renal involvement, often in the context of secondary antiphospholipid syndrome. Finally, as recently suggested, autoantibodies, T lymphocytes and deregulation of the cytokines network can affect bone marrow erythropoiesis leading to anemia.

**Key words:** systemic lupus erythematous, anemia, pathogenesis, diagnostic approach

**Short title:** anemia in lupus
Introduction

By incorporating haemocytopenias into the revised American College of Rheumatology criteria for systemic lupus erythematosus (SLE), the experts of the field have acknowledged that the “haematologic system” is frequently attacked in the setting of this disease. Although lymphopenia is the most frequent haematological disturbance in SLE, clinicians are often faced with the common problem of an anemic SLE patient (1-4).

Although it was initially suspected that anemia in SLE was a result of mainly antibody-induced damage of erythrocytes, evidence to date indicates that the causes of anemia in SLE vary and may be of immune or non-immune pathogenesis (1, 5-7) (Table).

Table. Causes of anemia in patients with SLE

<table>
<thead>
<tr>
<th>Category</th>
<th>Causes</th>
</tr>
</thead>
<tbody>
<tr>
<td>Anemia of chronic disease</td>
<td></td>
</tr>
<tr>
<td>Blood loss</td>
<td>Gastrointestinal loss, menorrhagias</td>
</tr>
<tr>
<td>Nutritional deficiencies</td>
<td>Iron, folate, B12</td>
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<tr>
<td>Immune-mediated</td>
<td>Haemolysis, red cell aplasia, haemophagocytosis, aplastic anemia, pernicious anemia</td>
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<tr>
<td>Myelofibrosis</td>
<td></td>
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<tr>
<td>Uremia</td>
<td></td>
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<tr>
<td>Treatment induced</td>
<td></td>
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<tr>
<td>Microangiopathic haemolysis</td>
<td>Disseminated intravascular coagulation, thrombotic thrombocytopenic purpura, drugs</td>
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<tr>
<td>Hypersplenism</td>
<td></td>
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<tr>
<td>Infection</td>
<td></td>
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<tr>
<td>Myelodysplasia</td>
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Anemia of chronic disease (ACD), iron deficiency anemia (IDA), autoimmune hemolytic anemia (AHA), anemia of chronic renal insufficiency and cyclophosphamide-induced myelotoxicity are the most frequent causes. It is noteworthy that ACD often coexists with anemia caused by other mechanisms. Iron deficiency is common in SLE patients as a result of menorrhagia and increased gastrointestinal blood loss, caused by the use of non-steroidal anti-inflammatory drugs, aspirin and oral anticoagulants.

In this review, we focus on the nature, pathophysiologic mechanisms and causes of anemia, as well as the clinical approach of anemia in patients with SLE.

Autoimmune haemolytic anemia

Antibody-induced damage of blood cells by complement-dependent or independent mechanisms has long been considered a common pathogenetic mechanism for cytopenias in SLE. Evidence which restricts bona fide AHA in 5-10% of SLE patients with anemia, appears to contradict this hypothesis (1). The overestimation of the prevalence of AHA in many studies is the result of the inclusion
of obscurely defined cases of anemia, since a positive Coombs’ test without actual haemolysis is found in 18-65% patients with SLE (1).

The pathogenesis of AHA should be reviewed within the fundamental immune disturbance of SLE. Interpreting how self-intracellular antigens become immunogens, so effective as to trigger and maintain a strong and prolonged autoantibody response, is both challenging and crucial. Genetic predispositions and defects in apoptosis, T cell function and complement or complement receptors are but a few of the numerous abnormalities which have been proposed to underlie lupus pathogenesis, predispose loss of self-tolerance and allow the production of pathogenic autoantibodies (8-11). In SLE the anti-erythrocyte antibody is mainly IgG of warm type and usually displays non-Rhesus specificity (1). Such antigens are expressed normally by human fetal erythrocytes, as early as 10 to12 weeks of life. It has been shown in NZB mice, an animal model for SLE, that autoreactive B cells can be sheltered from host erythrocytes entering the peritoneal cavity, an immune privileged compartment that allows them to escape deletion and later produce anti-erythrocyte antibodies, with the appropriate T-cell assistance (12).

The precise specificity of the anti-erythrocyte antibodies for the majority of patients with SLE and AHA is undefined. The non-Rhesus specific IgG autoantibodies in patients with primary AHA have been found to react with either the band 3 anion transporter protein of membrane erythrocytes or with an epitope formed by band 3 protein and glycophorin A (13). NZB lupus prone mice produce anti-erythrocyte autoantibodies that exhibit anti-band 3 specificity (14). Interestingly, anti-band 3 IgG antibodies are naturally formed in healthy individuals, possibly functioning as eliminators of senescent erythrocytes, which on aging express band 3 protein-derived neo-antigens (15). The relation between the naturally occurring and pathologic anti-band 3 autoantibodies remains an important, yet unanswered question. One could hypothesize that such antigenic neo-epitopes, when exposed on senescent red cells, drive autoantibody responses, thereby triggering auto-hemolytic process. This concept stems from considerable data suggesting that the generation and clearance of dead cells are important events that underlie the immunopathology of SLE in general (11).

Deficient clearance of dead cells is a critical pathogenetic feature of SLE. Macrophages from SLE patients were proven to be less phagocytic by prolonged clearance half time of Cr-labeled anti-IgG sensitized autologus erythrocytes (16). An interesting, yet unexplained, finding was that patients with SLE and AHA showed an acquired deficiency of either or both CD55 and CD59 erythrocytic expression, while SLE patients without AHA positively exhibited these molecules (17). Although the deficiency of these GPI-anchored proteins, whose role is to control complement activity, might contribute to hemolytic process by increasing the susceptibility to complement-mediated lysis, this defect seems to play an enhancing rather than a triggering role. Erythrocytes from patients with paroxysmal nocturnal hemoglobinuria are deficient in a membrane regulatory protein of complement, called decay-accelerating factor. Moreover, a functional defect in a second membrane regulatory protein of complement, CR1 has also been hypothesized. In this context there are also some data about the loss of CR1 on erythrocytes of SLE patients (18, 19). The loss of GPI-anchored structures might be responsible for some Coombs-negative haemolytic anemia cases in these patients.

In SLE patients, antibodies which react with negatively charged phospholipids such as cardiolipin and putative co-factors have been shown to correlate with venous and arterial thromboses, thrombocytopenia and recurrent fetal loss, a syndrome called...
secondary antiphospholipid syndrome (APS) (20). Among SLE patients, the prevalence of antiphospholipid antibodies is high, ranging from 12% to 30% for anticardiolipin (ACL), and 15% to 34% for lupus anticoagulant antibodies. Several studies of patients with SLE demonstrated a significant correlation between ACL or lupus anticoagulant and Coombs’ positive hemolytic anemia (20-25). There is increasing evidence that ACL autoantibodies are not just a secondary phenomenon caused by haemolysis. They could also contribute to the pathogenesis of AHA by acting as anti-erythrocyte autoantibodies (26, 27).

It is still unclear whether the presence of AHA worsens the outcome of SLE patients (3, 28). It has been suggested by one study group that lupus patients with AHA may have a more benign course, yet others found differences only in the prevalence of serositis (29, 30). Severe haemolytic anemia is rather rare and has been significantly associated with other organ involvement including the kidneys and central nervous system (31). In a retrospective case-control study of our department, when assessing the clinical picture, the immunological characteristics and the survival of 41 SLE patients with AHA, 2/3 of these patients were found to display autoimmune haemolysis at the onset of SLE (32). It was further revealed that patients with AHA secondary to SLE were more likely to have IgG ACL than controls. The frequency of IgG ACL in this SLE cohort with AHA was 74%, remarkably higher than that previously noted in unselected patients with SLE (33). In addition, many of these patients displayed renal involvement, thrombocytopenia and other manifestations of the APS. Thus, autoimmune haemolysis seems to be a marker for a subset of SLE patients with a higher prevalence of APS (34, 35).

The AHA in SLE patients is seldom severe and rarely fatal as prednisolone is usually sufficient in controlling haemolysis. Should this treatment fail other forms of immunosuppression such as azathioprine and cyclophosphamide as well as danazol, intravenous immunoglobulin or anti-CD20 monoclonal antibody should be tried. Splenectomy should be considered only as a last resort given the poor response reported (36).

**SLE: another syndrome of immune-mediated haemopoietic failure?**

The concept of the haemopoietic failure being the aftermath of an immunologically damaged bone marrow (BM) gathered momentum in the light of evidence produced by the study of BM biopsies from SLE patients. Overall hypocellularity, morphologic dysplasia, increased fibrosis and BM necrosis were frequent findings in SLE patients with haemocytopenias, suggesting a primary BM involvement in the disease’s tissue pathogeny, most likely mediated by autoantibodies, immune-complexes and immunopotent T cells (37, 38).

Solid proof of humoral immune mechanisms’ participation in haemopoietic dysfunction was obtained from SLE patients with aplastic anemia, a BM failure syndrome of a definite immune pathogenesis. In these rare cases, complement-dependent or independent autoantibodies were found to suppress erythroid and granulocytic colony formation of BM progenitor cells (39-41). In a subsequent study by Liu et al, IgG fractions of patients with active SLE and hemocytopenias suppressed BM progenitor growth in vitro, by directly binding CD34+ primitive haemopoietic cells but not more differentiated cells. The nature of the antigen on CD34+ cells still remains an enigma. Furthermore, correlation was not established between the severity of the peripheral cytopenias and the inhibitory capacity of the serum autoantibody on haemopoiesis (6).
Targeted by autoantibodies, the progenitor BM cells lead to various syndromes of haemopoietic failure, such as aplastic anemia, hypoplasia of myeloid line, amegacaryocytic thrombocytopenia and the extremely rare entity of the pure red cell aplasia (PRCA) (42-48). The presence of the inhibitory autoantibody is typically related to SLE activity and can be suppressed by successful therapy, i.e. by immunosuppression. However, PRCA can occur in the absence of disease activity or even precede the appearance of SLE (44). In most cases laboratory investigation unmasks the presence of inhibitory autoantibodies against erythroid progenitor cells, proerythroblasts and erythropoietin (EPO) (48).

Numerous studies have supported the view that T-cell mediated inhibition of haemopoiesis is the major culprit for BM failure in SLE (45, 49-51). As anecdotal reports were initially providing laboratory evidence of a haemopoietic cell inhibition by reactive T cells, larger studies employing co-culture experiments and colony-formation techniques later confirmed this hypothesis. It has been suggested that the homing of autoreactive lymphocytes in the BM of SLE patients may affect the hemopoietic capacity of BM stroma and also damage hemopoietic stem cells through direct cytotoxic destruction. Yamasaki et al described a series of 25 SLE patients with anaemia attributed to the suppressor activity of T lymphocytes, which were found to inhibit autologous or allogeneic BM erythroid colony formation in vitro. Removal of T lymphocytes from SLE marrow samples has been reported to result in a significant increase in the progenitor cell clonogenic potential (42).

That the hematologic abnormalities of SLE might not only reflect a process of intramedullary cellular destruction but more specifically apoptosis or programmed cell death, was first proposed by Papadaki et al (52). The authors found that patients with SLE displayed low numbers of CD34+ cells compared with controls, which correlated with an increased Fas expression by these cells and high apoptotic indices in the compartment of CD34+/Fas+ cells. Based on these findings, the authors proposed a Fas-mediated apoptotic stem cell depletion of SLE marrow, distinctively resembling the apoptotic exhaustion of bone marrow reserve in other syndromes of haemopoietic failure (53, 54). Fas upregulation on CD34+ cells has been well documented in aplastic anemia and has been ascribed to increased production of inhibitory cytokines, like TNFα and IFNγ, by BM cytotoxic T lymphocytes. An increased expression of TNFα mRNA has been reported in SLE patients’ BM, yet the possibility remains that Fas is over-induced in SLE along with several other genes such as CD69 and T cell class II MHC, as part of the broad cellular activation associated with SLE (55-57). Investigating the root cause of the SLE haemopoietic progenitors’ apoptotic behavior in a more recent study, Tiefenthaler et al, pointed to the possible involvement of humoral factors (58). Specific sera of SLE patients with active disease induced apoptosis of allogeneic CD34+ cells, an effect that was found to be independent of complement inhibition and Fas-blockade. Unfortunately, the investigators failed to connect the apoptotic process with autoantibody activity since removal of the IgG fraction from the pro-apoptotic sera did not protect CD34+ cells.

Evidence for the culpability of BM stroma in SLE haemopoietic failure derived from culture experiments in which stomal cells from SLE patients failed to support allogeneic progenitor cells growth (52). It has also been shown that, as a result of a diminished activity of monocytes, the production of hemopoietic growth factors by BM fibroblasts is insufficient, a fact that could explain SLE hematologic abnormalities (59).
Pathophysiologic mechanisms underlying anemia of chronic disease in SLE

Patients suffering from chronic inflammatory disorders, commonly display ACD, a mild to moderate normocytic-hypochromic anemia the pathogenesis of which remains obscure (60). Insufficient supply of haemopoietic cells with EPO, along with their resistance to its proliferative action constitutes an important pathogenetic mechanism of ACD in several autoimmune diseases (61-63). The phenomenon can be attributed to the impaired EPO resulting from the inhibiting action of inflammatory cytokines such as IL-1, TNF-α, INF α, β and TGF-β (64). Experiments have shown that rat kidneys produce less EPO when exposed to IL-1 (65). In addition, over production of these cytokines has been associated with primary resistance of haemopoietic progenitors to the action of EPO (66, 67).

In a study of our department, assessing whether EPO production is appropriate in SLE patients with anemia, hemoglobin levels were correlated with the logarithmic concentrations of EPO measured in SLE patients with different types of anemia (68). A significant increase of EPO was observed with decreasing values of haemoglobin in patients with IDA, but EPO levels, at different values of haemoglobin in patients with ACD and AHA remained unchanged. Moreover, the slope of EPO response was blunted in ACD and AHA compared with controls, indicating severely impaired EPO production in SLE patients with ACD and AHA. It has been previously shown that in SLE nephritis, CD4 lymphocytes and macrophages infiltrate the interstitial renal area producing cytokines inhibiting the production of EPO (69). Since patients with lupus nephritis often display ACD, inadequate production of EPO in these patients might be due to this mechanism.

The presence of autoantibodies against EPO (anti-EPO) has been proposed as another possible cause of EPO deficiency (70). Although a correlation between anti-EPO antibodies and EPO levels was not detected in this study, underestimation of EPO measurement due to autoantibodies interference can not be excluded in SLE patients, as proposed by Schett et al (71).

Recent studies have shown that resistance to EPO action can be attributable to autoantibodies against EPO (72). The possible role of anti-EPO antibodies in the pathophysiology of ACD in SLE patients was studied by measuring the levels of EPO, as well as the presence of anti-EPO antibodies (68). Anti-EPO antibodies were detected in 21% of patients with SLE and anemia, with higher incidence in patients with ACD. This data suggested that patients with anti-EPO antibodies suffered from a more active disease, despite the fact that negative correlation between hemoglobin levels and presence of anti-EPO antibodies was not proven. However, the presence of anti-EPO antibodies was associated with active SLE and severe anemia in a previous study (73). Anti-EPO antibodies were more frequently detected in patients with severe anemia, compared to those without. Moreover SLE patients with severe anemia had higher titers of anti-EPO antibodies compared to SLE patients with moderate anemia. The frequent presence of anti-EPO antibodies, particularly in patients with active SLE, ACD and severe anemia implies that anti-EPO antibodies possibly constitute a mechanism of resistance to the action of EPO, justifying anti-EPO antibodies’ role in the pathogenesis of ACD.

Clinical considerations. Conclusions.

Distinctly different therapeutic approaches are required for the multiple causes of anemia in patients with SLE. ACD is the most common form of anemia in these patients; AHA, IDA, drug-induced myelotoxicity, and anemia due to chronic renal failure are also often detected (1-3, 5, 68). Aplastic anemia, PRCA, pernicious
anemia, myelofibrosis, sideroblastic anemia, hemophagocytic syndrome, and thrombotic microangiopathy occur less frequently (39, 74-80). Given the complexity of these patients’ illnesses, a thorough history and physical examination is essential for placing the anemia in its proper context.

In clinical practice, simple tests may help to diagnose the underlying cause of anemia in SLE patients. For example, if the reticulocyte count is raised, a hemolytic process or acute bleeding should be suspected. Diagnosis of warm-type hemolysis relies on the positive direct Coombs’ test combined with reduction of haptoglobin. Increased serum creatinine and blood urea nitrogen levels would indicate poor renal function due to renal disease, which leads to poor secretion and lower serum levels of EPO. Frequently, however, the anemia in SLE patients is accompanied by a low reticulocyte count, reflecting a hypoproliferative state. An elevated MCV may be an indication of either vitamin B12, folate deficiency or the toxic effects of immunosuppressive agents. A low MCV typically indicates IDA or ACD. Patients with the ACD are characterized by reduced plasma iron and transferrin concentrations, while iron stores, as reflected by plasma ferritin levels, are normal or even increased. The differential diagnosis between IDA and the ACD can now be readily made by measurement of the plasma transferrin receptor concentration and, ideally, determination of the plasma transferrin receptor-ferritin index. BM aspiration can be helpful in evaluating the hypoproliferative anemia, revealing deregulation of cellularity, absence of iron stores or megaloblastoid maturation, hemophagocytosis, PRCA, or sideroblastic anemia.

Based on published clinical trials, recombinant human erythropoietin (rHuEPO) therapy can be proved beneficial outside the settings of uremia. In every day practice, the use of rHuEPO should be limited to patients with symptomatic anemia and those who are transfusion-dependent or candidates for blood transfusion. However, few SLE patients have haemoglobin levels lower than 8 to 9 gr/dl and practically none of these patients is transfusion-dependent. Thus, although SLE patients with ACD may show excellent hematologic response to rHuEPO, there is little rationale for widespread treatment. Evidence has been provided that rHuEPO therapy is occasionally associated with anti-rHuEPO antibodies not only capable of inhibiting the exogenously administered rHuEPO but also of inhibiting endogenous EPO, causing PRCA (81). Moreover, previous studies of the in vitro effects of rHuEPO on T and B cells and studies of lymphocytes subsets in dialysis patients receiving rHuEPO suggest that rHuEPO might augment immune responses (82). Should rHuEPO therapy increase autoimmune responses, caution is advised when administering it to SLE patients.

References.


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