The diagnosis and management of the haematologic manifestations of lupus

Abstract

Haematological manifestations in systemic lupus erythematosus (SLE) are frequently observed. They are diverse and range from mild to severe. Therefore, different treatment approaches are needed from simply keeping vigilant to significant immunosuppression. Most treatment evidence is based on case-reports or small retrospective studies, as few randomized controlled trials have been performed. The development of biological therapy has opened new possible ways to treat the most severe cases but further clinical trials are necessary.

In this review we consider the most common and characteristic haematological manifestations of SLE patients, focusing on their pathogenesis and management.

Highlights

- Haematological manifestations are common in Systemic Lupus Erythematosus.
- Some of them are part of the classification criteria.
- They can be related to the disease, concomitant blood disorders or iatrogenic.
- These manifestations have a wide range of severity, from mild to life threatening.
- Biological therapy has shown promising results, but more studies are necessary.

1. Introduction.

Haematological manifestations in systemic lupus erythematosus (SLE) are common and diverse. Their frequency varies in different populations[1]. These manifestations can be due to the disease itself, another concomitant disease or iatrogenic. Haemolytic anaemia, leukopaenia, lymphopaenia and thrombocytopaenia are incorporated into both the 1997 update of the 1982 American College of Rheumatology (ACR) [2] and the 2012 Systemic Lupus International Collaborating Clinics (SLICC) [3] classification criteria for SLE.

Most of these manifestations are caused by increased peripheral destruction of blood cells associated with circulating autoantibodies. The major haematological manifestations of SLE are anaemia, leukopaenia, thrombocytopaenia, and the antiphospholipid syndrome (APS). The bone marrow (BM) may also be a target in SLE and features such as myelofibrosis, aplastic anaemia and pure red cell aplasia can also occur. In this review we will consider the pathogenesis and management of these specific manifestations.

2. Red Blood Cell Associated Pathologies

A summary of red blood cell associated pathologies in SLE patients is shown in table 1.

2.1. Anaemia

Anaemia is frequent, affecting more than 50% of patients throughout the course of the disease [4, 5].

Anaemia is defined as haemoglobin of less than 12 g/dl in women and 13.5 g/dl in men [6]. It can be both immune and non-immune mediated in SLE patients.

2.1.1. Anaemia of Chronic Disease

2.1.1.1. Introduction

Anaemia of chronic disease (ACD) is the most common type of anaemia in SLE patients, responsible for about one third of the cases[7].

It usually presents as normocytic and normochromic anaemia, with normal or elevated serum ferritin levels and a normal BM.

2.1.1.2. Pathogenesis

The aetiology of ACD in SLE is still not fully understood but it seems to be related to changes in iron homeostasis, inadequate erythropoietin (EPO) response or activity and impaired erythropoiesis. A schematic explanation is presented in figure 1.

<u>Iron Homeostasis</u>

During inflammation, iron homeostasis is significantly affected, as hepcidin production is regulated by iron. Hepcidin is a hormone produced in the liver that prevents iron from entering into the plasma compartment, by inhibiting iron absorption in the duodenum and its release from hepatocytes and macrophages. It is tightly regulated by the levels of serum iron and its production increases when iron is

abundant, preventing further absorption and release from stores. Its production diminishes or ceases when iron is deficient [8]. It prevents iron efflux by interacting with ferroportin 1 at the cell surface, leading to internalization and degradation of ferroportin 1 protein [9].

Hepcidin production also seems to be regulated by inflammatory cytokines. IL-6 induces the production of hepcidin and a consequent hypoferraemic state [10]. Similarly adding IL-6-neutralizing antibodies to hepatocyte cultures ablated hepcidin production. IL-6 levels were significantly higher in SLE patients with active haematological disease and in those patients with anaemia the mean levels of IL-6 were significantly higher than in those patients without it [11]. The Signal Transducer and Activator of Transcription 3 (STAT3) binding site at position -64/-72 of the hepcidin promoter controls IL-6-dependent transcriptional activation and knockdown of STAT3 by RNAi reduces hepcidin mRNA expression, implying that stimuli which activate hepatic STAT3 may also enhance hepcidin expression[12]. Inflammatory immune regulators such as IFN-γ and lipopolysaccharides seem to increase monocytes iron acquisition, by stimulating divalent metal transporter 1 (DMT-1) expression and to retain the metal within the cells by inhibiting ferroportin synthesis [13]. In contrast, pro-hepcidin levels do not seem to correlate with disease activity, cytokine levels or serum iron levels in SLE patients [14].

Other cytokines, including TNF-α, IFN-γ and IL-1, are part of iron homeostasis by reducing the concentration of transferrin receptor on cell surface and increasing ferritin synthesis[9].

Erythropoietin

The pathogenesis of ACD in different autoimmune diseases is related to reduced EPO activity, due to reduced production and erythroid cells resistance. EPO is fundamental in maintaining an adequate haematocrit level in human blood and its concentration increases with a low haematocrit level and vice versa.

Absolute EPO levels were reduced in SLE patients, even in those without anti-EPO antibodies, but its correlation with haemoglobin and haematocrit variations was maintained [15]. The average levels of EPO in patients with SLE and anaemia did not vary according to the aetiology of anaemia[7].

Anti-EPO antibodies have been detected in SLE patients in some studies [7, 15] and were more common in ACD [7]. However, in patients with anti-EPO antibodies there was no correlation between EPO levels and haemoglobin or haematocrit [15]. In contrast, there is conflicting evidence about the relationship between anti-EPO antibodies and the severity of the anaemia. In some studies, the decrease in haemoglobin levels in patients with anti-EPO antibodies was not statistically significant [7] and the anaemia was not more pronounced in SLE patients with anti-EPO antibodies (as the EPO levels were also extremely low in these patients there may be some interference in the measurement of EPO due to the antibodies)[15]. In another study of 92 patients with SLE, the prevalence of anti-EPO antibodies in patients with more severe anaemia (6 out of 21 patients had a Haemoglobin <10.0

g/dl) was statistically significantly higher when compared with patients without anaemia and its antibodies titres were also more elevated [5].

Anti-EPO antibodies might also reflect SLE disease activity as they are associated with lower serum complement (C3 and C4) and higher anti-dsDNA autoantibody levels[15]. European Consensus Lupus Activity Measurement (ECLAM) scores were statistically significantly higher in patients with anti-EPO antibodies compared to those without them [5].

Impaired erythropoiesis

The mechanisms of impaired erythropoiesis depend on the inhibition of progenitor cells. Several mechanisms are involved, one consists of type I and II interferons inhibiting this proliferation and through caspase and ceramide-dependent pathways also induce apoptosis [16]

Both the sera and IgG isolated from four active SLE patients also suppressed progenitor cell growth in vitro [17].

2.1.1.3. Clinical implications

ACD is a normochromic and normocytic anaemia that is usually mild (haemoglobin 9 to 10 g/dl) to moderate (haemoglobin below 9 g/dl) [7]. The reticulocyte count, the serum concentration of iron and the transferrin saturation are low. In contrast, transferrin and ferritin levels are normal and the latter may be elevated, due to retention in the reticuloendothelial system [18].

As in the evaluation of any cytopaenia, a peripheral blood smear should be performed to distinguish between peripheral destruction in isolated cytopaenias and BM failure in pancytopaenia[19].

To distinguish ACD from iron-deficiency anaemia (IDA), the ferritin concentration level is widely used and if it is >20 µg/dl, IDA is unlikely. A BM biopsy might be performed but its diagnostic yield is low [7]. In another study levels >30 µg/l gave a positive predictive value of 92% [20]. Moreover, soluble transferrin receptor (sTfR) has been studied as a possible indicator of iron deficiency, without any connection to chronic disease and inflammation. Patients with ACD+IDA or IDA alone had significantly higher sTfR values [21]. An sTfR Index (sTfR/log ferritin index) above 2 is indicative of true iron deficiency in ACD, while a index below <1 suggests functional iron deficiency [16]. A differential diagnostic approach between ACD and IDA is shown in figure 2.

A nutritional deficiency of iron, folate or vitamin B12 should be considered If the reticulocyte count is inadequate [7]. Measurement of haptoglobin and lactate dehydrogenase to screen for haemolysis and renal function tests should also be performed [16]. Assays to measure hepcidin are not widely available [22]. The evaluation of EPO is not fully established and is only of value when the haemoglobin level is below 10 g/dl [18].

2.1.1.4. Treatment

Treatment of the underlying disease is the main goal in SLE patients with ACD [18], but mild cases often do not warrant treatment.

Treatment with an erythropoiesis-promoting agent might benefit patients who are symptomatic. The most widely used are epoetin α (recombinant human EPO) or darbepoetin α which also play a part in treating anaemia of chronic renal insufficiency [19].

Transfusions can be an option in the case of severe or life-threatening anaemia, especially if bleeding conditions are also involved [18]. Long-term transfusions are not recommended due to iron overload and sensitization to HLA antigens [18].

Iron supplementation is not recommended if ferritin level is normal or increased, due to possible adverse outcomes [18], especially in intravenous supplementation, as iron might be linked to atherosclerosis and has an anaphylactic risk [23].

Underlying deficiencies, such as folate or vitamin B12, should be corrected [16].

SLE patients with ACD have a significantly higher disease activity than patients with other types of anaemia, probably due to more frequent concomitant lupus nephritis [7]. Remission of ACD is rare and its recovery is especially slow in patients treated with immunosuppressives [7].

2.1.2. Iron deficiency anaemia

2.1.2.1. Introduction and Pathogenesis

IDA is common in SLE patients, affecting about one third of 132 patients with anaemia[7]. It is usually due to chronic gastrointestinal haemorrhage secondary to medication, notably nonsteroidal anti-inflammatory medications and glucocorticoids[24].

2.1.2.2. Clinical implications

IDA patients should be screened for a source of bleeding [19]. It may coexist with ACD, leading to a diagnostic challenge, as the serum ferritin is positively influenced by iron loading and inflammation [16].

2.1.2.3. Treatment

After a bleeding source has been excluded or identified and treated, patients may begin oral or intravenous iron supplementation [19]. Iron therapy is the first line treatment for patients with IDA or ACD+IDA [18]. However, oral iron supplementation can lead to inadequate iron absorption in patients with ACD, because iron transfer from enterocytes to the circulation is impaired [18].

There are few data about erythropoietic agents and their possible side effects on the course of the underlying disease, as they can interfere with cytokine cascades. If they are introduced, haemoglobin levels should be determined after four weeks of therapy and subsequently at approximately 4 weekly intervals. Iron should be administered simultaneously as there will be no haemoglobin increase if iron deficiency is not corrected [18].

2.1.3. Autoimmune haemolytic anaemia (AIHA)

2.1.3.1. Introduction

Haemolytic anaemia with reticulocytosis is included in both the ACR and the SLICC classification criteria. Autoimmune haemolytic anaemia (AIHA) involves anti-red blood cell (RBC) antibodies which damage erythrocytes in either a complement-dependent or independent manner [19]. AIHA may be the first manifestation of SLE and can appear several years before an SLE diagnosis is made[4]. In one study 27 out of 41 cases of SLE-related AIHA occurred at SLE onset[25].

AIHA can be either primary or secondary. Secondary AIHA is considered a manifestation or a complication of an underlying disorder when: there is an overt immunological link between them; the association of AIHA and the concomitant disease occurs more frequently than the disease itself and the correction of the concomitant disease reverses the AIHA [26].

Its prevalence varies, probably due to the different diagnostic criteria for AIHA. Ten patients out of a cohort of 305 SLE patients had severe haemolytic anaemia (defined by haemoglobin <8.0 g/dl, in the presence of a positive direct antiglobulin test (DAT), a raised reticulocyte count and haemoglobin reduction by 3.0 g/dl since the last evaluation) [27]. In another cohort of 533 SLE patients, haemolytic anaemia (haemoglobin decrease of 3g/dl, rise in conjugated bilirubin and a reticulocyte count >5%) was identified in 50 (8%) patients [28] and in a multi-ethnic cohort of 1251 of female SLE patients 76 patients (6%) had haemolytic anaemia [29].

2.1.3.2. Pathogenesis

RBC antibodies in SLE are mainly warm-type IgG, but doubts about its antigen specificity remain. The type of antibody is identified, based on the optimal temperature of antigen-antibody reaction. Warm-acting-AIHA and cold-acting-AIHA are distinguished by the optimal temperature of antigen–antibody reactivity [30], the latter being usually mediated by IgM[31]. RBC coated with warm IgG undergo changes in their membranes as they pass through the spleen, resulting in spherocytes, which are removed by phagocytosis [31].

There may be an association between haemolytic anaemia and anticardiolipin antibodies (ACA) [27]. ACA were also statistically significantly more common in SLE patients with AIHA than with IDA [7]. Even in idiopathic AIHA, IgG and IgM ACA are present in higher titres than in normal controls, irrespective of SLE, suggesting it might play a bigger part in RBC destruction [32]. Others have described an increase in ACA IgM levels in SLE patients with AIHA, but no difference in the level of ACA IgG between groups was found[33], while in a study with 41 SLE patients with AIHA IgG, but not IgM, ACA antibodies were associated with AIHA [25]. Antiphospholipid antibodies (aPL) in SLE patients were also related to complement activation and anti-RBC activity, with the levels of aPL IgG correlating to hypocomplementaemia and immunoglobulin binding on RBC [34]. Complement levels (both C3 and C4) are also lower in AIHA [7].

In NZB mice, the RBC autoantigen for IgG autoantibodies was identified as Band 3 protein, the RBC anion channel [35], but the autoantigen in humans has yet to be identified.

There are conflicting data as to whether anti-dsDNA antibodies are associated with AIHA [7, 27].

A possible role for CD55 and CD59, glycosylphosphatidylinositol-anchored proteins with complement inhibitory properties, in AIHA has been postulated. The diminished expression of CD55 and, more importantly, CD59 in RBC from many SLE patients with AIHA (and in some patients with primary AIHA), showed no correlation with the presence of aPL in SLE sera. However, acquired deficiency of CD55 and CD59 was seen more consistently in AIHA secondary to SLE than in primary AIHA, probably due to multiple alterations of the immune system. It might play a part in facilitating cell destruction and cytopaenia [36]. Another study confirmed a decreased expression of CD59 in SLE patients with AIHA when compared with SLE patients without it, primary AIHA and normal controls, but CD55 was not significantly decreased in SLE patients with AIHA [37].

2.1.3.3. Clinical implications

Studies of 76 patients with haemolytic anaemia revealed that pericarditis, pleuritis, lupus nephritis and seizures were more common than in those without haemolytic anaemia [29]. Similarly, in another large cohort group (870 SLE patients), leukopaenia, thrombocytopaenia and anti-dsDNA levels were predominant in patients with AIHA[38]. Severe AIHA has also been associated with damage accrual [39].

In one cohort of 305 SLE patients, none of the 10 patients with AIHA died as a direct result of the haematological manifestation [27]. In AIHA, the median time necessary to correct the anaemia is 3 months, and it has a low recurrence rate [7].

2.1.3.4. Treatment

AIHA therapy is mostly based in isolated case reports and a few retrospective studies. There is a lack of established definitions of treatment goals, especially when to switch to second-line therapies. There are few clinical trials of first-line treatments. Many of the available studies were conducted in idiopathic AIHA and their results extrapolated to SLE patients with AIHA [31].

Treatment options are varied, but one study showed oral prednisone (mean dose 1mg/kg) was used as the first-line treatment in 50% of patients and high dose methylprednisolone (1.5mg/kg/day) in the other 50% [40]. Twenty five per cent of patients with primary AIHA require at least 2 types of therapy, 13% require 3 and 4% > 4 types [41].

The treatment usually used is glucocorticoids as first line of treatment (prednisone up to 1 mg/kg/day) and the haemoglobin response usually takes up to 3 weeks, [31]. When a sustained response is achieved, the dosage should be slowly tapered, although the optimal rate has not been established. If a patient fails to respond (defined by lack of response after 3 weeks treatment), significant doses of oral

prednisolone (around 15 mg/day) plus second line therapy are likely to be required [31]. In these cases, both conventional immunosuppressive therapy and splenectomy can be used. The pros and cons of the various therapeutic options are shown in Table 2.

New therapies

There have been some case reports of new therapies or combinations in the management of patients with mostly primary AIHA. Rituximab (RTX), which binds the CD20 molecule present on many B cells, and alemtuzumab, a monoclonal antibody that binds to CD52, were used in 21 consecutive adult patients, 8 of whom had AIHA, and beneficial responses were observed in all patients. The median duration of complete remisison was 46 weeks and 3 of 8 patients with AIHA maintained complete remission at 33, 46, and 89 weeks. CD4+ T cells were still at low levels at week 24, whereas B and NK cells partially recovered to basal levels [42].

In a 29-year-old woman with warm IgM AIHA secondary to eosinophilic granulomatosis with polyangeiitis, eculizumab and RTX was successfully administered after treatment failure to 3 previous treatments. A single administration of eculizumab 600 mg IV, a humanized monoclonal antibody against terminal complement C5, lead to a 3 g/dl increase in haemoglobin levels, without further need to treatment or transfusion. Subsequently, RTX was successfully administered as maintenance therapy[43]. In 64-year-old female patient with AIHA, secondary diffuse large B-cell non-Hodgkin lymphoma, was given C1 esterase inhibitor (C1-INH) to enhance the efficacy of the RBC transfusion. This lead to attenuated complement deposition on RBCs and reduction in haemolysis rate. Therefore, the response to RBC transfusion was higher and more sustained over time [44].

2.1.4. Thrombotic microangiopathic haemolytic anaemia

In 1952, the name thrombotic microangiopathic haemolytic anaemia (TMHA) was introduced by Symmers to describe a diverse clinical presentation with localized or diffuse microvascular thrombosis, thrombocytopaenia, microangiopathic haemolytic anaemia, but with a negative Coombs' test. Simultaneously, patients could have fever, neurological symptoms, and kidney involvement. [45]

Its physiopathology is not fully understood, but it might be associated with aPL and TMHA could be a manifestation of APS. In a review, 61% of patients were categorized as having primary APS, and TMHA was the first manifestation of the APS in 93% of them. Thus, aPL might be a part of the development of TMHA in patients either with or without SLE. [46] In another study with 15 patients with TMHA (out of 114 SLE patients), the presence of anti-Sm and anti-RNP antibodies was associated with a higher incidence of TMHA[47].

The differential diagnosis of TMHA in SLE patients is extensive and challenging, as it can be a presentation of malignant hypertension, APS, catastrophic APS (cAPS), TTP or even a SLE flare. TTP and malignant hypertension have consumptive thrombocytopaenia with TMHA, in contrast to the immune-mediated processes seen

in APS and SLE. [48] In a study, 15 out of 114 SLE patients had TMHA and all of whom had a negative DAT result, with mild thrombocytopaenia and anaemia [47].

Patients should be systematically screened for lupus anticoagulant and ACA due to the association between TMHA and APS [46].

Disease activity as assessed by the SLE Disease Activity Index (SLEDAI) score was increased in patients exhibiting features of TMHA.[47]

Choosing the optimal treatment is complicated due to the difficult differential diagnosis and often therapy for all possible diagnoses is given simultaneously [48]. In one study 7 out of 15 patients responded to methylprednisolone alone, 4 responded to methylprednisolone with plasmapheresis, 2 to methylprednisolone and immunosuppressive therapy, and 2 methylprednisolone with both plasmapheresis and immunosuppressive therapy [47].

2.1.5. Pure red cell aplasia or hypoplastic anaemia

Pure red cell aplasia (PRCA) is characterized by normochromic normocytic anaemia and reticulocytopaenia, with aplasia or severe hypoplasia of the red cell line, while leukocyte and megakaryocyte lines in the BM remain normal [49]. In most cases, PRCA is diagnosed concomitantly or shortly after SLE diagnosis [50].

The pathogenesis of PRCA is varied including genetic defects affecting the erythropoietic lineage, viral infections (such as parvovirus B19) and autoimmune-related factors [49].

As previously described in ACD, anti-EPO antibodies can also lead to PRCA by neutralizing erythropoiesis [51]. It has also been shown that sera from SLE patients can suppress granulocytic and erythroid colony formation *in vitro.* The IgG fraction has been isolated from these sera and its action is probably related to binding CD34+ haematopoietic progenitor cells, but not to CD33+ cells [17]. However, in vivo reports are conflicting [52].

The disease responds to prednisone in the majority of cases, but tapering is difficult [50].

Aplastic anaemia is characterized by pancytopaenia with a low reticulocyte count and hypocellular BM. It is rare in SLE [53].

It is thought that antibodies to BM progenitor cells lead to BM aplasia and resultant pancytopaenia. This idea is supported by data showing that the suppression of colony forming units-granulocyte monocyte (CFU-GM) was mediated by the serum or IgG complement-dependent fraction of a SLE patient's serum in the acute phase. In contrast the IgG fraction obtained six weeks after that acute phase showed no CFU-GM suppression[54]. Knowing that cytopaenias may occur on the basis of inhibited myelopoiesis in SLE flares should lead to ANA screening in patients with BM acellularity of unknown cause [55].

Some case reports describe the use of cyclosporine (CSA) (300 mg/day) [53] and CYC in refractory cases with remarkable success[56].

2.1.6. Others

SLE patients can obviously be affected by nutritional deficiency anaemia. Low serum vitamin B12 levels have been reported in SLE patients [57]. Few cases of SLE patients with pernicious anaemia have been described [58-60].

3. White Cell Associated Pathologies

A summary of white cell associated pathologies in SLE patients can be found in table 3.

3.1. Leukopaenia

In the 1997 update of the ACR criteria leukopaenia is defined as levels <4000/mm^3 on at least 2 occasions and lymphopaenia as levels <1500/mm^3 on at least 2 occasions [61]. Similarly, in the SLICC classification criteria, leukopaenia (<4000/mm^3 on at least one occasion) and lymphopaenia (<1000/mm^3 at least once) are also part of the SLE classification criteria [3].

3.1.1. Neutropaenia

Neutropaenia in autoimmune diseases can be either primary or secondary. Antineutrophil antibodies can induce neutropaenia, which may also result from peripheral sequestration, BM inhibition, or apoptosis. The specific target of the autoantibodies is unknown and it is quite common for these cases to develop thrombocytopaenia or haemolytic anaemia simultaneously [62].

In SLE patients, thrombocytopaenia, central nervous system involvement and immunosuppressive medications were identified as independent risk factors for developing neutropaenia and, in some cases, the BM showed signs of myeloid hyperplasia, suggesting that BM failure is not the cause of neutropaenia [63].

Another study linked severe neutropaenia with the presence of IgG on myelocytes and promyelocytes, as well as mature cells. Patients with moderate neutropaenia had IgG only on neutrophils and metamyelocytes whereas in the control group IgG did not deposit on peripheral blood or earlier precursors [64]. There was a significant decrease in the number of granulocytic precursors cells (CFU-C) in SLE patients. In neutropaenic patients the numbers of CFU-C were lower than in non-neutropaenic patients, suggesting that a decrease in BM CFU-C plays a part in neutropaenia in SLE. Simultaneously, T cells from SLE patients inhibited *in vitro* CFU-C colony formation in the BM from healthy individuals [65].

It is also likely that an autoimmune aetiology also contributes directly to neutropaenia in SLE patients. There is an inverse relationship between the neutrophil count and the ability of patients' sera to opsonize granulocytes for recognition and clearance by human monocytes. The level of membrane-binding IgG significantly correlated with opsonic activity while the level of Clq-binding immune complexes was also elevated in some patients and correlated with opsonic activity[66]. Another study revealed that neutrophil count significantly correlates negatively with the C3-binding ability in patients with SLE [67]. These data suggest the possibility of complement activation, driven by antineutrophil antibodies.

In a study of 72 SLE patients, anti-Ro/SSA antibodies were linked to a lower average neutrophil count than those lacking these antibodies. The sera from the former bound neutrophils more than either sera from anti-Ro negative SLE patients or from healthy controls. It was also shown that anti-Ro antibody can fix complement on the surface of neutrophils, but its antigen was a 64-kD protein instead of 60-kD Ro[68].

In SLE patients with neutropaenia, the possibility of concomitant infection and medication side effects, particularly immunosuppressants must be considered [31, 63]. BM aspiration and biopsy may be necessary in acute and severe cases[31].

Treating the underlying condition and close monitoring are sufficient in most patients. Neutropaenia is only treated specifically if it is severe with concomitant infection [30]. In these cases, there are some limited data regarding the possible benefit of human recombinant granulocyte colony stimulating factor (rhG-CSF). There is a case report of severe neutropaenia in SLE patient who was treated with rhG-CSF (100 pg/d, *5* days) together with methylprednisolone pulse therapy, with a good outcome [69]. There is also a small study of 9 SLE patients with refractory infection who received rhG-CSF without corticosteroids. The average PMN count increased in two days, but 3 patients had a haematological flare [70].

3.1.2. Lymphopaenia

Out of 158 SLE patients with active disease studied from the time of diagnosis, lymphopaenia was present in 75%. After disease reactivation, another 18% also developed lymphopaenia[71].

Autoantibodies to lymphocytes seem to be part of the pathogenesis as titers of IgG antibodies to lymphocytes (but not IgM) have been shown to correlate inversely with lymphocyte and complement levels [72]. Another study reported that the sensitivity and specificity of IgG antilymphocyte antibodies in SLE was 42.3% and 96.7%, respectively and the antibodies were independently associated with lymphopaenia [73]. In a study conducted in human T and B cell lines as antigens (P12 (CD4-, CD8+), Jurkat (CD4-, CD8-), Hut78 (CD4+, CD8-) and Wa (B cell), respectively), serum antibody titres were higher than those in normal control subjects and it seems that autoantibodies react with both T and B cells [72]. The titers of anti-Ro/SSA, anti-dsDNA and anti-ribosomal P were also more elevated in the lymphopaenic group. No correlation was found for anti-La/SSB, anti-RNP, anti-Sm or anti-histones [74].

Antibodies against galectin 8 (Gal-8) are highly specific as they do not cross-react with other galectins (multifunctional proteins which mediate cell contact, migration, growth and apoptosis), and they are also associated with lymphopaenia in SLE patients, notably in those with a malar rash. However, these antibodies are also present in rheumatoid arthritis and sepsis [75].

As far as peripheral T cells are concerned, a reduction in T cell numbers relates to the presence of anti-T cell antibodies especially in acutely ill patients. B cells numbers, measured through lymphocytes bearing surface Ig, also frequently correlate with disease activity[76]. In patients with different subtypes of lupus erythematosus, T-

helper lymphocyte cells are more often diminished in patients with more severe presentations [77].

The CD55 and CD59 proteins have complement inhibitory properties. In SLE patients CD55 surface density was higher in CD19+ cells than in CD3+ cells, while CD 59 was higher in CD3+ cells. In SLE patients with lymphopaenia, CD55 and CD59 were less expressed in both CD3+ and CD19+ lymphocytes [74]. The surface expression of Fas antigen is also higher on both naïve and memory CD+ and CD8+ T cells from SLE patients, however only CD4+ cells levels have an inverse correlation with the expression of Fas antigen [78].

CD27+ memory B cells seem to be the most common population in SLE, but in lower numbers than in healthy controls. After immunosuppressive therapy, CD27+ cells remain stable, while naïve CD27- B cells and $CD27^{high}$ plasma cells are diminished [79].

The relationship between disease activity, as measured by SLEDAI and lymphopaenia, has been studied. At the beginning of one study SLEDAI score correlated positively with lymphopaenia (84) and after 1 year, a high SLEDAI score was similarly associated with lymphopaenia. Nonetheless, after 2 years the SLEDAI score only correlated with anti-dsDNA antibodies. Lymphopaenia is, together with other haematological changes, a predictor of flare during the following year [80]. SLEDAI levels were significantly higher in SLE patiens with anti-lymphocytic antibodies [73].

As for neutropaenia, the underlying disease should be managed appropriately. Although controversial [81], prophylactic antibiotic therapy against *Pneumocystis jiroveci* might be considered in more severe cases of lymphopaenia and it seems to have a good safety profile [82].

3.2. Leukocytosis

The major effect of glucocorticoids on neutrophils appears to be the inhibition of neutrophil adhesion to endothelial cells, which leads to neutrophilia [83]. Obviously, the main reason to an increased leukocyte count is infection, which requires swift action in terms of diagnosis and treatment.

4. Platelet associated pathologies

A summary of platelet associated pathologies in SLE patients can be found in table 4.

4.1. Thrombocytopaenia

4.1.1. Introduction

Thrombocytopaenia is a frequent manifestation in SLE patients though it is often mild. Three different mechanisms are linked to thrombocytopaenia: impaired production in the BM, sequestration in the spleen and accelerated destruction [31]. The most common is immune mediated platelet destruction in the peripheral circulation by antiplatelet antibodies [84-86].

4.1.2. SLE immune Thrombocytopaenia

4.1.2.1. Introduction

Immune thrombocytopaenia is an immune-mediated disorder occurring as a primary event (P-ITP) characterized by isolated thrombocytopaenia or associated with other conditions, such as SLE (SLE immune thrombocytopaenia) [87].

SLE immune thrombocytopaenia is a common clinical manifestation, ranging from 7 to 30% [88-91], which is defined as a platelet count <100x10^9/mm^3 without any other identifiable cause [2, 3, 61]. In a large single-centre cohort study 50 out of 632 patients had thrombocytopaenia [92]. Of these 50 patients, 54% had platelet counts of 50 to 100 x10^9/mm^3, 18% had counts between 20 and 50 x10^9/mm^3, and 28% had a platelet count of less than 20x10^9/mm^3.

4.1.2.2. Pathogenesis

Mechanisms implicated in immune SLE thrombocytopaenia are summarized in figure 3.

4.1.2.2.1. Antiplatelet Autontibodies

Antiplatelet autoantibodies bind to platelets, leading to platelet phagocytosis in the spleen. This is often associated with a normal or increased number of megakaryocytes in the BM. These autoantibodies can be found in both P-ITP and SLE immune thrombocytopaenia, but immunological differences have been described between the two conditions [31] and even among SLE patients with immune thrombocytopaenia there are differences in the autoimmunity directed against platelets [93].

Antiplatelet autoantibodies are present up to 60% of SLE patients, the majority being IgG (60%) (23% are IgM) [94, 95]. In P-ITP, the antigens for antiplatelet antibodies are glycoprotein IIb/IIIa (GpIIb/IIIa) and membrane glycoprotein (αIIaβ3integrin), and they can also be seen in SLE patients. In addition, glycoprotein Ia/IIa (GpIa/IIa) and glycoprotein IbIX (GPIbIX) antigens have also been described in SLE immune thrombocytopaenia. The presence of antiplatelet antibodies does not automatically lead to the development of thrombocytopaenia. Other factors for thrombocytopaenia development in seropositive patients are active disease and complement activation [92].

4.1.2.2.2. Other causes of immune-related platelet destruction

<u>The role of antiphospholipid antibodies</u>

SLE patients with thrombocytopaenia are more often positive for lupus anticoagulant and higher levels of IgM ACA have been related to a potential role of aPL in its pathogenesis. López-Soto *et al* related ACA, antiphosphatidic, antiphosphoserine, antiphosphoinositol and lupus anticoagulant antibodies with thrombocytopaenia [96]. Moreover, the presence of ACA has been found to increase the relative risk for thrombocytopaenia four-fold [97]. Nevertheless, the exact pathophysiology of the relationship between the presence of aPL and thrombocytopaenia remains unknown.

<u>The role of antibodies against thrombopoietin and antibodies against thrombopoietin receptor</u>

Although immune thrombocytopaenia is often related to a normal or increased number of megakaryocytes, amegakaryocytic hypoplasia has been described in a few cases [98-100]. This feature has been related to antibodies against thrombopoietin (TPO) and against the TPO receptor (c-mpl receptor). The binding of TPO (the c-mpl ligand) to its receptor is the key regulator of platelet production. SLE patients have higher serum levels of TPO, as well as autoantibodies against TPO and its receptor. Moreover, the presence of autoantibodies against the TPO receptor may be associated with a poorer response to steroids [86, 101, 102]. Despite these findings, the exact role of these antibodies in the pathogenesis of lupus thrombocytopaenia is uncertain.

The role of genetics

A genetic contribution to thrombocytopaenia was suggested in a study of 159 SLE co-affected siblings, in which the risk of thrombocytopaenia was increased when compared with a cohort of 709 non-related SLE patients [103]. A genetic linkage genetic linkage at 1q22 and 11p13 was found and also correlated with a severe SLE form [104, 105]. However, it remains unclear whether these findings are directly related to the mechanism of thrombocytopaenia or a marker of severe SLE.

4.1.2.3. Clinical Implications

The presentation of immune thrombocytopaenia in SLE patients can be divided in three main forms: acute, chronic and prior P-ITP. An overview of these manifestations can be found in table 5. The acute form occurs as part of a generalised exacerbation of SLE. It is usually severe (platelet count $<25x10^9/mm^3$) and may be associated with life-threatening haemorrhage risk. It often responds to steroid treatment and improves when the SLE comes under control. The chronic form is more common and it rarely causes major symptoms. It is not related to disease activity. Although it is less responsive to steroid therapy, this is not a major concern because the platelet count is usually modestly decreased ($75\text{-}125x10^9/mm^3$) and it may not require specific therapy. P-ITP can precede SLE diagnosis in 3% to 16% of patients and may occur up to 10 years before SLE becomes clinically apparent[106].

In most patients, thrombocytopaenia follows a benign course, platelet counts down to $30x10^9/mm^3$ rarely cause more than a prolonged bleeding time. However counts of less than $20x10^9/mm^3$ are usually associated with petechiae, purpura, ecchymosis, epistaxis and other clinical bleeding. Severe thrombocytopaenia is uncommon so major bleeding only occurs in a minority of patients. It can be fatal if it involves the central nervous system or the gastrointestinal tract [87].

The presence of thrombocytopaenia is an important independent risk factor for mortality in SLE. This has been validated for many, though not all European, North ans South American, Hispanic, Chinese and African American patients with SLE [107, 108]. SLE patients with late-onset thrombocytopaenia have a greater risk than those whose thrombocytopaenia occurred early in the course of their disease [94].

SLE patients with thrombocytopaenia are also more likely to have significant organ damage, such as heart, kidney and central nervous system involvement [90, 92, 109-

111]. Thrombocytopaenia has also been correlated with low levels of complement (C3), higher SLE disease activity, haemolytic anaemia and the presence of other cytopaenias [27, 92].

4.1.2.4. Treatment

When treatment is required, the approach is similar to P-ITP. Immune thrombocytopaenia treatment is usually recommended for symptomatic patients with platelet counts $<25x10^9/mm^3$ [112]. Platelet transfusions should be avoided in immune thrombocytopaenia and only considered when the platelet count is less than $10x10^9/mm^3$ or invasive procedures are needed [113].

Conventionally, high-dose glucocorticoids are considered first-line therapy in severe thrombocytopaenia [114, 115]. Hydroxychloroquine (HCQ) or danazol can be added in refractory patients or in an effort to taper glucocorticoids. If there is no significant improvement within three weeks or the side effects are intolerable, other immunosuppressive treatments should be considered [113], such as azathioprine (AZA), CSA, CYC, vincristine or vinblastine, but they have limited success [116]. Splenectomy used to be an alternative therapeutical option for SLE patients with refractory immune thrombocytopaenia [117-119]. However, in addition to concerns about high rate of infection [120] and the long-term outcome [119], the introduction of RTX has rendered this operation almost obsolete. RTX may be used in both SLE immune thrombocytopaenia and P-ITP. It is preferred to splenectomy in SLE patients, because its B lymphocyte depleting approach may be beneficial for other manifestations of SLE [121]. Moreover, a significant decrease of antiplatelet antibodies, especially IgG isotype, occurs after RTX treatment [122].

In emergency situations several therapies are used simultaneously, such as high-dose glucocorticoids and IVIg. IL-11 could be considered if the patient does not response adequately to the first two choices. Plasmapheresis can be effective in patients with refractory thrombocytopaenia and active bleeding [123].

Additional treatments such as other anti-other monoclonal therapies, including at CD40L, targeting co-stimulatory molecules (such as CTLA4-Ig) and immune complexes (such as intravenous anti-D) may be considered though there are few data.

The main treatments available with their targets can be found in figure 4, and a summary of their indications in table 6.

4.1.3. Thrombocytopaenic thrombotic purpura.

4.1.3.1. Introduction

TTP is a thrombotic microangiopathy first described in 1924 by Moskowitz. It is characterized by the pathological findings of platelet thrombi in the microcirculation of several organs. Patients with TTP typically develop microangiopathic haemolytic anaemia, thrombocytopaenia and organ failure of variable severity

Various clinical subtypes of TTP exist, including hereditary forms. TTP can be primary/idiopathic or secondary/acquired, associated with other conditions (autoimmune disorders, malignancies…) [124]. A number of autoimmune disorders have been reported in association with TTP and SLE is the most frequent autoimmune disease associated with TTP [125]. However, TTP is a rare complication in the context of SLE and generally occurs in patients with severe lupus activity and renal involvement [126]. The estimated incidence of TTP in SLE is 2 –3% [127]. However, post-mortem examination of patients suggest an even higher incidence [128].

4.1.3.2. Pathogenesis

TTP can result from a severe deficiency in ADAMTS13 (a disintegrin and metalloprotease with ThromboSpondin type 1 repeats) [129] but additional genetic and/or environmental triggers are thought to be required to trigger acute illness. ADAMTS13 limits platelet aggregation by cleaving Von Willebrand factor (VWF) multimers into less adhesive forms. Its deficiency leads to persistence of ultra-large VWF (ULVWF) multimers, widespread formation of microvascular platelet thrombi, and subsequent microangiopathic haemolytic anaemia and organ ischaemia [130]. Severe ADAMTS13 deficiency in acquired TTP is caused by autoantibodies against ADAMTS13, which either neutralize ADAMTS13 activity or accelerate its clearance [131, 132]. High titres of anti-ADAMTS13 antibodies may be associated with refractory disease in SLE patients [133].

Anti-nuclear (ANA), anti-Ro and anti-dsDNA antibodies may develop many years before the diagnosis of SLE [134]. This association has been also published in a TTP population, where anti-Ro and anti-dsDNA antibodies at the time of TTP diagnosis were significant risk factors for the later development of SLE [125]. This finding could lead to early identification of an associated autoimmune disease allowing early targeted management.

The predisposing role of aPL in TTP is controversial and remains unclear [125, 135].

4.1.3.3. Clinical implications

Prominent clinical features of TPP include severe thrombocytopaenia (<50x10 mm^3), microangiopathic haemolytic anaemia, fever, renal failure and neurological dysfunction. There is considerable overlap between the characteristic features of TTP and some SLE features [136]. Although the diagnosis of SLE usually precedes that of TTP [137], it can develop simultaneously or after the diagnosis of TTP [126, 138-142]. In paediatric populations, there are some data that suggest that primary TTP may evolve to SLE [143].

Prolonged follow-up is necessary after an acute primary TTP, especially when anti-Ro or anti-dsDNA antibodies are present at the time of TTP diagnosis [125].

TTP in SLE is often life-threatening and in spite of earlier and more aggressive therapy in SLE patients, the mortality is higher and the time to complete remission longer than in idiopathic TTP, suggesting a refractory and more severe disease [126, 144].

4.1.3.4. Treatment

Plasmapheresis can successfully remove the autoantibodies and replenish ADAMTS13, thereby restoring functional ADAMTS13 levels. Since its introduction, mortality from TTP has decreased dramatically from 90% to 25% [145]. Hence, it is the first-line treatment in TTP [146, 147]. However, SLE patients seem more refractory to this treatment [126].

In case of a poor response to plasmapheresis in SLE patients with TTP, other treatments to be considered include high-dose glucocorticoid, CYC and IVIg [138, 148].

RTX has been successfully used in refractory TTP in SLE [136, 149-153]. Since TTP can precede SLE onset, it has been hypothesized that if RTX was used for the treatment of TTP this could prevent or delay the appearance of overt clinical SLE. However, the results of a cross-sectional study do not support this hypothesis since acute TTP patients previously treated with RTX did not have a lower incidence of autoimmune disorders than those not given the treatment [125].

4.1.4. Other causes of thrombocytopaenia in SLE patients

Drug therapy used in SLE can induce thrombocytopaenia often by leading to impaired production of platelets in the BM. This is well recognized with drugs such as AZA and CYC and rarely with drugs such as HCQ, MMF and CSA.

Platelet consumption may also occur in association with microangiopathic haemolytic anaemia. Pseudo-thrombocytopaenia, secondary to platelet aggregation or platelet adherence to leukocytes, must always be considered and excluded by examining the peripheral smear [31]. BM examination, especially in older patients, should also be considered to rule out occult myelodysplasia.

4.2. Thrombocytosis.

Platelet count >400x10^9/mm^3 constitutes thrombocytosis. It is much less common than thrombocytopaenia in SLE. It may occur as a result of active disease or reactive to the underlying inflammatory process. Hyposplenism (or functional aesplenia or autosplenectomy) has been reported as a cause of thrombocytosis in SLE patients [154, 155] and may be related with aPL or with associated APS [156, 157].

5. Pancytopaenia.

There are a number of potential causes of pancytopaenia in SLE patients, notably drugs, peripheral destruction, other concomitant diseases, autoimmune myelofibrosis and uncommon causes such as macrophage activation syndrome (MAS).

However, when a SLE patient presents with a reduced number of red cells, leukocytes and platelets, this often suggests haematopoietic failure as the result of immune-mediated BM damage. When performing a BM biopsy in SLE patients with an unexplained pancytopaenia, hypocellularity was described as a predominant finding [158, 159].

5.1. Autoimmune myelofibrosis and SLE.

5.1.1. Introduction

Myelofibrosis is rare and characterized by deposition of reticulin fibres in the BM stroma, which usually occurs in response to clonal proliferation of myeloid stem cells in myeloproliferative disorders [160]. It has been described in association with several haematologic malignancies and metastatic solid malignancies [161, 162]. However, it can be also a manifestation of endocrine or inflammatory conditions. The association between myelofibrosis and autoimmune disorders was first described in 1978 [163]. Twenty years later Paquette *et al* [164] proposed and defined the term autoimmune myelofibrosis. Since then, around 40 cases have been reported in association with SLE [165]. The spleen is often, but not always, enlarged. However, this pathology may be more common, with cases being incorrectly characterized as blood peripheral cytopaenias in patients previously diagnosed with SLE, and cases being misdiagnosed as primary myelofibrosis in patients not previously diagnosed with SLE [165].

5.1.2. Pathogenesis

The aetiology of autoimmune myelofibrosis remains unknown. One hypothesis proposes that circulating autoantibodies and immune complexes of SLE stimulate the megakaryocytes Fc-receptors, resulting in the release of growth factors. Platelet-derived growth factor, found in megakaryocytes and platelets, stimulates fibroblast growth [160]. Consequently, transforming growth factor β and epidermal growth factor promote collagen synthesis [166]. Fibroblast proliferation, increased collagen synthesis or altered collagen turnover lead to an increase in reticulin, which is deposited in BM stroma [167]. It has been suggested that autoantibodies against CD34+ and cytotoxic T cells act as causal agents in BM damage and also perpetuate damage [168].

5.1.3. Clinical implications

Autoimmune myelofibrosis can be diagnosed at the time or after the diagnosis of SLE. Most patients present with either bicytopaenia (anaemia and thrombocytopaenia) or pancytopaenia [165]. It is more frequent in younger patients than primary myelofibrosis (median age 29 years vs 66 years in primary myelofibrosis) [30, 165, 169]. The majority of case reports are among Caucasian or Mexican-American, followed by African-American and Arab patients [30, 165].

BM biopsy in patients with suspected immune SLE myelofibrosis is virtually indistinguishable from primary myelofibrosis [167]. The biopsy of immune SLE myelofibrosis usually shows increased reticulin fibres and fibroblasts whereas significant marrow fibrosis is not often seen. However, the finding of BM reticulin fibrosis in a SLE patient should not immediately prompt the diagnosis of autoimmune myelofibrosis. Mild degrees of reticulin fibrosis can be observed in other pathologies such as immune thrombocytopaenia and may be found in many patients with SLE when routine BM biopsies are performed (132). Some authors conclude that BM biopsy should only be recommended if cytopaenia does not recover after conventional therapy [158].

The prognosis of autoimmune myelofibrosis in SLE patients is generally favourable and usually responds well to first line treatment [165]. However, in patients who eventually do not respond to treatment, transformation to acute leukaemia or other complications may occur.

5.1.4. Treatment

Glucocorticoids are the first line of treatment and often very successful unlike the situation with primary myelofibrosis. Early diagnosis and prompt treatment can lead to an improvement in the cytopaenia and in the BM architecture with a resolution of the pathology. However, well-established myelofibrosis has a poorer response and may be fatal [165].

In primary myelofibrosis, activating mutations in Janus kinase (JAK) have been recognized to play an important part in its pathogenesis [170]. A JAK inhibitor, Ruxolitinib, was shown to be effective in the treatment of primary myelofibrosis [171-173]. However, these mutations have not been found in immune SLE myelofibrosis and Rituxinib has not been used for its treatment as far as we are aware.

IVIg proved to be effective in some patients [165, 174] and there is one case report with combined treatment of glucocorticoids and MMF which reports a good outcome[175].

5.2. Other causes of pancytopaenia

5.2.1. Secondary macrophage activation syndrome in SLE patients.

MAS is characterized by increased proliferation and activation of benign macrophages and T cells, with increased secretion of proinflammatory cytokines[176]. It is classified as primary/familial and secondary/reactive in the context of malignancy, systemic autoimmunity, infection, or drug-hypersensitivity reaction.

Secondary MAS is a rare cause of cytopaenia in SLE [176] but may be life-threatening. Although it is more frequent in paediatric populations, it also can occur in adults where it is usually more severe [177]. Given its complex clinical features, MAS can be missed in SLE patients [176, 178]. Secondary MAS in SLE patients is often present at disease onset. However, it can be triggered by an infection (mostly viral) and, sometimes, both flare and infection coexist in SLE patients [179].

Its pathogenesis remains unclear. It has been proposed that autoantibodies and immune complexes sensitize BM cells to macrophages, which subsequently engage in uncontrolled phagocytosis. Moreover, T cell–derived cytokines (IL-1, IL-6, IFN-γ, TNF-α) enhance the inappropriate activation of macrophages. This cytokine storm might be a result of primary uncontrolled T-cell activation in SLE patients [31].

It is often manifested with pancytopaenia associated to hyperferritinaemia, anti-DNA antibodies, low C-reactive protein (<30 mg/L), a falling erythrocyte sedimentation rate (ESR), hypocomplementaemia and elevated triglycerides. Its clinical characteristics include non-remitting high-fever, weight loss, arthritis, pericarditis,

rash, myocarditis, nephritis, splenomegaly, hepatomegaly and lymphadenopathy [180].

The therapeutic strategies for MAS in SLE are not well established and to treat this syndrome effectively, it is vital to identify the trigger. In SLE patients in which MAS is driven by disease activity in the absence of obvious infection, immunosuppressive therapy is the main treatment, including high-dose glucocorticoids, CYC or CSA [179, 181]. When a flare is present along with a concomitant infection, IVIg in addition to anti-infective agents should be considered, as well as glucocorticoids [182]. If it is triggered by an obvious microbial infection, antibiotics should be initiated and immunosuppressive therapy decreased as much as possible.

6. Evans syndrome.

Evans syndrome is characterised by the presence of autoimmune haemolytic anaemia (AIHA) associated with concomitant or sequential development of autoimmune thrombocytopaenia [183]. Therefore, when a SLE patient is diagnosed with AIHA a carefully monitoring of the platelet count is mandatory. However, secondary Evans syndrome in SLE patients is rare and it often precedes the onset of SLE [184]. High-dose glucocorticoids might be used, but once they are stopped or tapered, relapses are common. In refractory cases, other drugs used in either AIHA or immune SLE thrombocytopaenia have been successfully used, such as danazol [185], romiplostim [186] and RTX [187].

7. Haemostasis alterations

7.1. Thrombosis in SLE patients.

Thrombosis causes substantial morbidity and mortality in SLE patients. In a 10-year prospective cohort study, thrombosis was the second most frequent cause of death [188]. Moreover, SLE patients have an increased risk of thrombosis, even in the absence of aPL which increases the risk even further [94]. Thrombotic complications can be found in > 10% of SLE patients and this risk increases up to 50% in patients with lupus anticoagulant [189, 190]. Therefore a risk-stratified approach to thrombosis risk factors is required in order to prevent their occurrence.

The pathogenesis of thrombosis in SLE is multifactorial and several risk factors have been identified [191]. Three major conditions have been described historically: hypercoagulability, premature atherosclerosis and vasculitis[192]. A hypercoagulable state secondary to aPL is the most common mechanism[193]. Other disease-associated risk factors and predisposing conditions have been reported, e.g. hypertension, glucocorticoid treatment, hyperhomocysteinaemia, decreased protein S concentration and changes in fibrinolysis [194-196].

A summary of the main mechanisms involved in the pathogenesis of thrombosis in SLE patients can be found in figure 5.

7.2. Epidemiology and traditional thrombosis risk factors in SLE.

The age at onset of thrombosis in SLE patients is lower than in the general population and its incidence is increased in the first year after diagnosis. This might

be related to higher SLE activity during this period, higher levels of circulating autoantibodies and immune complexes [197]. Moreover, when focusing on cardiovascular thrombotic events, older age at the time of diagnosis and longer duration of disease also increase risk for thrombosis [198, 199]. This might be explained by the fact that older SLE patients tend to have more accumulated damage and more vascular morbidity.

The role of ethnicity has also been studied. A large prospective study was undertaken to determine the incidence and risk factors for arterial and venous thromboembolic events in different ethnicity populations [191]. In this study, 625 patients who fulfilled the ACR criteria for SLE were enrolled (258 Chinese, 140 African Americans, and 227 Caucasians). The cumulative hazard of arterial events at 60 months after the diagnosis of SLE was lower in the Caucasians compared to the Chinese and African Americans. There was a significantly lower risk of venous thrombosis events in the Chinese group when compared with Caucasians. These differences could not be fully explained by differences in the prevalence of well-known risk factors (including age, smoking, diabetes mellitus and hypertension). Genetic and immunologic factors may also play a role [191].

Smoking is a significant risk factor for vascular events in SLE and has been associated with worse outcomes in SLE patients [198, 200]. Hypertension, diabetes mellitus, and dyslipidaemia are common in SLE patients and these factors have been associated with thrombosis in numerous studies [195, 201]. Given that SLE patients have a greater risk of thrombosis than general population, traditional risk factors should be assessed regularly and treated appropriately.

Glucocorticoids, which are commonly used for SLE treatment, are associated with thrombosis risk and have also been associated with abnormalities in the coagulation cascade when administered in higher doses [202, 203].

7.3. SLE, antiphospholipid antibodies and antiphospholipid syndrome.

aPL are associated with several clinical features notably venous and arterial thrombosis, thrombocytopaenia and pregnancy loss. However, aPL may only be transiently positive. To be considered significant, they should be persistently positive on two occasions, at least 12 weeks apart.

ACA, lupus anticoagulant and anti-ß2-glycoprotein I (ß2GPI) are well-known risk factors for cardiovascular mortality in SLE and are strongly associated with an increased incidence of thrombotic events in these patients [204, 205]. For example, the presence of lupus anticoagulant has been described as a significant risk factor for myocardial infarction [206] and stroke [207]. APS is defined as the presence of at least one clinical criteria (vascular thrombosis or pregnancy morbidity) and one laboratory criteria (lupus anticoagulant, ACA, anti-β2GPI antibodies) [208]. When controlling for other explanatory variables, aPL-positive SLE patients were found to have more than three times the odds of having a thrombotic event compared with those lacking aPL antibodies [204]. Love *et al* found that among SLE patients, 42% of the lupus anticoagulant-positive and 40% of the aPL-positive patients had a history of thrombosis. In contrast, the prevalence of thrombosis in lupus anticoagulant or

ACA negative SLE patients was only 10–18% [190]. Other antibodies to negative-charged phospholipids (such as phosphatidic acid, phosphatidylinositol and phosphatidylserine) have been identified but it remains doubtful whether these are actually predictive of thrombosis in SLE patients [209].

The mechanism by which aPL increase the risk of venous and arterial thrombosis is not well understood and several hypothesis have been proposed e.g. they might contribute to increased thrombosis by various possible haemostatic mechanisms such as: clot formation, inhibition of natural anticoagulant mechanisms and impairment of fibrinolysis [210-216].

Although they are called anti-phospholipid antibodies, the most important antigen identified is the protein co-factor β2GPI [217, 218], aPL can also interact with other phospholipid-binding proteins, particularly with prothrombin [216]. This interaction may disturb endothelial cell function [219]. Moreover, antiprothrombin antibodies have been suggested to possess prothrombinase activity leading to increased fibrin production [217].

aPL react with phospholipid-binding proteins (in particular β2GPI and prothrombin) expressed on cell membranes of different cell types, such as monocytes, endothelial cells and platelets. The complex binding between aPL and the corresponding cell membrane protein leads to cell activation by disturbing the cell membrane and promoting signals to the nucleus. Platelet activation (by the anti-β2GPI complex) increases the synthesis of thromboxane and the expression of platelet-membrane glycoproteins, such as GPIIb/IIIa, which promotes platelet aggregation. Both activated monocytes and endothelial cells upregulate the production of tissue factor and promote endothelial leukocyte adhesion, cytokine secretion and prostaglandin E2 (PGE 2) synthesis [210, 212, 213].

aPL might also induce complement activation, which generates split products that then attract inflammatory cells and initiate thrombosis and tissue injury [220, 221]. Increased complement deposition (C1q, C3d and C4d) on platelets has also been described in SLE, especially in patients with a previous history of venous thrombosis [222, 223]. They also disrupt the fluid phase of coagulation by affecting Protein C cleavage system (see Protein C and S section), by disturbing fibrinolysis (see Fibrinolysis section) and displacing the binding of the natural anticoagulant annexin A5 to anionic structures.

7.4. Impaired fibrinolysis.

Impaired fibrinolysis has been reported in patients with SLE and APS. It contributes to hypercoagulability and an increased risk of thrombosis. Different mechanisms have been described as aetiological agents of impaired fibrinolysis in SLE and APS patients. Some are not related to aPL, such as the increased plasminogen activator inhibitor type 1 (PAI-1) activity that may inhibit tissue plasminogen activator (tPA) activity, while others are related to aPL such β_2GPI antibodies, which interferes with the interaction of β_2GPI with tPA activator and the enhancement of tPA activity [224].

7.5. Proteins S and C.

The protein C system is one of the most important anti-thrombotic pathways mediated by the vessel wall. Activated protein C (APC) inhibits thrombin generation by cleaving procoagulant protein factors Va and VIIIa, in the presence of protein S [225]. Protein S exists in plasma either bound to C4b-binding protein or as a free form - the latter being the necessary cofactor for activating Protein C. In SLE there is a reduction of free protein S, despite normal total protein S levels. This may be due to increased C4b-binding protein or an increased binding affinity between it and protein S [226]. Moreover, aPL may lead to protein C dysfunction by binding to it via its β_2GPI antigens [227].

7.6. Prevention and treatment of thrombosis in SLE patients

7.6.1. Prevention treatment.

The use of low dose aspirin as primary thromboprophylaxis has had conflicting results. Tektonidou *et al* reported that treatment duration with aspirin and HCQ is associated with decreased thrombosis in aPL-positive patients [228]. Another study also reported a lower incidence of thrombosis in APLA-positive patients when given ASA prophylaxis [229]. Nevertheless, the Antiphospholipid Antibody Acetylsalicylic Acid (APLASA) study found that low dose aspirin was not effective in preventing thrombosis in positive aPL patients, when compared to placebo [230]. However, this study excluded high-risk groups and has been criticized for being underpowered to detect a beneficial effect of aspirin. However, low-dose aspirin is still used as primary thromboprophylaxis in patients with SLE and persistently positive aPL antibodies [231].

HCQ reduces SLE-related morbidity and mortality and it may also reduce thrombotic risk [195, 204]. Its antithrombotic potential could be explained by multiple mechanisms including: inhibition of platelet aggregation and adhesion [232], inhibition of arachidonic acid release from stimulated platelets [233], cholesterol-lowering [232], blockade of aPL production [234] and inhibition of the antiphospholipid-induced receptor (GPIIb/IIIa) expression [235]. Moreover, HCQ might decrease thrombus size and time of development in a dose-dependent manner [236]. It should be considered in SLE patients who are also positive for aPL, in addition to aspirin.

7.6.2. Thrombosis treatment

The risk of recurrent thrombosis in positive aPL patients is between 22% and 69% [237-239] and this risk increases in the first year after the discontinuation of anticoagulation treatment [240, 241]. The duration of tfreatment length depends on the presence of aPL, the site of the thrombosis, recurrence and co-existing precipitating factors. Prolonged anticoagulation therapy may be necessary.

Life-long anticoagulation is recommended in APS patients after a thrombotic event. This recommendation applies to patients with persistently positive moderate to high titres of aPL or with lupus anticoagulant and a definite diagnosis of APS. The target international normalized ratio (INR) for APS syndrome patients is 2-3 for venous events [242, 243] and 3-4 for arterial or recurrent venous events [244]. Patients with thrombosis and aPL positivity who do not fulfil the APS criteria should be managed

as the general population. Therefore, patients with venous thromboembolism or stroke and a single positive aPL test should be treated with warfarin at a target INR of 2.0-3.0 and low-dose aspirin[245].

The majority of recurrences occur when INR is sub-therapeutic. Hence INR values should be monitored regularly. Moreover, patients with positive lupus anticoagulant can have a spurious increase in INR result, which leads to underdosing of vitamin K antagonists. If a recurrent event occurred while INR was in therapeutic levels, increasing the target INR to higher than 3.0 should be considered, as well as adding aspirin or switching to low–molecular-weight heparin (LMWH) [241, 246, 247]. The use of direct anticoagulants, namely Rivaroxaban and Apixaban, in patients with APS was successful in some case reports [248] but there were also other unsuccessful reports with Rivaroxaban [249]. A prospective, randomized controlled phase II/III clinical trial of Rivaroxaban in patients with APS (RAPS trial) and previous VTE, with or without systemic lupus erythematosus (SLE) has just been completed. It has sought to determine whether the intensity of anticoagulation achieved with rivaroxaban is not inferior to that of warfarin in these patients [250]. The results indicate that Rivaroxaban is not inferior to warfarin [251]. Recently, other designs of randomised clinical trials with Apixaban (ASTRO-APS) [252] and Rivaroxaban (TRAPS trial) [253] in APS have also been published.

RTX lead to a significant reduction in aPL among SLE patients [254]. Moreover, a high response rate in refractory APS has been described in various case reports and series. The clinical resolution of thrombosis and haematologic manifestations was reported in the majority of these patients and aPL antibodies levels became negative or were significantly reduced [255, 256].

8. Conclusion

Haematological disorders in SLE patients are very diverse and their pathogenesis is still not fully understood. They can be a manifestation of SLE itself, a concomitant disease or secondary to SLE therapies. The clinical presentation ranges from mild to life-threatening. In the mildest presentations, specific targeted treatment is usually unnecessary, but, in the most severe cases, immunosuppressive therapy with multiple drugs might be lifesaving. However, most evidence is based on case-reports or small retrospective studies, with very few randomized controlled trials reported.

The development of biological therapy in the past 15 years has opened new possible ways to treat the most severe cases more effectively but, as in the other immunosuppressive drugs, more randomized prospective studies and longer follow-up periods are necessary to establish their optimal role in treating these manifestations.

TABLES

Red cell alterations in SLE patients

Main causes of anaemia

- **Chronic disease anaemia.**
- **Iron deficiency anaemia.**
  - **Blood loss (GI, menorrhagia).**
  - **Nutritional deficiencies of iron**
- **Autoimmune haemolytic anaemia.**
- **Microangiopathic haemolitic anaemia.**
- **Pure red cell aplasia (hypoplastic anaemia).**
- **Other causes of anaemia:**
  - **Other nutritional deficiencies (vitamin B12, folate).**
  - **Other concomitant immune disorders (pernicious anaemia).**
  - **Iatrogenic: AZA, CYC.**
  - **Anaemia in pancytopaenia related disorders: Myelofibrosis, thrombotic thrombocytopenic purpura…**

Table 1. Red cell alterations in Systemic lupus erythematosus patients.

AZA: azathioprine, CYC: cyclophosphamide, GI: gastrointestinal, SLE: systemic lupus erythematosus.

Treatment of Autoimmune Haemolytic Anaemia			
Treatment	**Indications**	**Comments**	**Ref**
GCs	First line therapy. Initial dose: 1mg/kg/day.	Refractory: • After 3 weeks of treatment **OR** • Prednisone >15 mg/day **OR** • >0.1 mg/kg/day of prednisone equivalent for maintenance.	[31]
AZA	To induce remission. Steroid-sparing agent.	Limited evidence in AIHA.	[31]
MMF			[257]
CSA	To induce remission in refractory cases. Discontinuation may be difficult.	Evidence in refractory AIHA, immune thrombocytopaenia and Evans syndrome.	[258]
CYC	To induce remission in refractory cases.	In a study of high-dose treatment of CYC in AIHA refractory patients: • All became transfusion independent. • 2/3 patients went into complete remission (5 P-AIHA and 1 S-AIHA).	[259]
Splenectomy	Controversial results. Cautious use in DAT-positive AIHA.	Out of 28 patients with AIHA (21 I-AIHA and 7 S-AIHA). • 2 of S-AIHA had a positive response. • No criteria to predict response.	[260]
		30 SLE patients with TTP or AIHA. • No difference between splenectomised and non-splenectomised.	[261]
		30 AIHA patients who underwent splenectomy: • I-AIHA: effective and safe in refractory patients. • S-AIHA: increased response to medical therapy.	[262]
IVIg	Possible adjunctive treatment when significant toxicity to other drugs.	Acute benefit in 1/3 AIHA patients. Related to good response: • Hepatomegaly. • Low pre-treatment Hb (6-7g/dl).	[263]
Danazol	Sporadic use as an addition to first line therapy.	17 patients (10 with warm antibody AIHA, 5 who relapsed after initial response to prednisone and 2 with refractory AIHA): • Better responses. • Unsuccessful in the relapse group.	[264]
Plasma exchange	Use before transfusion of RBC without benefits.	9 patients and total 38 sessions of plasma exchange: • No significant increase in Hb in patients who underwent plasma-exchange before transfusion of RBC.	[265]
RTX	To induce remission in refractory cases.	15 children (9 with AIHA and 6 with Evans syndrome). • 13 patients had >1.5 g/dL increase of Hb and >50% reduction in reticulocyte count.	[266]

		• 2 non-responder patients had AIHA with warm-reactive IgG autoantibodies. • DAT positive in 14 children at pre-treatment but became negative in 6 after 2 months of treatment.	
		Other reports in paediatric population.	[267] [268]
		Reports in adult population.	[269] [270]
		Phase III RCT: • Prednisolone and RTX: response increased gradually over 6 months. • GCs alone: maximum response at 3 months.	[271]

Table 2. Treatment of Autoimmune Haemolytic Anaemia

AIHA: autoimmune haemolytic anaemia, AZA: azathioprine, CSA: Cyclosporine, CYC: Cyclophosphamide, DAT: direct antiglobulin test, GCs: Glucocorticoids, Hb: haemoglobin, I-AIHA: idiopathic autoimmune haemolytic anaemia, IVIg: Intravenous Immunoglobulin, MMF: Mycophenolate mofetil, RBC: red blood cells, RCT: randomized clinical trial, RTX: Rituximab, Ref: references, S-AIHA: Secondary autoimmune haemolytic anaemia, TTP: Thrombotic thrombocytopaenic purpura.

White cell alterations in SLE patients

Main causes of leukopaenia

- **Neutropaenia.**
  - **Immune mediated.**
  - **Infections.**
  - **Iatrogenic (AZA, CYC).**
- **Lymphopaenia.**
  - **Immune mediated.**
  - **Infection (viral).**
  - **Iatrogenic.**

Main causes of leukocytosis

- **Infections.**
- **Disease activity.**
- **Iatrogenic: use of GC.**

Table 3. White cell alterations in Systemic lupus erythematosus patients.

AZA: azathioprine, CYC: cyclophosphamide, GC: glucocorticoids, GI: gastrointestinal, SLE: systemic lupus erythematosus.

Platelet alterations in SLE patients

Main causes of thrombocytopaenia

- **Immune mediated thrombocytopaenia.**
- **Infections.**
- **Iatrogenic (AZA, CYC).**
- **Antiphospholipid syndrome.**
- **Anaemia in bi/pancytopaenia related disorders: Thrombotic thrombocytopenic purpura, myelofibrosis...**
- **Pseudothrombocytopaenia.**

Main causes of thrombocytosis

- **Infections.**
- **Disease activity.**
- **Hyposplenism**

Table 4. Platelet alterations in Systemic lupus erythematosus patients.

AZA: azathioprine, CYC: cyclophosphamide, GI: gastrointestinal, SLE: systemic lupus erythematosus.

SLE IMMUNE TRHOMBOCYTOPAENIA PRESENTATION

Prior P-ITP	ACUTE PRESENTATION	CHRONIC PRESENTATION
• 3-16% patients. • Up to 10 years before SLE debut. • Usually mild disease.	• Platelet count may be < 30 $x10^9/mm^3$ • SLE exacerbation. • Life-threating haemorrhage. • GCs: good response.	• Platelet count usually < $100x10^9/mm^3$ • SLE well-controlled. • No/minor symptoms. • GCs: bad response.

Table 5. Systemic lupus erythematosus immune thrombocytopaenia presentation.

GCs: glucocorticoids, P-ITP: primary immune thrombocytopaenia, SLE: systemic lupus erythematosus.

Treatment of Immune Thrombocytopaenia			
Treatment	**Indications**	**Comments**	**Ref**
GCs	First line therapy.	• Response within 1-8 weeks. • High rate of long-term failure. <u>Oral high-dose dexamethasone</u> (40mg/day for 4 days x 4-8 cycles with intervals of 2-4 weeks). <u>Prednisone</u> (tapering from 0,5-1mg/kg/day) can be used, but dexamethasone has similar remission rates and better long-term responses. <u>Pulse methylprednisolone</u> has an increased risk of serious adverse effects (mainly avascular necrosis) without added benefits when compared to high-dose oral GCs.	[114] [115] [116]
Danazol	In combination with GCs when refractory to first line therapy. Steroid-sparing agent.	Variable dosing between studies.	[116] [272] [273]
		16 patients: • Initially treated with 200 mg/day and then increased by 200 mg every four weeks until response. • All patients had a good response within two months. • In an average follow-up of 18.2 months, danazol was tapered down to 200/400 mg/day without recurrence.	[185]
		6 patients refractory to GCs were successfully treated with: • High initial dose (800 mg/day for 8 weeks). • Lower maintenance dose (ranging from 200 to 600 mg/day). Danazol could not be discontinued without recurrence.	[274]
		• Danazol is safe and well tolerated. • It may be used during pregnancy.	[275]

HCQ		• Increased rate of sustained response.	[116]
CYC	Refractory immune thrombocytopaenia.	10–15 mg/kg intravenous CYC, monthly for at least 4 months: • Platelet count increases within 2-18 weeks. • High rates of sustained response. • Maintenance therapy rarely needed	[276]
		Concerns about adverse effects led to the proposal of new regimen: • Induction dose 500 mg, biweekly for 3 months. • Followed by MMF or AZA, improving tolerability without loss of efficacy.	[277]
MMF	Refractory immune thrombocytopaenia. Steroid-sparing agent.	Case report of its use as maintenance therapy [277].	[278] [279] [280]
AZA	Steroid-sparing agent.	Case report of its use as maintenance therapy [277].	[281] [282]
CSA	Refractory immune thrombocytopaenia. Steroid-sparing agent.	• Risk of nephrotoxicity • Lower doses have been successfully used.	[283] [284] [285]
Vincristine		• Severe side effects: neuropathy and bone pain.	[286]
IVIg	Preferred if rapid rise in platelet count is necessary due to: • Active bleeding. • Emergent surgery.	Initial dose: 400 mg/kg daily for 5 consecutive days. Maintenance: 400 mg/kg monthly in an intermittent or continuous manner. Not enough evidence analysing long-lasting response.	[287] [288] [289] [290] [291] [292]
Splenectomy	Refractory immune thrombocytopaenia.	Good short term response in SLE patients.	[117, 293] [118] [294]
		Controversial results in long-term response when compared with P-ITP. Lack of follow-up in studies	
		Less durable response.	[119]

		In a cohort 17 splenectomised patients sustained response was found in 65%, with a mean of 65 months of follow-up (ranging from 3 to 209 months).	[116]
		Controversial results in infection risk	
		Increased risk of infection due to: • *Streptococcus pneumoniae*. • *Neisseria meningitides*. • *Haemophilus influenzae* Vaccination with anti-pneumococcal, Haemophilus influenza type B, meningitis C and Influenza. Prophylactic antibiotics, particularly in concomitant chronic hypocomplementaemia.	[120] [261]
		9 SLE splenectomised patients without increased risk of infection (mean follow-up of 93 months).	[295]
RTX	Refractory immune thrombocytopaenia.	Significantly decreases antiplatelet antibodies, especially IgG isotype. Preferred to splenectomy because it is beneficial for other manifestations of SLE. Case report evidence of its success.	[122] [296] [297] [298] [299] [300] [301]
IL-11	Life-threatening refractory immune thrombocytopaenia	Case report of a patient with intrabronchial haemorrhage refractory to IVIg, high-dose GCS, CYC and plasma exchange. Response to IL-11 over a 5-day period, achieving a platelet count of $50x10^9/mm^3$	[302]
Eltrombopag	Refractory immune thrombocytopaenia.	Thrombopoeitin receptor agonists.	[303]
Romiplostim	Steroid-sparing agent.	Increase platelet production.	[304]

Table 6. Treatment of Immune Thrombocytopaenia.

AZA: azathioprine, CSA: Cyclosporine, CYC: Cyclophosphamide, GCs: Glucocorticoids, HCQ: Hydroxychloroquine, IVIg: Intravenous immunoglobulin, SLE: Systemic lupus erythematosus.

FIGURES

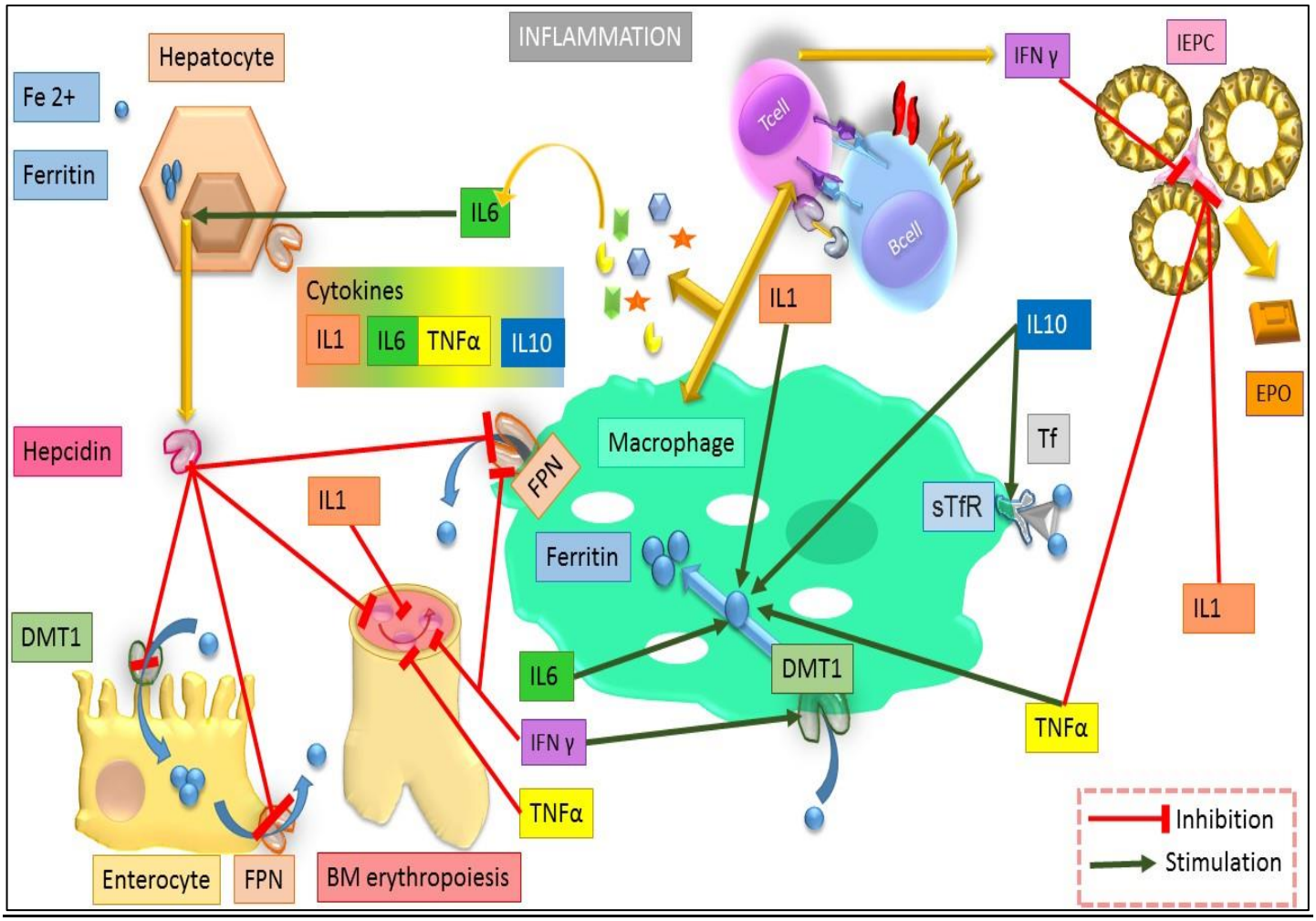

Figure 1 Chronic disease anaemia pathogenesis.

Righ corner: Hepcidin role in chronic disease anaemia.Left corner: Cytokin role in chronic disease anaemia. Red lines indicate inhibition and green lines indicate stimulation.

BM: bone marrow, DMT1: divalent metal transporter 1, EPO: erythropoietin, Fe 2+: iron, FPN: ferroportin, IEPC: intersticial epo producting cells, IFN γ: interferon γ, IL1: interleukin 1, IL6: interleukin 6, IL10: interleukin 10, Tf: transferrin, TNF α: tumour necrosis factor α, sTfR: soluble transferrin receptor.

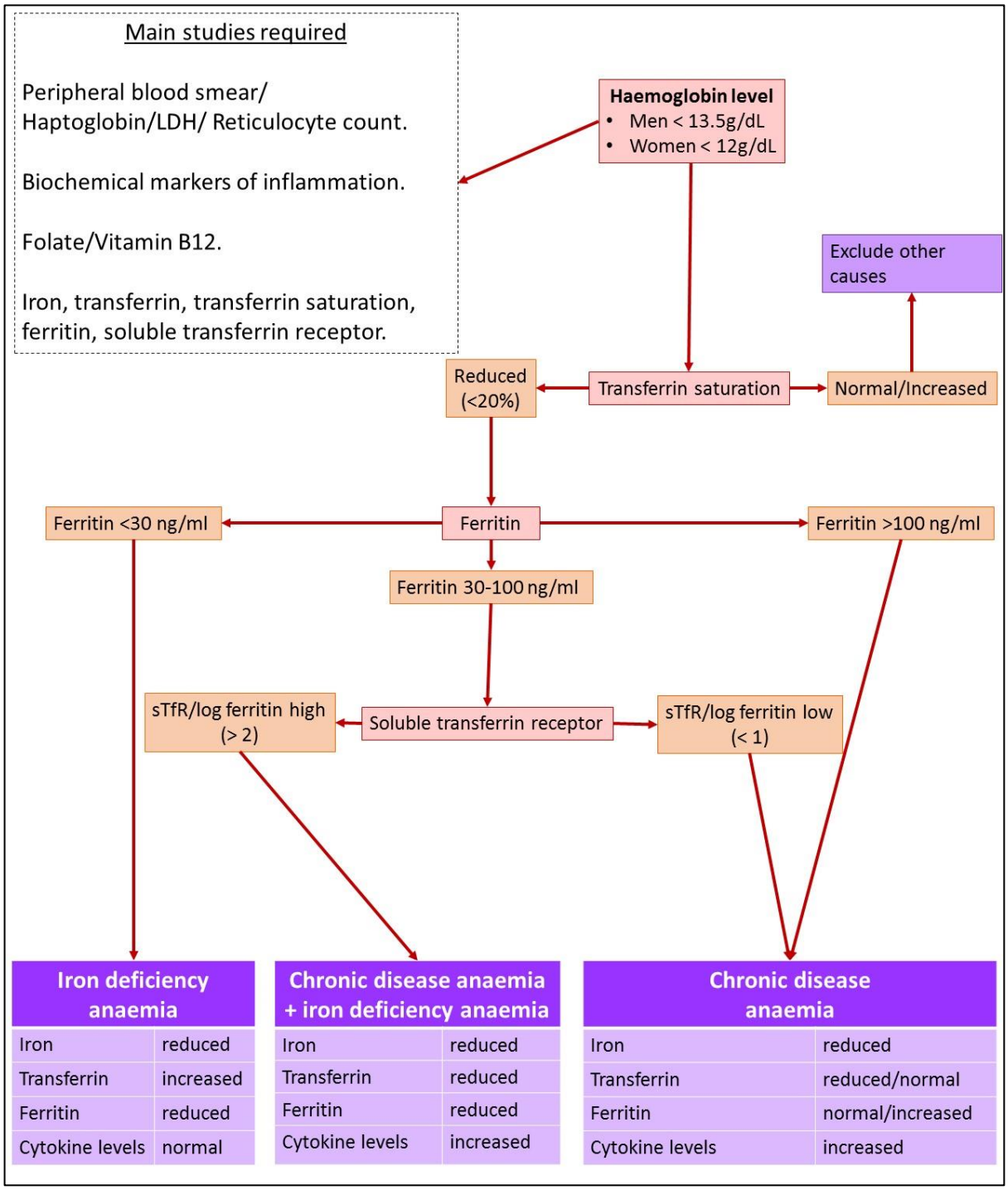

Figure 2. Differential diagnose of systemic lupus erythematosus anaemias: iron deficiency anaemia and chronic disease anaemia.

sTfR: soluble transferrin receptor.

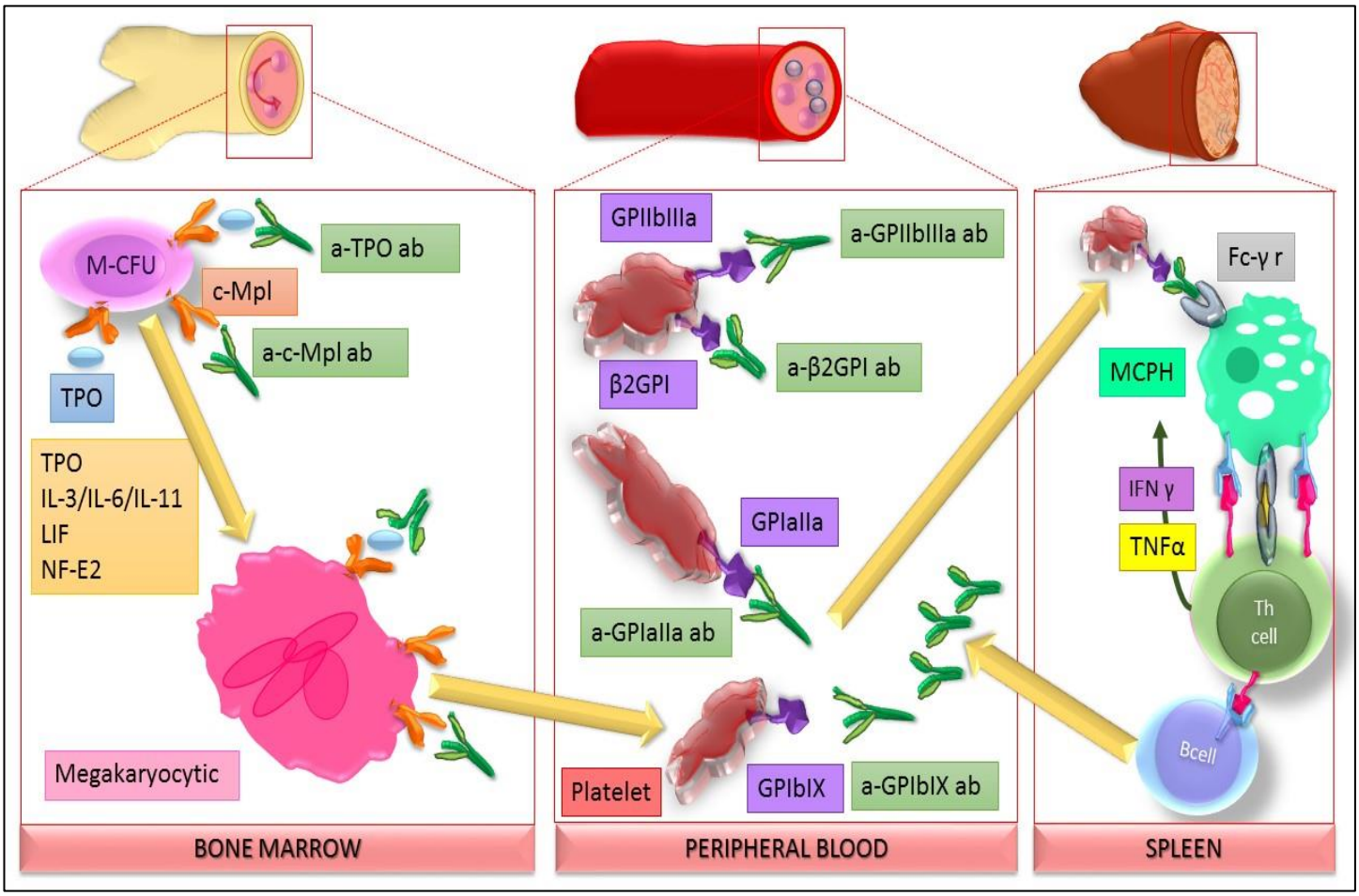

Figure 3. Systemic lupus erythematosus immune thrombocytopaenia pathogenesis. A summary of autoantibodies interactions in bone marrow, peripheral blood and spleen.

a- β2GPI ab: anti β2 glycoprotein I antibody, a-c-Mpl ab: anti thrombopoietin receptor antibody, a- GPIaIIa ab: anti glycoprotein IaIIa antibody, a-GPIbIX ab: anti glycoprotein IbIX antibody, a-GPIIbIIIa ab: anti glycoprotein IIbIIIa antibody, a-TPO ab: anti thrombopoietin antibody, β2GPI: β2 glycoprotein I, c-Mpl: thrombopoietin receptor, Fc-γ r: fragment, crystallisable γ receptor, GPIaIIa: glycoprotein IaIIa, GPIbIX: glycoprotein IbIX, GPIIbIIIa: glycoprotein IIbIIIa, IFN γ: interferon γ, IL-3: interleukin 3, IL-6: interleukin 6, IL-11: interleukin 11, LIF: leukemia inhibitory factor, M-CFU: Megakaryocytic colony forming units, MCPH: Macrophage, NF-E2: transcription factor NF-E2 45 kDa subunit, TNF α: tumour necrosis factor α, TPO: thrombopoietin.

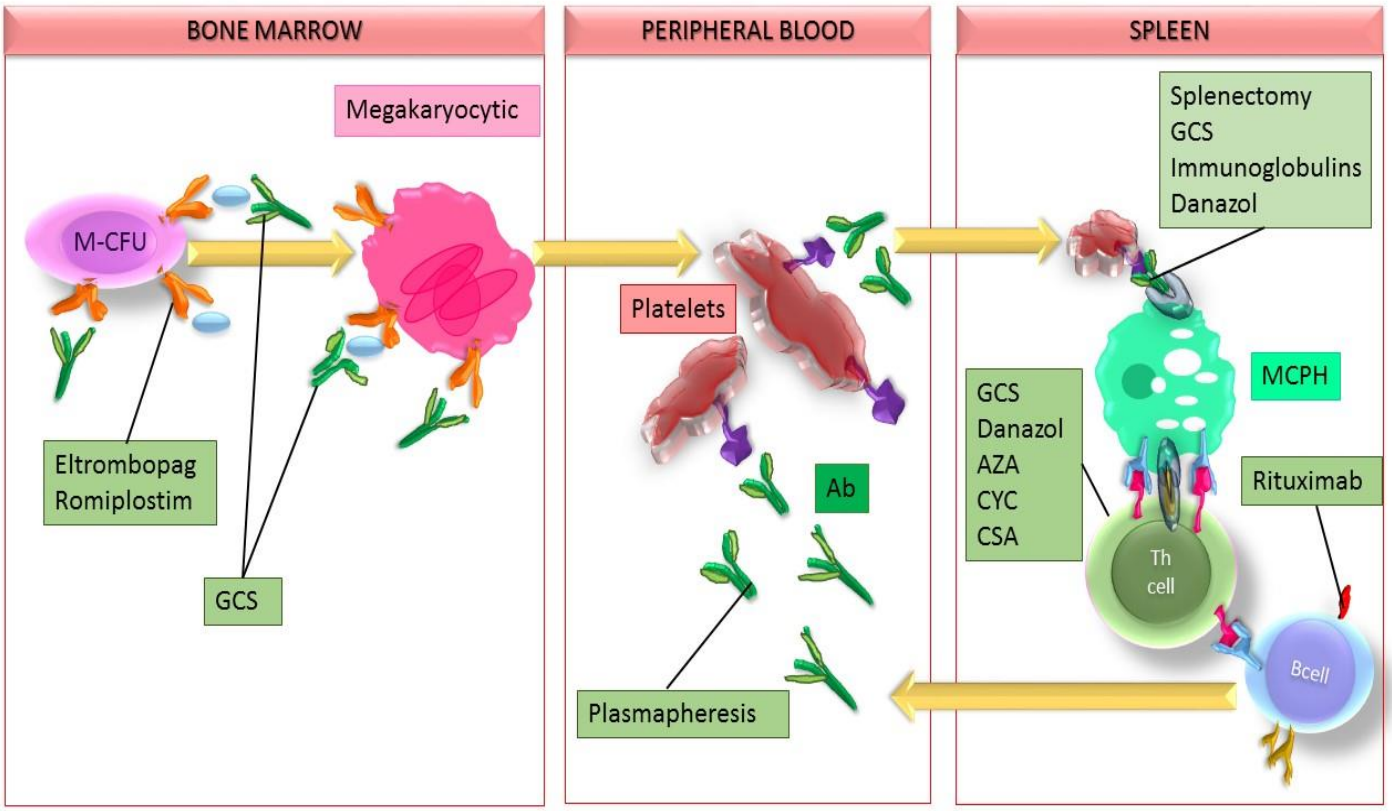

Figure 4. Systemic lupus erythematosus immune thrombocytopaenia: treatments and targets.

Ab: autoantibodies, AZA: azathioprine, CSA: cyclosporine, CYC: cyclosphosphamide, GCS: glucocorticoids, M-CFU: Megakaryocytic colony forming units, MCPH: Macrophage.

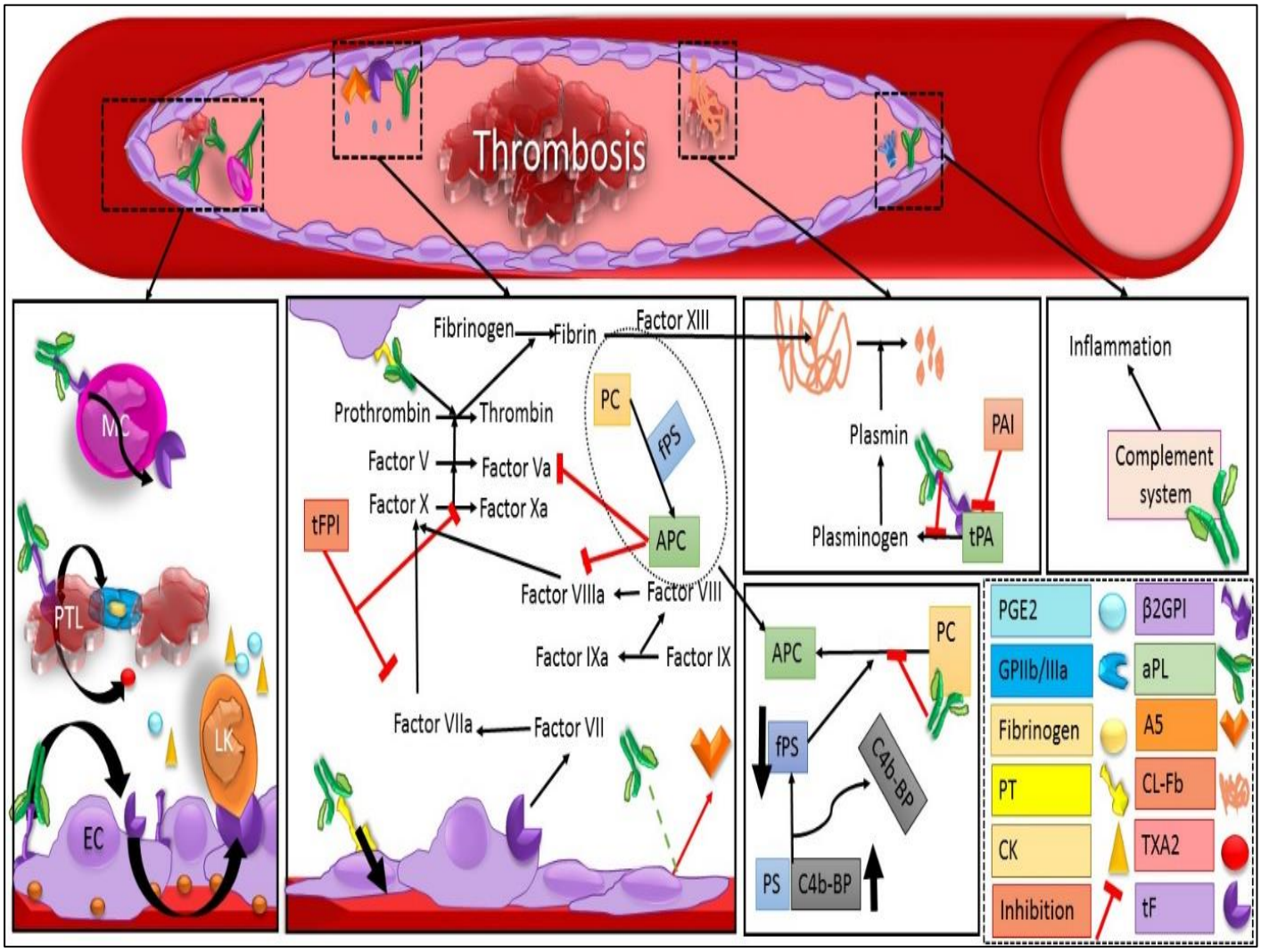

Figure 5. Main mechanisms in the pathogenesis of thrombosis in systemic lupus erythematosus patients.

1. Interaction of antiphospholipid antibodies with cell membranes of monocytes, endothelial cells and platelets.
2. Alterations in the coagulation cascade.
3. Impairment of fibrinolysis and interaction with protein S and protein C.
4. Complement system role.

A5: annexin A5, APC: activated protein C, aPL: antiphospholipid antibody, β2GPI: β2 glycoprotein I, C4b-BP: C4b-binding protein, CK: cytokines , CL-Fb: cross linked fibrin, EC: endothelial cell, GPIIb/IIIa: glycoprotein IIb/IIIa, LK: leukocyte, MC: monocyte, PAI: plasminogen activator inhibitor type 1 PC: protein C, PGE2: prostaglandin E2, PS: protein S, PT: prothrombin antigen, tF: tissue factor, tFPI: tissue factor pathway inhibitor, tPA: tissue plasminogen activator, TXA2: Thromboxane A2.

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