CRITICAL REVIEW

Autoimmune cytopenias in chronic lymphocytic leukemia

Carlo Visco,[1]* Wilma Barcellini,[2] Francesco Maura,[2,3] Antonino Neri,[2,3] Agostino Cortelezzi,[2,3] and Francesco Rodeghiero[1]

Chronic lymphocytic leukemia (CLL) is frequently complicated by secondary autoimmune cytopenias (AIC) represented by autoimmune hemolytic anemia (AIHA), immune thrombocytopenia (ITP), pure red cell aplasia, and autoimmune granulocytopenia. The distinction of immune cytopenias from cytopenias due to bone marrow infiltration, usually associated with a worse outcome and often requiring a different treatment, is mandatory. AIHA and ITP are more frequently found in patients with unfavorable biological risk factors for CLL. AIC secondary to CLL respond less favorably to standard treatments than their primary forms, and treating the underlying CLL with chemotherapy or monoclonal antibodies may ultimately be necessary.
Am. J. Hematol. 89:1055–1062, 2014.

Introduction

The clinical course of chronic lymphocytic leukemia (CLL) is frequently complicated by autoimmune cytopenias (AIC), which can affect a significant proportion of patients [1]. AIC in CLL include autoimmune hemolytic anemia (AIHA), immune thrombocytopenia (ITP), pure red cell aplasia (PRCA) and autoimmune granulocytopenia (AIG) [2–7]. The most frequent forms are AIHA (7–10%) and ITP (1–5%). PRCA is much rarer (<1%) [6,8] followed by AIG, which has been reported in only 3 out of 1750 patients (0.17%) from a single institution [8].

CLL is generally characterized by profound immunosuppression and, as a consequence, patients are subject to a high rate of infections whose severity may be increased by concomitant AIC [4,9–12]. AIC may occur at any time during the course of the disease but in a fraction of cases AIC may precede or be concomitant to CLL diagnosis. It is important to distinguish cytopenias related to non-immune causes (i.e. bone marrow infiltration) from AIC secondary to the disease, since these latter forms have different prognosis and treatment.

Pathogenesis of Secondary AIC

Secondary AIC associated to CLL are usually mediated by IgG auto-antibodies that coat red blood cells, granulocytes or platelets, thus promoting their accelerated clearance by the spleen and the liver. Auto-antibodies or self-reacting T-cells can also impair blood cells production by interfering with erythroblasts or megakaryocytes maturation in the bone marrow as in PRCA and ITP. Moreover, in vitro studies have shown that natural killer (NK) cells are able to directly lyse erythroblasts. About 90% of AIC in CLL are due to polyclonal high-affinity immunoglobulin G (IgG) antibodies produced by non-malignant B-cell clones directed against red blood cell (RBC) or platelet membrane antigens [13]. Malignant CLL clone may also produce autoimmune antibodies, usually of the immunoglobulin M class (IgM), in less than 10% of AIC [14–16]. In addition to autoreactive antibodies, several other immune mediated mechanisms have been described that may interfere with blood cells production, such as direct inhibition of erythro- or megakaryo-cytopoiesis by cytotoxic T-cells and NK-cells, also secreting inhibitory cytokines. Dysregulation of the microenvironment induced by soluble factors secreted by malignant cells and consequent T-cell dysfunction are variably involved [2,4].

Historically, CLL has been viewed as a tumor caused by the accumulation of long-lived resting lymphocytes carrying intrinsic apoptotic defects. However, in recent years, this model has been challenged, after many experiments have shown that CLL contains a sizeable fraction of actively proliferating cells [4,17]. These cells are released in the peripheral blood after recirculating through the microenvironment of the proliferative centers of the lymph-nodes where they are "recharged" and endowed with increased migratory potential before becoming quiescent again [18,19]. It is emerging that the capability of a CLL cell to participate in the proliferation centers is strongly influenced by the characteristics of its B receptor (BCR) and by its interaction with (auto)antigens, aspects that are strongly influenced by the mutational status of the immunoglobulin heavy-chain variable (IGHV) gene [9,17,19,20]. The IGHV mutational status defines two disease subgroups: the first characterized by no or very few somatic mutation of the IGHV gene (U-CLL); the second displaying several somatic mutations (M-CLL) [21,22]. While M-CLL and U-CLL share a largely similar gene expression profile (GEP), differences in clinical behavior exist and are relevant [23]. In fact, U-CLL predicts for a worse outcome and a higher prevalence of AIC [24–29]. Unlike M-CLL, whose BCR is quiescent and unable to transmit external signals to the cell nucleus, U-CLL B-cells are responsive to external stimuli by binding (auto)antigens, thus promoting their survival and proliferation [20]. U-CLL B-cells have an increased ability to phosphorylate p72 (SYK) and ZAP-70 molecules in response to surface IgM ligation by an antigen, providing evidence for an intact downstream signaling pathway of their BCR [30]. This translates in a more aggressive clinical behavior of U-CLL, which might be induced

[1]Department of Cell Therapy and Hematology, Ospedale San Bortolo, Vicenza; [2]Fondazione IRCCS Ca' Granda Ospedale Maggiore Policlinico, Milan; [3]Department of Clinical Sciences and Community Health, University of Milan, Milan

Conflict of interest: The authors declare no potential conflicts of interest.
***Correspondence to:** Carlo Visco, Department of Cell Therapy and Hematology, San Bortolo Hospital, Vicenza, Italy. E-mail: carlovisco@hotmail.com
Contract grant sponsor: AViLL/AIL (Associazione Vicentina per le Leucemie, i Linfomi e il Mieloma/Associazione Italiana Leucemie) (Vicenza, Italy), Hematology Project Foundation (HPF, Fondazione Progetto Ematologia, Vicenza, Italy), Assistenza Studio Malati Ematologici (ASME-Milano), and Associazione Italiana Leucemie Milano (to A.C.).
Contract grant sponsor: Associazione Italiana Ricerca sul Cancro (AIRC); Contract grant number: IG4569 (to A.N.).
Received for publication: 9 May 2014; **Revised:** 28 May 2014; **Accepted:** 6 June 2014
Am. J. Hematol. 89:1055–1062, 2014.
Published online: 10 June 2014 in Wiley Online Library (wileyonlinelibrary.com).
DOI: 10.1002/ajh.23785

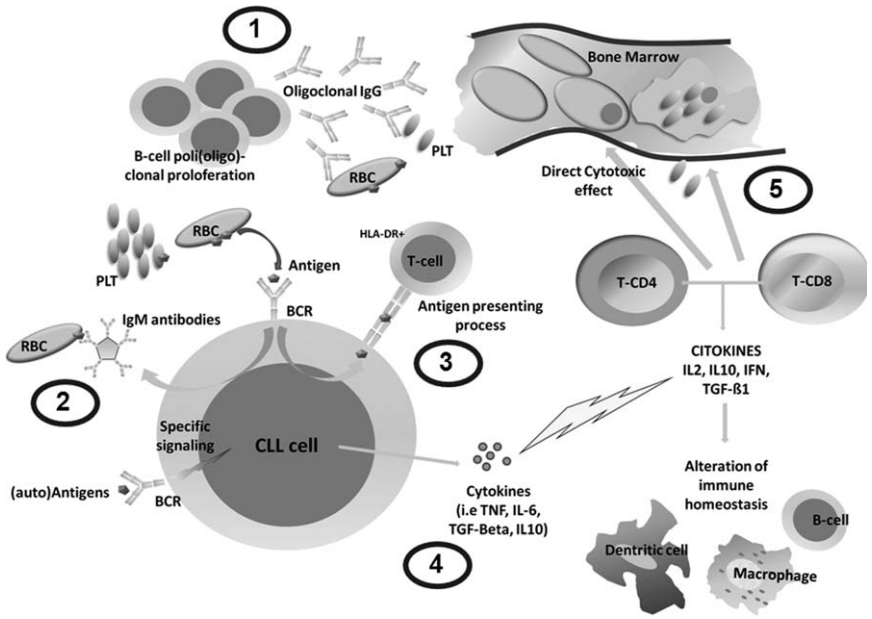

Figure 1. Secondary AIC associated to CLL are usually mediated by IgG auto-antibodies produced by non-malignant B-cell clones (1), that promote accelerated clearance of blood cells in the spleen and liver. Malignant CLL clone may also produce autoimmune antibodies, usually of the immunoglobulin M class (IgM), in less than 10% of AIC (2). CLL cells can act as antigen-presenting cells, favoring the emergence of autoreactive T helper cells and non-functional T regulatory cells (T-regs), through CD27-CD70 interaction (3). T-regs are usually increased in number but result functionally impaired. CLL cells may behave as immunoregulatory B-cells, by producing immunosuppressive cytokines, including TGF-beta and IL10, when stimulated by external signals (4). Autoreactive T/NK cytotoxic cells, on their own, may interfere with megakaryocyte maturation and erythroid precursors (as in PRCA), by releasing inhibitory cytokines or with a direct cytotoxic effect (5).

by the frequent (auto)antigenic interactions with their BCR, thus activating otherwise quiescent CLL B-cells. Similarly, the preferential occurrence of ITP and AIHA, as well as of isolated positive direct anti-globulin test (DAT) in U-CLL, may also reflect propensity of this clone to interact with other cells of the immune system and with the microenvironment [24,31].

The role of antigen stimulation on BCR configuration and activity is further highlighted by the evidence that more than 20% of CLL patients exhibit closely homologous "stereotyped" heavy chain complementary-determining region 3 (HCDR3) sequences and approximately 1% of these also carry virtually identical IGHV amino-acid sequences [32–36]. These findings suggest that clones sharing stereotyped BCRs may have expanded through stimulations by a restricted set of epitopes, which may play an important role in CLL pathogenesis [17]. Importantly, an association has been reported between specific stereotyped BCR configurations and occurrence of ITP and AIHA, further stressing the potential role of (auto)antigen stimulation in the pathogenesis of AIC [25,29]. In particular it has been shown that the risk of developing ITP was higher among patients with stereotyped subset #1 (IGHV1–5-7/IGHD6–19/IGHJ4) and #7 (IGHV1–69 or IGHV3–30/IGHD3-3/IGHJ6); instead, a higher risk of developing AIHA was associated with subset #3 (IGHV1–69 and IGHV4–30/IGHD2-2/IGHJ6).

Beside the role of BCR on CLL clone, a series of additional cellular events are likely to be implicated in the induction of AIC. It is hypothesized that CLL cells themselves may act as antigen-presenting cells (APC), inducing the formation of auto-reactive T helper cells. This would occur through the production of B-cell activator factor and a proliferation-inducing ligand, which is facilitated by the presence of nonfunctional T regulatory cells (T-regs), which impairs immune surveillance [37,38]. The impaired function of T-regs, that in normal conditions have a key role in preventing autoimmune phenomena, is typical of patients with CLL and is even worsened by the use of cytotoxic drugs [39–42]. Although several authors reported that T-regs are increased in CLL patients few data are available describing T-regs functions in patients with CLL in the course of autoimmune phenomena [43,44]. The interaction between neoplastic B-cells and T-regs through CD27-CD70 linking apparently lowers T-regs susceptibility to apoptosis and confers functional impairment [45]. The functional impairment of T-regs may prevent the elimination of non-neoplastic autoreactive T- and B-cells, as witnessed by reports indicating that T-regs have the potential to treat immune-mediated disease [46,47].

More recently, Toll-like receptors (TLR), which represent major agents of innate immunity and play an important role in the activation of normal B-lymphocytes, were found to be abnormally expressed in CLL, with a reduction of TLR2 and TLR4 and an increase of TLR7, TLR9, and TLR10 [48,49]. Patients with reduced TLR4 had an increased risk of development of autoimmune complications, suggesting that impaired expression of these molecules may be associated with reduced ability to silence autoreactive phenomena [50].

Historically, the association between fludarabine used as a single agent and AIHA onset has been a major concern, so that treatment guidelines [51–54] considered treatment with fludarabine contraindicated in patients with previous episodes of AIC. This paradigm has changed after fludarabine has been routinely used in association with other drugs (i.e. cyclophosphamide) and monoclonal antibodies (i.e. rituximab), which strongly lowered the risk of secondary AIC [55]. However, cytotoxic treatments may still complicate the homeostasis of the immune system, as frequently reported for cladribine, pentostatin or alemtuzumab [56–58]. Recently it was reported that alemtuzumab treatment may facilitate ITP development outside CLL [59].

Figure 1 summarizes the main pathogenetic mechanisms involved in AIC development.

Diagnostic Criteria for Secondary AIC

AIHA

All common causes of anemia should be taken into account in the differential diagnosis (i.e. iron or vitamins deficiencies, occult

 doi:10.1002/ajh.23785

bleeding, chronic inflammatory conditions, sepsis etc) particularly in patients with an indolent course of the disease. In patients with more advanced or active disease, bone marrow failure, hypersplenism and recent chemotherapy should be excluded.

Once alternative causes of anemia have been ruled out, the diagnosis of AIHA is confirmed in the presence of all the following criteria [27,60].

1. Hb levels lower than or equal to 110 g/L, in the absence of any cytotoxic treatment in the preceding month;
2. One or more laboratory signs of hemolysis (increased unconjugated bilirubin, elevated lactic dehydrogenase, reduced haptoglobin);
3. Either reticulocytosis or positive direct antiglobulin test (DAT).

Laboratory signs of hemolysis may be misleadingly altered in CLL. This makes the diagnosis of AIHA sometimes difficult, especially in cases with negative DAT. Alteration of LDH levels may be related to disease progression, haptoglobin may be influenced by underlying inflammation, while increased bilirubin levels may result from recent administration of cytotoxic therapy. Reticulocytosis may be absent when bone marrow function is conditioned by infiltration of leukemic cells, or inhibited by chemotherapy.

While DAT represents the most important and useful diagnostic tool, its negativity does not necessarily exclude an antibody mediated pathogenesis of the anemia. In fact DAT negative AIHA have been documented in CLL [61], as in 1 to 10% of patients with primary AIHA [58]. Despite the nearly equivalent clinical characteristics between DAT positive and negative AIHA [62], it was suggested an hypothetic difference in cytokine pattern [63] and/or IgG subclass [64]. DAT negative AIHA may be due to the presence of autoantibodies of IgA class or of low-affinity, or to reduced amount of RBC-bound IgG molecules below the threshold of the test (around 400 molecules per erythrocyte). In these cases, the use of mono-specific antisera against IgA, low ionic strength solutions or cold washings, or more sensitive tests (microcolumn and solid-phase) can be indicated. More sophisticated techniques (enzyme-linked and radiolabeled tests, flow-cytometry and mitogen-stimulated DAT), that are not routine in the majority of laboratories, can detect up to 30 to 40 molecules of anti-RBC autoantibodies. In particular, the latter have been proposed as functional methods able to detect anti-RBC antibodies in about 1/3 of secondary AIHA associated to CLL [65].

The distinction between "warm" and "cold" AIHA is of great importance in terms of diagnosis, treatment and prognosis. Cold forms are rarer and are generally classified as cold hemoagglutinin disease (CHD). CHD is due to IgM, which are able to fix complement (I/i system) more efficiently than other isotypes, display an optimal temperature of reaction at 4°C, and prevalently cause intravascular hemolysis. Warm AIHA are instead generally due to IgG directed against epitopes of the Rh system, which react at 37°C, and determine extravascular hemolysis. The hemolytic process occurring in vivo in the intravascular compart, mainly due to IgM autoantibodies, is estimated to destroy ten times more red cells compared to the IgG-mediated extravascular hemolysis that takes place in the spleen [61].

Rare cases of AIHA caused by IgM "warm" autoantibodies exist. They present with severe hemolysis and are clinically more insidious compared to patients with other types of AIHA. A correct diagnosis is often difficult because it requires serologic work-up that should include the dual DAT to detect both IgM and IgG antibodies [66]. Finally, it is worth reminding that DAT positivity in the course of CLL may be due not only to autoantibodies, but also to alloantibodies, especially in patients with history of red cells transfusions and in multiparous females. Such antibodies are sometimes responsible for severe hemolytic reactions following transfusion [61].

In such a complex scenario, bone marrow evaluation is strongly suggested when diagnosis remains questionable in order to exclude neoplastic infiltration or development of myelodisplastic syndrome/secondary acute leukemia, especially in older patients with a long history of CLL. In addition, peripheral blood smear examination represents always an easy and fast diagnostic tool that adds important information on cell morphology, including the presence of schistocytes, that should be absent in AIHA.

ITP

The lack of a sufficiently sensitive and specific auto-antibody test, able to parallel the performance of DAT for red blood cells, represents a major limitation in the interpretation of thrombocytopenia developing in the course of CLL. The following criteria have been proposed [27,67,68] in order to make a diagnosis of secondary ITP associated to CLL, recently renamed secondary ITP (CLL-associated) according to the new standard terminology [69].

1. Fall (at least half of the initial level and below 100×10^9/L) of the platelet count that is otherwise unexplained (e.g. not drug-induced);
2. Normal or augmented number of megakaryocytes in the bone marrow aspirate or biopsy;
3. No or limited (not palpable) splenomegaly;
4. No cytotoxic treatment in the last month;
5. Exclusion of other causes of thrombocytopenia.

Common causes of thrombocytopenia in CLL are splenomegaly, bone marrow failure secondary to tumor infiltration, recent chemoimmunotherapy, failure to recover a normal count due to megakaryocyte dysplasia [70]. Peripheral blood smear examination should be part of the diagnostic tool in order to rule out pseudo-thrombocytopenia. Exclusion of disseminate intravascular coagulation, thrombotic thrombocytopenic purpura and/or heparin-induced thrombocytopenia is also required. Acute infections (bacterial, viral, fungal) or coexisting chronic infection such as helicobacter pylorii, human immunodeficiency virus (HIV), hepatitis C, and drug induced thrombocytopenia should be ruled out by clinical and laboratory analysis [69,71].

Bone marrow evaluation remains crucial for diagnosing secondary ITP. However, in patients with Binet C stage and extensive bone marrow involvement, the interpretation of the number of megakaryocytes is sometimes problematic. In these cases, essential requisites for the diagnosis of ITP will be the lack of response to platelet transfusion (in patients without known refractoriness to platelet concentrates) and/or a rapid (<1 week) response to high-dose intravenous Ig (IVIg). Lack of response to platelet transfusion will be defined as the failure to obtain satisfactory responses in terms of bleeding or platelet number to two or more platelet transfusions [71]. For patients whose ITP was diagnosed at the time of CLL presentation the same diagnostic criteria should be applied, although the time of the fall of the platelet count cannot be established in patients presenting with no data on their previous platelet count.

As mentioned before, platelet antibody assays aimed at measuring the autoantibodies bound to platelets have limited diagnostic utility mainly because of very low specificity, but can be useful in selected complicated cases. Finally it should be noted that about 30% of ITP cases also have simultaneous AIHA (Evans Syndrome) [72].

PRCA

The diagnosis of secondary PRCA associated to CLL should be considered in any patient with anemia and reticulocytopenia [5,73]. When PRCA is suspected, signs of hemolysis and peripheral blood smear examination should be investigated in order to rule out

hemolysis. PRCA is characterized by profound reticulocytopenia with normal bilirubin and LDH levels, and it should thus be distinguishable from AIHA. It should be noted that the reticulocyte count expressed as a percentage of red cells (and not as absolute number) can be misleadingly normal in the presence of severe anemia.

Definite diagnosis relies on bone marrow biopsy which will show selective red cell aplasia with virtual total absence of erythroid precursors. Characteristic defects of erythroblast maturation may also be present on residual erythroid precursors. Anti-glycophorin immunohistochemistry may facilitate identification of red cell precursors when the bone marrow is overcrowded by lymphocytes.

PRCA can be seen in association with other AIC making the diagnosis sometimes difficult. Viral infections such as Epstein–barr virus and Parvovirus B19 should be excluded [74], as well as HIV and viral B and C hepatitis. Blood levels of folic acid and vitamin B12 will need to be ascertained. Finally, the concomitant presence of a thymoma should be excluded by means of patient history and radiographic examinations. Acquired forms of secondary PRCA associated to CLL will be easily distinguished from congenital forms, such as Diamond-Blackfan anemia, Fanconi anemia, and congenital dyserythropoietic anemias by patient age and history. Finally, a distinction between myelodysplastic syndrome with erythroid hypoplasia and PRCA should be considered in the differential diagnosis, especially in elderly patients with CLL. Again, in the PRCA setting more than in any other AIC, bone marrow aspirate and biopsy are important for a correct diagnosis.

AIG

Secondary autoimmune granulocytopenia (AIG) occurs rarely in CLL, and should be suspected in patients with isolated neutropenia and no other apparent cause [5]. AIG diagnostic criteria still remain elusive and AIG can be considered a diagnosis of exclusion.

Initial diagnostic work up should include patient history. A history of recurrent infections years before CLL diagnosis may point to the presence of a congenital or acquired disturbance of immunity, which should be ruled out with ad-hoc investigations. Detailed review of peripheral smear is mandatory in order to look for infection marks (i.e. Dohle bodies), dysplastic elements and nutritional deficiencies. It is important to exclude any other cause of secondary immune neutropenia such as systemic lupus erythematosus, rheumatoid arthtritis, Sjogren syndrome, celiac disease, hyperthyroidism (Graves disease), Chron disease and other (auto)immune diseases [75]. Concomitant large granular lymphocyte leukemia (T-LGL), which is a common cause of secondary AIG, should be excluded by means of flow cytometry and blood smear morphology [76]. Rituximab therapy has been associated with a late onset (>4 weeks after exposure) neutropenia [77]. The pathogenesis of this alteration is still unknown. Voog et al. [78] have described IgG antibodies able to bind neutrophils while others consider AIG a consequence of disturbances in B cell recovery [79]. Recent reports suggested a higher incidence of this complication among HIV, in patients treated with purine analogs such as fludarabine, and those with marked B-lymphocyte depletion and low serum IgM [80].

Although not conclusive, the presence of normo/hyper cellular bone marrow with late maturation arrest of the granulocytes may favor the diagnosis of secondary AIG. The anti-neutrophil antibody test represents a potentially useful diagnostic tool both in primary and secondary AIG. The direct anti neutrophil antibody test is characterized by limited discriminating power and it is not still well standardized being affected by high false positive rate [81,82]. Conversely, indirect anti neutrophil antibody test seems more precise and reproducible [75]. Lack of coverage of the full human neutrophil antigens pattern in neutrophil suspension is the major determinant of the limited sensitivity of this test. In the largest study conducted in primary AIG, sensitivity of the indirect test was 74% at first assessment and further increased on repeated determination [83]. In case the first test is negative or border line, it is recommended to repeat the test up to four or more times over a time-span of 4 to 6 months or longer [84].

TABLE I. Clinical and Biological Characteristics of CLL Patients Developing AIHA or ITP

	AIHA	ITP
Active disease	Usually yes	Usually not
Rai and Binet stage	Advanced	Any
Median time from CLL diagnosis (mo)	39	20
Refractory to standard treatment (%)	16	32
Prognostic significance on OS	Unclear	Possible
Unfavorable cytogenetic deletion (%)	40	40
Un-mutated IGHV (%)	60–70	80
Stereotyped HCDR3 (%)	29	50

AIHA: autoimmune hemolytic anemia; ITP: immune thrombocytopenia; IGHV: immunoglobulin heavy-chain variable region; HCDR: heavy-chain complementarity determining region-3.

Impact of AIC on patients outcome

CLL is characterized by a marked clinical heterogeneity, ranging from patients with several years of stable disease and a normal life expectancy to patients with rapidly progressive disease [1,9]. Next-generation sequencing techniques have recently added new independent and reliable markers that can predict tumor aggressiveness [85–87]. However, although less stylish, the "old fashioned" classifications described by Rai and Binet about 30 years ago are still the most widely used easy-to-make prognostic scores [88,89]. In both classifications, cytopenias are associated with poor prognosis in CLL, yielding an advanced stage of the disease. The cause of the cytopenias was not considered in the original Rai and Binet classifications, while the recently modified staging systems of the National Cancer Institute Working Group (NCIWG) do require that the cytopenias are related to bone marrow failure caused by CLL [1]. Thus, while not all cytopenias are associated with poor prognosis once reverted to normal values according to current classification (i.e. iron or vitamin B12/folate deficiency), the prognostic implications of AIC still represent a matter of debate and remain controversial [27,60,67,68,90].

Some of the major reasons for this uncertainty rely in the time of AIC presentation, which may variably extend before or after CLL diagnosis, the high variability of cytotoxic treatments and their interplay with tumor/AIC response; the lack of consistency in the way overall survival is measured, whether from the time of CLL diagnosis (which may be several years before the onset of AIC), or from the time of AIC onset. Furthermore, clinical presentation and association of secondary ITP and AIHA with biological characteristic of the underlying CLL can vary greatly, as summarized in Table I. The known association between AIC and adverse biological prognostic factors such as U-CLL or adverse cytogenetic has often hampered the significance of univariate survival analysis. Finally, time to onset of AIHA and ITP from CLL diagnosis is also different, as shown in Fig. 2, with ITP occurring earlier, when CLL is usually not active or quiescent, while AIHA is more frequently associated to advanced or progressive disease.

Despite all these uncertainties, in a recent study from Moreno et al., patients with AIC at diagnosis (stage C "immune") have been associated with better outcome than those in whom cytopenia was due to bone marrow failure (stage C "infiltrative"), though their survival was inferior to patients with uncomplicated stage A [67]. In another series of CLL patients seen in the Division of Hematology at Mayo Clinic, survival from onset of cytopenia was reported to be significantly better for patients with AIC (median 9.1 years) compared to patients with BM failure (median 4.4 years, $P < 0.001$) [60]. Although authors concluded that cytopenia caused by AIC was not an adverse prognostic factor, the reported survival for patients developing early AIC was in

 doi:10.1002/ajh.23785

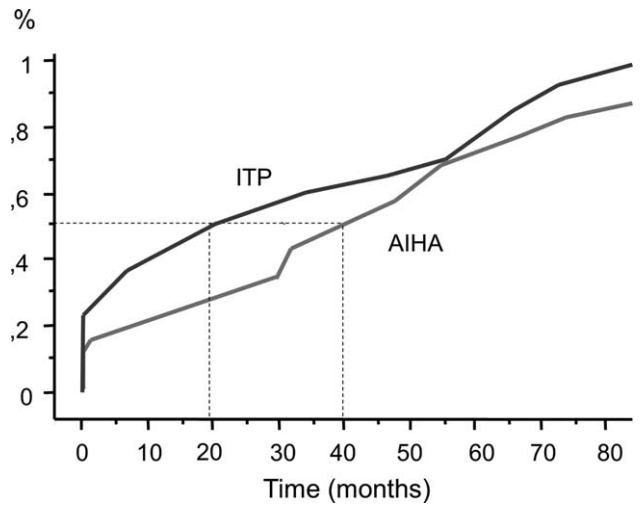

Figure 2. Time to AIHA and ITP development from CLL diagnosis (time 0), obtained from representative cohorts of patients with CLL [25,27,28].

the range of Binet stage B patients rather than stage A. In line with this, a recent study focused on AIC diagnosed at CLL presentation, reporting that patients with stage C "immune" had significantly worse overall survival than stage A patients ($P < 0.0001$), but not different from stage B ($P = 0.14$) [68]. Importantly, the latter series relied on patients treated with modern approaches that included the systematic use of monoclonal antibodies together with cytotoxic treatment.

An Italian study investigated clinical, therapeutic, and prognostic features of AIHA in the course of CLL reporting that AIHA had no independent effect on survival [91]. On the other hand, Dearden et al assessed the prognostic effect of a positive DAT in patients with CLL treated for the first time. A positive DAT predicted a poorer response to treatment and together with AIHA was associated with a lower overall survival, particularly among patients treated with fludarabine as single agent [92]. It is important to note, however, that the latter study was performed in a subset of patients with CLL that required treatment, which is a different group of patients than those who are diagnosed with a clinically apparent AIHA or than consecutive CLL patients presenting to a hospital. In terms of ITP, Visco et al. reported that this complication was associated with an inferior outcome, which could be partly explained by the association between ITP and U-CLL [27]. The prognostic impact of ITP was dependent on the time of onset of thrombocytopenia. The same group reported that later onset of AIHA in the course of CLL had a negative effect on outcome compared to early onset [28]. These results were replicated in a recent multicenter report, where occurrence of AIHA was significantly associated with U-CLL and/or unfavorable cytogenetic lesions [25].

The apparently inferior survival of CLL patients developing AIC may be explained by higher risk of infections, bleeding and cardiovascular complication associated with AIC onset and treatment, but a similarly plausible reason is that AIC represent a clinical expression of CLL clone aggressiveness. The latter theory is supported by the evidence that both AIHA and ITP were consistently associated with U-CLL, higher CD38 and ZAP-70 expression and unfavorable cytogenetic lesions (i.e. del11q23 and del17p13) in several independent series [25,67,68,90,91,93].

Treatment

No prospective randomized trials are available to guide the management of AIC secondary to CLL. The majority of information comes from retrospective studies or case reports. It is widely accepted that cases occurring with progression of the underlying disease will require CLL-specific cytotoxic treatment, and/or an anti-CD20 monoclonal antibody [94]. On the other hand, patients with isolated AIC, in absence of CLL progression, should be managed as indicated for patients with primary forms of autoimmune cytopenias.

AIHA, first line treatment

The decision to start a specific treatment of AIHA relies on hemoglobin level and associated symptoms, and transfusion is required only for symptomatic cases with severe anemia.

For AIHA caused by warm antibodies, the first line therapy is prednisone at a dose of 1 mg/kg per day given for 3 to 4 weeks, which is then slowly tapered off during the following 1 to 2 months. The alternative regimen of repeated courses of pulsed high dose dexamethasone (40 mg/day for 4 consecutive days), which is considered equivalent to standard prednisone in primary AIC, warrants further investigations in terms of efficacy and safety in the CLL setting. If a rapid response is needed (i.e. patients with massive hemolysis), immunoglobulin infusion (IVIg) may be administered (1 g/kg on days 1 and 2).

In case of AIHA caused by cold antibodies (CHD), corticosteroid treatment is much less effective. In fact, while corticosteroids are usually effective in about two-thirds of warm AIHA, they are able to control hemolysis in roughly only 15% of CHD. Accordingly, the use of corticosteroids is usually discouraged as first line therapy in CHD. Moreover, while warm and mixed cases usually display a severe onset with symptomatic anemia requiring prompt treatment, CHD may have a milder presentation. Thus the decision to treat CHD should be reserved to patients with transfusion dependent symptomatic anemia, and/or with disabling circulatory symptoms. In these cases rituximab is recommended as first-line treatment.

ITP, first line treatment

Specific treatment is indicated in patients with platelet count below 30×10^9/L or in case of bleeding signs or symptoms. Bleeding rarely occurs above 50×10^9/L [69,71]. Platelet transfusion could be required in case of life-threatening hemorrhages.

The first line therapy is prednisone at a dose of 1 mg/kg per day given for 3 to 4 weeks, which is then tapered off. A more prolonged course at full dosage with slow tapering (in terms of several weeks) is suggested by some authorities. As already stated for AIHA, pulsed high dose dexamethasone warrants further investigations in terms of efficacy and safety in the CLL setting. If a rapid response is needed (i.e. in ITP patients with significant bleeding or prior to splenectomy), IVIg may be administered (1 g/kg on days 1 and 2).

ITP response rates to specific therapies are lower in comparison to the figures generally reported for the idiopathic counterpart. Approximately one fifth of patients are refractory to standard approaches [27], which is greater than what has been reported for primary ITP (9%). Response to steroids occurs in roughly half of patients, which is in the lower part of the range of responses to steroids reported for primary ITP (50 to 75%). On the other hand, while approximately 80% of primary ITP patients usually respond to IVIg, only half of patients with secondary ITP respond to IVIg alone [6,27], making CLL related ITP a pretty insidious complication of the disease.

AIHA, second line treatment

Patients, who do not respond to conventional upfront therapy within 4 to 6 weeks should be considered for alternative treatment strategies. Refractory or relapsed patients should always be investigated again to exclude other causes of anemia such as marrow failure or CMV reactivation or parvovirus B19 infection [7].

Cyclophosphamide may be considered in patients who fail to respond to corticosteroids, require higher maintenance doses, or fail to sustain a prolonged response. Other immunosuppressive agents used in primary AIHA, such as low dose cyclosporine, mycophenolate mofetil, or azathioprine are possible alternative therapies.

Monoclonal antibodies able to target clonal and non-clonal CLL B-cells offer a valid therapeutic option in refractory cases [95]. Although this therapy as single agent is well tolerated and may induce significant responses, only a small fractions of patients achieve durable remissions [95–98]. Rituximab is generally administered at the dose scheduled for the treatment of lymphomas (375 mg/m^2/weekly for 4 weeks), while lower doses (100 mg fixed dose for 4 weekly infusions) have been tested only in the setting of primary AIHA [99]. In a cohort of steroid-refractory patients with AIHA secondary to CLL treated with rituximab as single agent [98], a complete (CR) and partial response (PR) were achieved in 22% and 50% of cases, respectively. At a mean follow-up of 17 months, about 40% of patients were transfusion-free.

Different groups tested the combination of rituximab with cytotoxic drugs. These approaches emphasize the role of "CLL-directed treatment" in AIC, targeting the non-neoplastic B/T-cell population and neoplastic CLL B-cells, thus inhibiting inflammatory cascades and the cross-talk between all these cells. Kaufman et al. treated with rituximab, cyclophosphamide, and dexamethasone (RCD) a cohort of CLL patients with refractory AIC [100]. All patients with AIHA responded to treatment with a median duration of response of 22 months. Patients converting DAT from positive to negative had longer response duration compared to patients never achieving negativity (41 months vs. 10 months). The same regimen was used in a cohort of 48 CLL patients including 26 AIHA, 9 ITP, and 8 Evan's syndrome. Overall, RCD achieved 89.5% response rate, irrespective of the AIC subtype, but relapse occurred in almost 40% of patients with a median response duration of 24 months [101,102]. Another effective regimen is rituximab, cyclophosphamide, vincristine and prednisone (R-CVP) that showed a significant activity in cohorts of CLL patients with refractory ITP/AIHA with a median time to next treatment for AIC of 21.7 months [103].

More recently, alemtuzumab has been reported as a possible alternative therapy, especially for patients with severe, refractory secondary AIHA, who have not previously responded to conventional therapy [104]. A number of new monoclonal antibodies, such as ofatumumab and obinotuzumab are currently investigated in CLL and their activity in CLL-related autoimmune cytopenias should be evaluated in future studies [94].

ITP, second line treatment

Alternative treatments for refractory or relapsed patients include monoclonal antibodies ± chemotherapy. These regimens induce a sufficiently rapid response in the majority of patients.

In refractory secondary ITP, rituximab monotherapy given at the usual dose of 375 mg/m^2/weekly for 4 weeks was associated with an encouraging overall response of 86%, with 57% of patients achieving CR [105]. These rates compare favorably with refractory secondary AIHA associated to CLL (see previous paragraph), making rituximab an appealing option in this setting. The mean duration of response was 21 months. The combination of rituximab with cytotoxic regimens (RCD and R-CVP), as for AIHA, were also associated with a high rate of responses (nearly 90%) that lasted for an average of 20 to 24 months [101–103].

Although splenectomy efficacy is well established in primary ITP, this procedure may worsen immunodeficiency and risk of infections in CLL patients. Furthermore, many CLL patients cannot be candidate to surgery due to their old age and/or important comorbidities. For this reason, although effective (50–70% of patients experiencing a long-term response) [27], splenectomy should be reserved to highly selected cases.

Few case reports suggest that TPO-mimetics may represent an efficacious therapeutic approach in refractory secondary ITP [106–109]. These novel ITP specific treatments might spare these patients not only from bleeding risk but also from toxic or inappropriate cytotoxic therapies, not otherwise demanded by the burden of the underlying disease. Two studies for the treatment of secondary ITP associated to CLL with eltrombopag are ongoing in Italy and United States, and preliminary results are awaited soon (ClinicalTrials.gov Identifier: NCT01610180; NCT01168921).

PRCA

The primary goal in PRCA is to induce the recovery of erythropoiesis avoiding an excess of transfusions. Several treatment regimens usually effective in autoimmune conditions fail in secondary PRCA, especially when this condition is associated to CLL or lymphoma.

PRCA is generally poorly responsive to corticosteroids, and cyclosporine represents the treatment of choice. Patients usually require prolonged treatment or combination therapy to maintain remission, thus increasing the risk of infections [7,70]. Most patients will require long-term low-dose maintenance therapy [110,111]. Response should be monitored by measuring the absolute reticulocyte count, which usually increases within 2 to 3 weeks from initiation of therapy while substantial improvements in the hemoglobin level can take up to months.

There are case reports of successful treatment of patients with PRCA with rituximab [73,112] or alemtuzumab [113,114] as single agents, but the response rate is lower than for AIHA or ITP. The mechanism of action of rituximab in PRCA is not entirely clear. The rapid response that has been observed in some patients raises the possibility of mechanisms different from those thought to be active in other AIC [112]. Interestingly, IVIg have been reported to be effective in some cases of PRCA associated to parvovirus B19 infection because they contain neutralizing antibodies against the virus [74].

Rossignol et al. [102], recently reported the efficacy of RCD combination in five PRCA patients. All five patients obtained CR and only one patient relapsed.

AIG

The course of AIG is usually benign. Most cases resolve spontaneously within one to three weeks, while other require specific treatment [81]. When the absolute neutrophil count is below 500/μL, the risk of infection is significantly increased. The standard approach to AIG is represented by the administration of granulocyte colony-stimulating factor (G-CSF), which usually improves or resolves neutropenia within few days. However, neutropenia often recurs after discontinuation of the treatment. For this reason patients should be monitored over time to prevent relapses or serious infective complications. Prophylactic antibiotics are indicated in persistent neutropenia, especially as secondary prophylaxis.

Splenectomy was commonly used before the availability of G-CSF but results were discouraging. Sirolimus, cyclosporine and IVIg have also been reported to be sometimes beneficial in AIG [76–78]. Monoclonal antibodies such as rituximab and alemtuzumab can provide long lasting remissions but reports are limited.

References

1. Hallek M, Cheson BD, Catovsky D, et al. Guidelines for the diagnosis and treatment of chronic lymphocytic leukemia: A report from the International Workshop on Chronic Lymphocytic Leukemia updating the National Cancer Institute-Working Group 1996 guidelines. Blood 2008;111:5446–5456.
2. Diehl LF, Ketchum LH. Autoimmune disease and chronic lymphocytic leukemia: Autoimmune hemolytic anemia, pure red cell aplasia, and autoimmune thrombocytopenia. Semin Oncol 1998;25:80–97.
3. Ebbe S, WittelS B, Dameshek W. Autoimmune thrombocytopenic purpura ("ITP" type) with chronic lymphocytic leukemia. Blood 1962;19:23–37.

4. Hamblin TJ. Autoimmune complications of chronic lymphocytic leukemia. Semin Oncol 2006;33:230–239.
5. Hodgson K, Ferrer G, Pereira A, et al. Autoimmune cytopenia in chronic lymphocytic leukaemia: Diagnosis and treatment. Br J Haematol 2011;154:14–22.
6. Visco C, Rodeghiero F. Immune thrombocytopenia in lymphoproliferative disorders. Hematol Oncol Clin North Am 2009;23:1261–1274.
7. Zent CS, Kay NE. Autoimmune complications in chronic lymphocytic leukaemia (CLL). Best Pract Res Clin Haematol 2010;23:47–59.
8. Zent CS, Ding W, Reinalda MS, et al. Autoimmune cytopenia in chronic lymphocytic leukemia/small lymphocytic lymphoma: Changes in clinical presentation and prognosis. Leuk Lymphoma 2009;50:1261–1268.
9. Chiorazzi N, Rai KR, Ferrarini M. Chronic lymphocytic leukemia. N Engl J Med 2005;352:804–815.
10. Dameshek W. Chronic lymphocytic leukemia–An accumulative disease of immunolgically incompetent lymphocytes. Blood 1967;29:84.
11. Galton DA. The pathogenesis of chronic lymphocytic leukemia. Can Med Assoc J 1966;94:1005–1010.
12. Kipps TJ, Carson DA. Autoantibodies in chronic lymphocytic leukemia and related systemic autoimmune diseases. Blood 1993;81:2475–2487.
13. Hamblin TJ, Oscier DG, Young BJ. Autoimmunity in chronic lymphocytic leukaemia. J Clin Pathol 1986;39:713–716.
14. Beaume A, Brizard A, Dreyfus B, et al. High incidence of serum monoclonal Igs detected by a sensitive immunoblotting technique in B-cell chronic lymphocytic leukemia. Blood 1994;84:1216–1219.
15. Broker BM, Klajman A, Youinou P, et al. Chronic lymphocytic leukemic (CLL) cells secrete multispecific autoantibodies. J Autoimmun 1988;1:469–481.
16. Stevenson FK, Hamblin TJ, Stevenson GT, et al. Extracellular idiotypic immunoglobulin arising from human leukemic B lymphocytes. J Exp Med 1980;152:1484–1496.
17. Chiorazzi N, Ferrarini M. Cellular origin(s) of chronic lymphocytic leukemia: Cautionary notes and additional considerations and possibilities. Blood 2011;117:1781–1791.
18. Burger JA, Ghia P, Rosenwald A, et al. The microenvironment in mature B-cell malignancies: a target for new treatment strategies. Blood 2009;114:3367–3375.
19. Deaglio S, Aydin S, Grand MM, et al. CD38/CD31 interactions activate genetic pathways leading to proliferation and migration in chronic lymphocytic leukemia cells. Mol Med 2010;16:87–91.
20. Zenz T, Mertens D, Kuppers R, et al. From pathogenesis to treatment of chronic lymphocytic leukaemia. Nat Rev Cancer 2010;10:37–50.
21. Fais F, Ghiotto F, Hashimoto S, et al. Chronic lymphocytic leukemia B cells express restricted sets of mutated and unmutated antigen receptors. J Clin Invest 1998;102:1515–1525.
22. Hamblin TJ, Davis Z, Gardiner A, et al. Unmutated Ig V(H) genes are associated with a more aggressive form of chronic lymphocytic leukemia. Blood 1999;94:1848–1854.
23. Klein U, Tu Y, Stolovitzky GA, et al. Gene expression profiling of B cell chronic lymphocytic leukemia reveals a homogeneous phenotype related to memory B cells. J Exp Med 2001;194:1625–1638.
24. Herve M, Xu K, Ng YS, et al. Unmutated and mutated chronic lymphocytic leukemias derive from self-reactive B cell precursors despite expressing different antibody reactivity. J Clin Invest 2005;115:1636–1643.
25. Maura F, Visco C, Falisi E, et al. B-cell receptor configuration and adverse cytogenetics are associated with autoimmune hemolytic anemia in chronic lymphocytic leukemia. Am J Hematol 2013;88:32–36.
26. Visco C, Giaretta I, Ruggeri M, et al. Unmutated IgVH in chronic lymphocytic leukemia is associated with a higher risk of immune thrombocytopenia. Leukemia 2007;21:1092–1093.
27. Visco C, Ruggeri M, Laura EM, et al. Impact of immune thrombocytopenia on the clinical course of chronic lymphocytic leukemia. Blood 2008;111:1110–1116.
28. Visco C, Novella E, Peotta E, et al. Autoimmune hemolytic anemia in patients with chronic lymphocytic leukemia is associated with IgVH status. Haematologica 2010;95:1230–1232.
29. Visco C, Maura F, Tuana G, et al. Immune thrombocytopenia in patients with chronic lymphocytic leukemia is associated with stereotyped B-cell receptors. Clin Cancer Res 2012;18:1870–1878.
30. Mockridge CI, Potter KN, Wheatley I, et al. Reversible anergy of sIgM-mediated signaling in the two subsets of CLL defined by VH-gene mutational status. Blood 2007;109:4424–4431.
31. Vanura K, Le T, Esterbauer H, et al. Autoimmune conditions and chronic infections in chronic lymphocytic leukemia patients at diagnosis are associated with unmutated IgVH genes. Haematologica 2008;93:1912–1916.
32. Bomben R, Dal BM, Capello D, et al. Molecular and clinical features of chronic lymphocytic leukaemia with stereotyped B cell receptors: results from an Italian multicentre study. Br J Haematol 2009;144:492–506.
33. Maura F, Cutrona G, Fabris S, et al. Relevance of stereotyped B-cell receptors in the context of the molecular, cytogenetic and clinical features of chronic lymphocytic leukemia. PLoS One 2011;6:e24313.
34. Stamatopoulos K, Belessi C, Moreno C, et al. Over 20% of patients with chronic lymphocytic leukemia carry stereotyped receptors: Pathogenetic implications and clinical correlations. Blood 2007;109:259–270.
35. Tobin G, Thunberg U, Johnson A, et al. Chronic lymphocytic leukemias utilizing the VH3–21 gene display highly restricted Vlambda2-14 gene use and homologous CDR3s: implicating recognition of a common antigen epitope. Blood 2003;101:4952–4957.
36. Widhopf GF, Rassenti LZ, Toy TL, et al. Chronic lymphocytic leukemia B cells of more than 1% of patients express virtually identical immunoglobulins. Blood 2004;104:2499–2504.
37. Galletti J, Canones C, Morande P, et al. Chronic lymphocytic leukemia cells bind and present the erythrocyte protein band 3: possible role as initiators of autoimmune hemolytic anemia. J Immunol 2008;181:3674–3683.
38. Hall AM, Vickers MA, McLeod E, et al. Rh autoantigen presentation to helper T cells in chronic lymphocytic leukemia by malignant B cells. Blood 2005;105:2007–2015.
39. Gorgun G, Holderried TA, Zahrieh D, et al. Chronic lymphocytic leukemia cells induce changes in gene expression of CD4 and CD8 T cells. J Clin Invest 2005;115:1797–1805.
40. Ramsay AG, Gribben JG. Immune dysfunction in chronic lymphocytic leukemia T cells and lenalidomide as an immunomodulatory drug. Haematologica 2009;94:1198–1202.
41. Riches JC, Ramsay AG, Gribben JG. T-cell function in chronic lymphocytic leukaemia. Semin Cancer Biol 2010;20:431–438.
42. Beyer M, Kochanek M, Darabi K, et al. Reduced frequencies and suppressive function of CD4+CD25hi regulatory T cells in patients with chronic lymphocytic leukemia after therapy with fludarabine. Blood 2005;106:2018–2025.
43. D'Arena G, Rossi G, Vannata B, et al. Regulatory T-cells in chronic lymphocytic leukemia and autoimmune diseases. Mediterr J Hematol Infect Dis 2012;4:e2012053.
44. Lad DP, Varma S, Varma N, et al. Regulatory T-cells in B-cell chronic lymphocytic leukemia: Their role in disease progression and autoimmune cytopenias. Leuk Lymphoma 2013;54:1012–1019.
45. Strati P, Caligaris-Cappio F. A matter of debate in chronic lymphocytic leukemia: Is the occurrence of autoimmune disorders an indicator of chronic lymphocytic leukemia therapy? Curr Opin Oncol 2011;23:455–460.
46. Ward FJ, Hall AM, Cairns LS, et al. Clonal regulatory T cells specific for a red blood cell autoantigen in human autoimmune hemolytic anemia. Blood 2008;111:680–687.
47. Wright GP, Ehrenstein MR, and Stauss HJ. Regulatory T-cell adoptive immunotherapy: Potential for treatment of autoimmunity. Expert Rev Clin Immunol 2011;7:213–225.
48. Grandjenette C, Kennel A, Faure GC, et al. Expression of functional toll-like receptors by B-chronic lymphocytic leukemia cells. Haematologica 2007;92:1279–1281.
49. Muzio M, Bertilaccio MT, Simonetti G, et al. The role of toll-like receptors in chronic B-cell malignancies. Leuk Lymphoma 2009;50:1573–1580.
50. Barcellini W, Imperiali FG, Zaninoni A, et al. Toll-like receptor 4 and 9 expression in B-chronic lymphocytic leukemia: Relationship with infections, autoimmunity and disease progression. Leuk Lymphoma 2014 [Epub ahead of print].
51. Barcellini W, Capalbo S, Agostinelli RM, et al. Relationship between autoimmune phenomena and disease stage and therapy in B-cell chronic lymphocytic leukemia. Haematologica 2006;91:1689–1692.
52. Leporrier M, Reman O, Troussard X. Pure red-cell aplasia with fludarabine for chronic lymphocytic leukaemia. Lancet 1993;342:555.
53. Montillo M, Tedeschi A, Leoni P. Recurrence of autoimmune thrombocytopenia after treatment with fludarabine in a patient with chronic lymphocytic leukemia. Leuk Lymphoma 1994;15:187–188.
54. Weiss RB, Freiman J, Kweder SL, et al. Hemolytic anemia after fludarabine therapy for chronic lymphocytic leukemia. J Clin Oncol 1998;16:1885–1889.
55. Hallek M, Fischer K, Fingerle-Rowson G, et al. Addition of rituximab to fludarabine and cyclophosphamide in patients with chronic lymphocytic leukaemia: a randomised, open-label, phase 3 trial. Lancet 2010;376:1164–1174.
56. Byrd JC, Hertler AA, Weiss RB, et al. Fatal recurrence of autoimmune hemolytic anemia following pentostatin therapy in a patient with a history of fludarabine-associated hemolytic anemia. Ann Oncol 1995;6:300–301.
57. Otton SH, Turner DL, Frewin R, et al. Autoimmune thrombocytopenia after treatment with Campath 1H in a patient with chronic lymphocytic leukaemia. Br J Haematol 1999;106:261–262.
58. Cuker A, Coles AJ, Sullivan H, et al. A distinctive form of immune thrombocytopenia in a phase 2 study of alemtuzumab for the treatment of relapsing-remitting multiple sclerosis. Blood 2011;118:6299–6305.
59. Reda G, Maura F, Gritti G, et al. Low-dose alemtuzumab-associated immune thrombocytopenia in chronic lymphocytic leukemia. Am J Hematol 2012;87:936–937.
60. Zent CS, Ding W, Schwager SM, et al. The prognostic significance of cytopenia in chronic lymphocytic leukaemia/small lymphocytic lymphoma. Br J Haematol 2008;141:615–621.
61. Petz LD, Garratty G. Immune Hemolytic Anemias, 2nd ed. Philadelphia: Churchill Livingstone; 2004.
62. Kamesaki T, Toyotsuji T, Kajii E. Characterization of direct antiglobulin test-negative autoimmune hemolytic anemia: A study of 154 cases. Am J Hematol 2013;88:93–96.
63. Toyotsuji T, Kamesaki T, Kajii E. Coombs-positive and negative autoimmune hemolytic anemia might immunologically differ. Rinsho Ketsueki 2011;52:461.
64. Engelfriet CP, von dem Borne AEG, Beckers D, et al. Immune destruction of red cells. In: Bell CA, editor. A Seminar on Immune-Mediated Cell Destruction. Washington, DC: American Association of Blood Banks; 1981. pp 93–130.
65. Barcellini W, Montesano R, Clerici G, et al. In vitro production of anti-RBC antibodies and

cytokines in chronic lymphocytic leukemia. Am J Hematol 2002;71:177–183.
66. Bartolmas T, Salama A. A dual antiglobulin test for the detection of weak or nonagglutinating immunoglobulin M warm autoantibodies. Transfusion 2010;50:1131–1134.
67. Moreno C, Hodgson K, Ferrer G, et al. Autoimmune cytopenias in chronic lymphocytic leukemia: prevalence, clinical associations, and prognostic significance. Blood 2010;96:752–761.
68. Visco C, Cortelezzi A, Moretta F, et al. Autoimmune cytopenias in chronic lymphocytic leukemia at disease presentation in the modern treatment era: Is stage C always stage C? Leuk Lymphoma 2013.
69. Rodeghiero F, Stasi R, Gernsheimer T, et al. Standardization of terminology, definitions and outcome criteria in immune thrombocytopenic purpura of adults and children: Report from an international working group. Blood 2009;113: 2386–2393.
70. Hodgson K, Ferrer G, Montserrat E, et al. Chronic lymphocytic leukemia and autoimmunity: A systematic review. Haematologica 2011;96: 752–761.
71. Neunert C, Lim W, Crowther M, et al. The American Society of Hematology 2011 evidence-based practice guideline for immune thrombocytopenia. Blood 2011;117:4190–4207.
72. Tandra P, Krishnamurthy J, Bhatt VR, et al. Autoimmune cytopenias in chronic lymphocytic leukemia, facts and myths. Mediterr J Hematol Infect Dis 2013;5:e2013068.
73. Ghazal H. Successful treatment of pure red cell aplasia with rituximab in patients with chronic lymphocytic leukemia. Blood 2002;99:1092–1094.
74. Mouthon L, Guillevin L, Tellier Z. Intravenous immunoglobulins in autoimmune- or parvovirus B19-mediated pure red-cell aplasia. Autoimmun Rev 2005;4:264–269.
75. Fioredda F, Calvillo M, Bonanomi S, et al. Congenital and acquired neutropenias consensus guidelines on therapy and follow-up in childhood from the Neutropenia Committee of the Marrow Failure Syndrome Group of the AIEOP (Associazione Italiana Emato-Oncologia Pediatrica). Am J Hematol 2012;87:238–243.
76. Viny AD, Lichtin A, Pohlman B, et al. Chronic B-cell dyscrasias are an important clinical feature of T-LGL leukemia. Leuk Lymphoma 2008; 49:932–938.
77. Wolach O, Bairey O, and Lahav M. Late-onset neutropenia after rituximab treatment: case series and comprehensive review of the literature. Medicine (Baltimore) 2010;89:308–318.
78. Voog E, Morschhauser F, Solal-Celigny P. Neutropenia in patients treated with rituximab. N Engl J Med 2003;348:2691–2694.
79. Dunleavy K, Tay K, Wilson WH. Rituximab-associated neutropenia. Semin Hematol 2010;47: 180–186.
80. Rao VK, Oliveira JB. How I treat autoimmune lymphoproliferative syndrome. Blood 2011;118: 5741–5751.
81. Hwang K, Park CJ, Huh HJ, et al. Flow cytometric detection of neutrophil-associated immunoglobulin in patients with or without neutropenia and establishment of the reference interval. Ann Clin Lab Sci 2011;41:144–149.
82. Ito T, Taniuchi S, Tsuji S, et al. Diagnosis of autoimmune neutropenia by neutrophil-bound IgG and IgM antibodies. J Pediatr Hematol Oncol 2011;33:552–555.
83. Bux J, Behrens G, Jaeger G, et al. Diagnosis and clinical course of autoimmune neutropenia in infancy: Analysis of 240 cases. Blood 1998;91: 181–186.
84. Fioredda F, Calvillo M, Bonanomi S, et al. Congenital and acquired neutropenia consensus guidelines on diagnosis from the Neutropenia Committee of the Marrow Failure Syndrome Group of the AIEOP (Associazione Italiana Emato-Oncologia Pediatrica). Pediatr Blood Cancer 2011;57:10–17.
85. Fabbri G, Rasi S, Rossi D, et al. Analysis of the chronic lymphocytic leukemia coding genome: role of NOTCH1 mutational activation. J Exp Med 2011;208:1389–1401.
86. Rossi D, Fangazio M, Rasi S, et al. Disruption of BIRC3 associates with fludarabine chemorefractoriness in TP53 wild-type chronic lymphocytic leukemia. Blood 2012;119:2854–2862.
87. Wang L, Lawrence MS, Wan Y, et al. SF3B1 and other novel cancer genes in chronic lymphocytic leukemia. N Engl J Med 2011;365: 2497–2506.
88. Binet JL, Lepoprier M, Dighiero G, et al. A clinical staging system for chronic lymphocytic leukemia: prognostic significance. Cancer 1977;40: 855–864.
89. Rai KR, Sawitsky A, Cronkite EP, et al. Clinical staging of chronic lymphocytic leukemia. Blood 1975;46:219–234.
90. Shvidel L, Tadmor T, Braester A, et al. Pathogenesis, prevalence, and prognostic significance of cytopenias in chronic lymphocytic leukemia (CLL): A retrospective comparative study of 213 patients from a national CLL database of 1,518 cases. Ann Hematol 2013;92:661–667.
91. Mauro FR, Foa R, Cerretti R, et al. Autoimmune hemolytic anemia in chronic lymphocytic leukemia: clinical, therapeutic, and prognostic features. Blood 2000;95:2786–2792.
92. Dearden C, Wade R, Else M, et al. The prognostic significance of a positive direct antiglobulin test in chronic lymphocytic leukemia: A beneficial effect of the combination of fludarabine and cyclophosphamide on the incidence of hemolytic anemia. Blood 2008;111:1820–1826.
93. Zanotti R, Frattini F, Ghia P, et al. ZAP-70 expression is associated with increased risk of autoimmune cytopenias in CLL patients. Am J Hematol 2010;85:494–498.
94. Laurenti L, Vannata B, Innocenti I, et al. The use of monoclonal antibodies in the treatment of autoimmune complications of chronic lymphocytic leukemia. Mediterr J Hematol Infect Dis 2013;5:e2013027.
95. Aleem A, Alaskar AS, Algahtani F, et al. Rituximab in immune thrombocytopenia: Transient responses, low rate of sustained remissions and poor response to further therapy in refractory patients. Int J Hematol 2010;92:283–288.
96. Bruserud O, Havardstein K. Should low-dose mycophenolate mofetil be used to prolong the response after rituximab therapy in patients with immune thrombocytopenic purpura? A case report. Hematology 2009;14:224–226.
97. Cooper N, Evangelista ML, Amadori S, et al. Should rituximab be used before or after splenectomy in patients with immune thrombocytopenic purpura? Curr Opin Hematol 2007;14: 642–646.
98. D'Arena G, Laurenti L, Capalbo S, et al. Rituximab therapy for chronic lymphocytic leukemia-associated autoimmune hemolytic anemia. Am J Hematol 2006;81:598–602.
99. Barcellini W, Zaja F, Zaninoni A, et al. Low-dose rituximab in adult patients with idiopathic autoimmune hemolytic anemia: clinical efficacy and biologic studies. Blood 2012;119:3691–3697.
100. Kaufman M, Limaye SA, Driscoll N, et al. A combination of rituximab, cyclophosphamide and dexamethasone effectively treats immune cytopenias of chronic lymphocytic leukemia. Leuk Lymphoma 2009;50:892–899.
101. Michallet AS, Rossignol J, Cazin B, et al. Rituximab-cyclophosphamide-dexamethasone combination in management of autoimmune cytopenias associated with chronic lymphocytic leukemia. Leuk Lymphoma 2011;52:1401–1403.
102. Rossignol J, Michallet AS, Oberic L, et al. Rituximab-cyclophosphamide-dexamethasone combination in the management of autoimmune cytopenias associated with chronic lymphocytic leukemia. Leukemia 2011;25:473–478.
103. Bowen DA, Call TG, Shanafelt TD, et al. Treatment of autoimmune cytopenia complicating progressive chronic lymphocytic leukemia/small lymphocytic lymphoma with rituximab, cyclophosphamide, vincristine, and prednisone. Leuk Lymphoma 2010;51:620–627.
104. Osterborg A, Karlsson C, Lundin J. Alemtuzumab to treat refractory autoimmune hemolytic anemia or thrombocytopenia in chronic lymphocytic leukemia. Curr Hematol Malig Rep 2009;4:47–53.
105. D'Arena G, Capalbo S, Laurenti L, et al. Chronic lymphocytic leukemia-associated immune thrombocytopenia treated with rituximab: A retrospective study of 21 patients. Eur J Haematol 2010;85:502–507.
106. Bussel JB, Provan D, Shamsi T, et al. Effect of eltrombopag on platelet counts and bleeding during treatment of chronic idiopathic thrombocytopenic purpura: A randomised, double-blind, placebo-controlled trial. Lancet 2009;373: 641–648.
107. D'Arena G, Cascavilla N. Romiplostim for chronic lymphocytic leukemia-associated immune thrombocytopenia. Leuk Lymphoma 2011;52:701–704.
108. Gudbrandsdottir S, Frederiksen H, Hasselbalch H. Thrombopoietin-receptor agonists in haematological disorders: The Danish experience. Platelets 2012;23:423–429.
109. Koehrer S, Keating MJ, Wierda WG. Eltrombopag, a second-generation thrombopoietin receptor agonist, for chronic lymphocytic leukemia-associated ITP. Leukemia 2010;24: 1096–1098.
110. Chikkappa G, Pasquale D, Zarrabi MH, et al. Cyclosporine and prednisone therapy for pure red cell aplasia in patients with chronic lymphocytic leukemia. Am J Hematol 1992;41:5–12.
111. Gotic M, Basara N, Rolovic Z, et al. Successful treatment of refractory pure red cell aplasia secondary to chronic lymphocytic leukaemia with cyclosporine A: Correlation between clinical and in vitro effects. Nouv Rev Fr Hematol 1994;36:307–309.
112. Narra K, Borghaei H, Al-Saleem T, et al. Pure red cell aplasia in B-cell lymphoproliferative disorder treated with rituximab: Report of two cases and review of the literature. Leuk Res 2006;30:109–114.
113. Rodon P, Breton P, Courouble G. Treatment of pure red cell aplasia and autoimmune haemolytic anaemia in chronic lymphocytic leukaemia with Campath-1H. Eur J Haematol 2003;70:319–321.
114. Ru X, Liebman HA. Successful treatment of refractory pure red cell aplasia associated with lymphoproliferative disorders with the anti-CD52 monoclonal antibody alemtuzumab (Campath-1H). Br J Haematol 2003;123:278–281.