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Autoimmune Complications in Chronic Lymphocytic Leukemia (CLL)

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Abstract

Patients with B-chronic lymphocytic leukemia/small lymphocytic lymphoma (CLL) have a 5–10% risk of developing autoimmune complications which primarily cause cytopenia. These autoimmune cytopenias can occur at any stage of CLL and do not have independent prognostic significance. The most common autoimmune complication is autoimmune hemolytic anemia with a lower frequency of immune thrombocytopenia and pure red blood cell aplasia and only rare patients with autoimmune granulocytopenia. Autoimmune cytopenia should always be considered in the differential diagnosis of cytopenia in patients with CLL. Patients with CLL can also have more than one form of autoimmune cytopenia which can occur together with bone marrow failure. Treatment is usually effective but rarely curative for autoimmune cytopenia complicating CLL. Optimal therapy will depend on a timely and accurate diagnosis of autoimmune cytopenia and should be individualized according to the severity of the cytopenia and the presence or absence of concomitant progressive CLL requiring therapy.

Keywords

Chronic lymphocytic leukemia; small lymphocytic lymphoma; autoimmune hemolytic anemia; immune thrombocytopenia; pure red blood cell aplasia

Introduction

Autoimmune cytopenias are important and relatively frequent complications of chronic lymphocytic leukemia/small lymphocytic lymphoma (CLL). In contrast, non-hematological autoimmune complications of CLL such as paraneoplastic pemphigus, glomerulonephritis, C1 esterase deficiency, and pernicious anemia are rare[1–4]. This review will thus focus on the epidemiology, pathogenesis, clinical features, and management of autoimmune cytopenia complicating CLL. The presentation and management of these autoimmune complications of CLL have changed because of the major improvements in diagnostic precision, development of accurate prognostic markers and more effective treatment modalities in CLL. Accordingly this review is focused on how these factors can be

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integrated into a more precise management of the CLL patients who have autoimmune cytopenias.

Epidemiology

For still unknown reasons, CLL is the most prevalent lymphoid malignancy in Europe and North America[5–7]. Although autoimmune cytopenia has been recognized as a complication of CLL for over 100 years[8], there is limited data on it's epidemiology and minimal data on true incidence and prevalence. Most prior epidemiological data are derived from tertiary care medical centers treating populations biased towards patients with advanced stage and extensively treated CLL compared to the general population of CLL patients seen in the medical community. In addition, most studies report the cumulative risk of developing autoimmune cytopenia in a defined CLL population rather than incidence or prevalence of these complications. The validity of some data from older studies can also be compromised because of the less accurate diagnostic methods available at the time of these investigations. The reported risk of autoimmune cytopenia is thus highest in the oldest studies with autoimmune hemolytic anemia (AIHA) rates of over 26%[9]. However, more recent studies have decreased these estimates to 10–15%9 and the most recent studies of less biased CLL populations using the modern diagnostic criteria suggest that the overall risk of autoimmune complications in patients with CLL is probably in the 5 – 10% range[4,10–12]. Nevertheless autoimmune cytopenia is still an important cause of anemia and thrombocytopenia in patients with CLL.

Cytopenia in patients with CLL can have multiple etiologies including progressive bone marrow (BM) infiltration by CLL cells resulting in inadequate hematopoiesis (BM failure), autoimmune disease, side effects of treatment, non-CLL related disorders, or a combination of these mechanisms. A recent study of 1750 patients with CLL seen over a period 10 years at the Mayo Clinic found that 24% had cytopenias that were not due to short term myelosuppression by treatment[10]. Although the common etiology of cytopenia was BM failure (54%), an appreciable number of patients had other causes of their cytopenia including autoimmune disease (18%), non-CLL related disorders (11%), long term complications of treatment of CLL (4%), and splenomegaly (3%)10. In this recent series of patients autoimmune cytopenia was thus responsible for 25% of cytopenias that could be attributed to CLL10.

Autoimmune cytopenia can occur at any time in the course of CLL and in some patients precedes the diagnosis of their CLL. In the recently reported Mayo Clinic study, the diagnosis of autoimmune cytopenia was made before the diagnosis of CLL in 9% of patients (at a median interval of 1.7 years) and 19% of patient had autoimmune cytopenia and CLL diagnosed within 1 month of each other10. In the high CLL prevalence regions of the world such as North America and Europe, chronic B cell lymphoproliferative disorders (CLPD) are the most common secondary cause of autoimmune cytopenias. As many as 18% of patients initially diagnosed with primary AIHA will subsequently develop CLPD (usually CLL) at a median interval of about 2 years[13,14]. The relationship between monoclonal B cell lymphocytosis (MBL) and autoimmune cytopenia has also been evaluated. MBL is considerably more frequent than CLL and can be detected in about 3.5% of otherwise healthy adults with normal blood counts in European and North American populations15. In one study of patients a with diagnosis of idiopathic autoimmune cytopenia, 19% had MBL with a CLL immunophenotype16. The biological and clinical significance of this finding has not yet been determined but could be important because MBL can progress to CLL.

The relative frequency of each of the clinical forms of autoimmune cytopenia in CLL remains uncertain with conflicting results from different studies. The older literature

reported the highest rates of AIHA with lower rates of immune thrombocytopenia (ITP) and multilineage autoimmune cytopenias[9]. In addition, non standardized definitions of autoimmune cytopenia have complicated the comparison of different studies. For example, the results of studies of acute onset ITP17 differ from those which include all patients with ITP irrespective of the rate of onset of thrombocytopenia10. In addition, earlier and more accurate diagnosis of CLL and more sophisticated methods of determining the etiology of cytopenias, have altered the reported frequencies of the different clinical manifestations of autoimmune cytopenia in CLL.

The consensus view remains that AIHA is the most common autoimmune complication of CLL with a frequency of 5%-10%10–12,[18]. AIHA alone or in combination with other forms of autoimmune cytopenia (Evans syndrome) occurs in about 55% −66% of CLL patients who have autoimmune cytopenia [4,]10. The frequency of ITP is more difficult to determine with accuracy because of the lack of reliable tests for anti-platelet antibodies19,20, lack of BM data in some studies, and absence of uniform diagnostic criteria for ITP for which is still a diagnosis of exclusion. The reported risk for ITP ranges from 1–5%[10,]12,17,18. Not surprisingly the proportion of ITP among CLL patients with autoimmune cytopenia is highest is patients in whom BM studies are routinely used to determine the etiology of thrombocytopenia. In our own studies where we did routinely perform BM studies, the diagnosis of ITP was made in 47% of patients with autoimmune cytopenia[10]. Pure red blood cell aplasia (PRBCA) is a much less common complication of CLL occurring in about 1% of patients and cases of autoimmune granulocytopenia are rare[10,]12,18,21. However it is important to recognize that autoimmune mechanisms can cause multilineage cytopenia in CLL patients and these are estimated to occur in approximately 12 – 17% of patients with autoimmune cytopenia[10,17].

These data show that although autoimmune cytopenia is an important complication of CLL, the incidence and prevalence of this problem is not yet completely defined. Obtaining this data will require prospective studies using state of the art diagnostic criteria in large community based populations.

Pathogenesis

Autoimmune cytopenia complicating CLL is usually caused by polyclonal T cell dependent mechanisms that result from the loss of self tolerance. The pathogenic antibodies responsible for about 90% of cases of AIHA and ITP are produced by non-malignant B cells and are polyclonal high affinity IgG[22] directed against red blood cell (RBC) or platelet antigens[4,]10–12,22,[23]. These antibodies can ligate antigens on RBC and platelets and the opsonized cells are then destroyed via an antibody dependent cellular cytotoxicity (ADCC) mechanism mediated predominantly by fixed macrophages in the spleen and liver[13]. Although CLL cells can produce monoclonal autoantibodies that are detectable in the serum, these are rare and responsible for less than 10% of cases of autoimmune cytopenia4,10–12,[22–25]. These monoclonal autoantibodies are usually IgM directed against the I antigen[11] and cause AIHA by both complement dependent cytotoxicity and ADCC [26]. The autoimmune mechanism in PRBCA is less well defined and can is believed to be generated either by antibody induced lysis or directly by T cell mediated mechanisms[9]. There is little data on the mechanism of autoimmunity in patients with AIG.

The etiology for production of polyclonal IgG anti-RBC and anti-platelet antibodies in patients with CLL is not fully understood and the loss of peripheral immune tolerance could involve both T cell dysfunction and pathological antigen presentation by CLL cells. Murine and human AIHA studies have shown that autoreactive T helper (T_H) cells are critical for the induction of AIHA[27–30]. Autoreactive T_H cells specific for Rh family antigens

(including the RhD and RhCcEe epitopes), which are the dominant RBC antigens in AIHA, have been demonstrated in CLL patients with AIHA[28,30]. These autoreactive T_H cells could be induced by the pathological autoantigen presentation mediated via CLL cells. Although CLL cells are usually considered to be inefficient antigen presenting cells (APC), they are able to present Rh antigens to T cells *in vitro*[30]. The anion exchanger known as band 3 (B3), which is the most abundant RBC membrane protein, is also frequently targeted in AIHA31. Because CLL cells bind to B3 with high avidity and phagocytose the protein *in vitro*, it is possible that CLL cells present B3 epitopes to autoreactive T cells[31]. Therefore, CLL cells could function as auto-antigen presenting cells which then trigger the T_H cell-mediated auto-antibody production against RBC antigens by normal B lymphocytes still resident in the CLL patient. The antibodies causing ITP in patients with CLL are most often polyclonal high affinity IgG directed against the platelet surface antigen GpIIb/IIIa. Although the mechanisms that result in the production of these pathological antibodies are believed to be similar to those proposed for AIHA, this has not yet been demonstrated.

Abnormal function or loss of regulatory T cells (Treg), a subset of $CD4^+$ $CD25^{hi}FoxP3^+T$ cells with a role in maintaining peripheral tolerance[32], could have significant involvement in the etiology of autoimmune cytopenias in CLL. There are as yet no published data on the relationship of Treg function and autoimmune cytopenia in CLL. However, patients with idiopathic AIHA have clones of Treg specific to Rh epitopes[33] and patients with idiopathic ITP have reduced numbers and defective suppressive capacity of Treg[34]. Although patients with advanced stage CLL have increased numbers of Treg the functional capacity of these cells is unknown. Importantly, Treg function has been shown to be decreased after treatment with fludarabine which is a risk factor for the development of autoimmune cytopenia in CLL[35]. These data suggest that defective Treg function could be a factor in the development of autoimmune cytopenia in patients with CLL and this is a subject of ongoing investigations.

Autoantibodies specific for RBCs detected by the direct antiglobulin test (DAT), which also detect evidence of complement activation(C3) by antibodies, have been reported in up to 70% of patients populations with CLL[8]. However, these higher rates from earlier studies have not been confirmed in more recent studies. A recent publication showed a DAT positive report a rate of about 10% in patients requiring initial therapy for CLL36. Most patients with CLL and a positive DAT do not have AIHA but nevertheless are at increased risk of subsequent development of AIHA12. However, clinically evident hemolysis only occurs in a minority of patients with a positive DAT12. The reasons why only some patients with a positive DAT develop overt AIHA are not known. Determining the other factors that contribute to the development of AIHA could be of value in preventing and treating this condition.

Anti-platelet antibodies can also occur in patients with CLL that do not have thrombocytopenia but there is no data on how many of these patients subsequently develop ITP. ITP could develop in only some patients with anti-platelet antibodies because impaired thrombopoiesis prevent the BM from compensating for increased platelet sequestration. In these patients impaired thrombopoiesis could be caused by antibody mediated damage of megakaryocytes and inadequate thrombopoietin responses to thrombocytopenia[37]. The role of these mechanisms in ITP associated with CLL appear to be complex but nevertheless need to be more fully elucidated as they could be amenable to therapeutic interventions.

Therapy-related autoimmune cytopenia

The association between treatment of CLL and the development of autoimmune cytopenia was recognized soon after the introduction of alkylator therapy[38]. The subsequent use of purine analogues and alemtuzumab which are highly toxic to T lymphocytes, appear to have

increased the risk of autoimmune cytopenias[39–46]. CLL patients treated with fludarabine monotherapy for the first time have an approximately 2% risk of developing AIHA with a higher risk reported for patients receiving re-treatment with fludarabine[47]. The mechanism by which treatment of CLL can increase the risk of autoimmune cytopenia is not known although one plausible explanation is that treatment is toxic to Treg resulting in loss of self tolerance[35]. A better understanding of the mechanism could enable clinicians to avoid using specific drugs in patients at risk and to develop new strategies for management of patients that have drug-induced immune cytopenia.

Clinical Features

As stated above autoimmune cytopenia can occur at any time in the course of CLL. Because the diagnosis of autoimmune cytopenia can precede or be concomitant with the diagnosis of CLL in an appreciable percent of patients (22 – 50%)[10–12,17,48], all patients presenting with autoimmune cytopenia should be screened for an underlying lymphoid malignancy. The majority of CLL patients who develop autoimmune cytopenia will do so prior to initial treatment for progressive CLL[11,49].

The risk of autoimmune cytopenia in patients with CLL is cumulative and thus increases with lymphocyte count, age, and the need for treatment for progressive disease4,11. Autoimmune complications are also more common in men10,[11]. The risk of autoimmune cytopenia is increased in patients with unmutated (UM) IGVH and these patients show a biased use of VH1 and VH3 gene families[17,50]. The risk of ITP has been reported to be increased in patients with CLL cells positive for the expression of ZAP-7017. These preliminary data from a limited number of patients provide promising new leads for predicting which patients with CLL will develop autoimmune cytopenia.

AIHA complicating CLL usually presents with symptomatic anemia which can be of sudden or gradual onset[49]. The diagnostic characteristics are an increase in RBC production as measured by the absolute reticulocyte count, and evidence of extravascular hemolysis (increased indirect bilirubin and LDH levels). The DAT for RBC bound IgG and/or complement component C3 is positive in over 90% of patients[11,49]. However, the diagnosis of AIHA can be difficult in patients with more advanced stage CLL who have higher rates of positive DAT,increased LDH levels because of high CLL burden, and in whom the etiology of anemia is more likely to be multifactorial. In these patients it is essential to do a BM study to establish the etiology of the anemia and plan treatment10. We suggest that AIHA should be part of the differential diagnosis of all CLL patients with anemia and that a careful diagnostic evaluation is required before initiation of therapy.

In contrast to AIHA, the majority of patients with CLL who develop ITP are asymptomatic at diagnosis[49]. Those patients who present with bleeding complications usually have lower median platelet counts ($< 15 \times 10^9$/L) compared to those that do not have bleeding[49]. Even when considering only those patients with rapid onset thrombocytopenia, only about 50% of patients will present with bleeding and only about 10% with severe bleeding[17]. As recognized by Rai et al[51] and Binet et al[52] in their widely used clinical staging classifications, patients with CLL and progressive BM failure usually develop symptoms of anemia before they have marked thrombocytopenia. ITP is thus an important consideration in the differential diagnosis of thrombocytopenia in patients with CLL, and especially for those patients who are not anemic or symptomatic. Unfortunately there is currently no reliable and clinically available test for anti-platelet antibodies[9,19,20]. The diagnosis of ITP thus requires a BM study to demonstrate adequate platelet production and exclude alternative etiologies including progressive CLL. An additional diagnostic difficulty is to distinguish between thrombocytopenia caused by splenic sequestration and antibody

mediated mechanisms. Fortunately this does not usually result in management problems because isolated splenic sequestration rarely causes severe thrombocytopenia and thus does not require treatment. However, the diagnostic classification of CLL patients with splenomegaly and thrombocytopenia can influence the results of clinical studies. For all these reasons, improvements in the diagnostic precision for ITP by reliable non invasive measurements of platelet production and pathological anti-platelet antibodies would be very useful in our overall evaluation and management of CLL patients.

PRBCA occurs less frequently than AIHA or ITP but is still an important diagnostic consideration in patients with CLL and anemia[21]. PRBCA is characterized by profound reticulocytopenia and normal bilirubin and LDH levels and should thus be easily distinguishable from AIHA. However, PRBCA can be difficult to differentiate from BM failure caused by progressive CLL. The diagnosis of PRBCA complication requires a BM study which will show the characteristic defects in RBC precursor maturation. Transient RBC production failure due to parvovirus B19 infection should be considered in all patients with CLL presenting with PRCA. Parvovirus B19 infection can be diagnosed in most patients by the polymerase chain reaction (PCR) test for viral nucleic acid in the blood or by examination of the BM for the characteristic morphological features of parvovirus B19 infection. The diagnosis of parvovirus B19 infection has major clinical importance because infected patients usually respond well to intravenous immunoglobulin (IVIG) infusion and do not require other immunosuppressive therapy[53].

Although AIG is a rare complication of CLL, this diagnosis should be considered in the differential diagnosis of prolonged neutropenia[10]. Diagnosis of AIG requires a BM study to demonstrate failure of neutrophil production and eliminate other possible causes. A small number of patients can develop prolonged neutropenia persisting for many months after treatment of progressive CLL with chemoimmunotherapy regimens that include rituximab and purine analogues. In these patients, distinguishing between immune mechanisms (AIG) and slow recovery from drug toxicity may not be possible and treatment options are limited.

Prognostic significance of autoimmune cytopenia in CLL

The clinical classifications described by Rai et al 51 and Binet et al 52 consider that all patients with CLL causing anemia or thrombocytopenia have advanced stage disease with poor prognosis. Several studies have subsequently shown that autoimmune cytopenia is not associated with the same adverse prognostic significance as BM failure in patients with CLL10[−12]. In a recently published study limited to rapid onset ITP (developing within 2 weeks) univariate analysis showed that ITP was associated with poorer prognosis but the association was not significant when other clinical and biological factors were included in a multivariate analysis[17]. These data suggest that patients with CLL and autoimmune cytopenia should only be considered to have clinically advanced stage disease if a BM study shows evidence of BM failure caused by progressive CLL.

Prognostic significance of a positive DAT test in patients without AIHA

Only a minority of patients with a positive DAT will develop AIHA or other forms of autoimmune cytopenia and there is no reliable method to predict which DAT positive CLL patients will develop autoimmune cytopenia. A positive DAT test does predict for subsequent development of AIHA[12,18] and the frequency of DAT positive patients does increase with the progression of CLL[18]. A positive pretreatment DAT could also increase the risk of treatment associated AIHA[36]. In contrast, among patients with CLL complicated by AIHA, a negative DAT which is more likely to occur in patients with a pathological monoclonal anti-RBC IgM, is associated with a poorer prognosis[11]. These data do not suggest that a positive DAT test is necessarily an independent marker of poor prognosis in

patients with CLL and a positive DAT in the absence of autoimmune cytopenia does not preclude the appropriate use of purine nucleoside based treatments for progressive CLL in these patients. .

Management

The number of patients who require treatment for autoimmune cytopenia complicating CLL is relatively small. There is thus limited data and no prospective randomized therapy trials to guide management. The available information is necessarily from retrospective analyses and case studies. These constraints limit the ability to provide data based and proven therapeutic recommendations. However it is still possible to come up with reasonable guidelines for the management of CLL patients with autoimmune cytopenia. .

Patients with CLL can be categorized as having “simple”, “complex”, or treatment related autoimmune cytopenia[54]. Those patients with autoimmune cytopenia complicating non progressive CLL that does not require treatment, can be considered to have “simple” autoimmune cytopenia and can usually be managed with therapies similar to those used to treat with primary autoimmune cytopenias. In contrast, patients with autoimmune cytopenia complicating progressive CLL meeting standard criteria for treatment of their CLL[55] have more “complex” treatment requirements . Treatment decisions for these patients with “complex” autoimmune cytopenia can be especially difficult when cytopenias are caused by both autoimmune mechanisms and BM failure.

Treatment of “simple” autoimmune cytopenia

AIHA—Corticosteroids are the usual first line of therapy and most patients respond rapidly to treatment with high dose oral prednisone (1 mg/kg for 10–14 days), followed by a gradual prednisone taper over the next 2–3 months[56]. However durable responses are only achieved in about one third of patients and the majority of responding patients will have evidence of recurrence of hemolysis, usually as the prednisone dose is decreased, and require either maintenance corticosteroids or combination therapy12،48،49،[56]. The mechanism of action of corticosteroids in the treatment of autoimmune cytopenia is not full understood but the initial effects are likely to be inhibition of macrophage mediated ADCC of antibody opsonized RBC with later decreases in the levels of the pathological anti-RBC antibodies[57]. A better understanding of the mechanism of action of corticosteroids could be useful to optimize the dose and duration of therapy.

Corticosteroid-sparing drugs can be useful in the management of patients requiring prolonged immunosuppression. Cyclosporin at a dose 5 to 8 mg/kg/d has been reported to be effective therapy for AIHA 58،[59]. Renal toxicity was the most common side effect. In our experience, the dose can be tapered to 3 mg/kg/d after initial response and patients can often be maintained at a target maximum blood cyclosporine level of 100 μg/L or less. IVIG (0.4g/kg/day for 3–5 days) can induce a rapid but usually short duration response in patients with CLL and AIHA and can be a useful adjunct to RBC transfusion in patients with severe or poorly tolerated hemolysis. IVIG is believed to block sequestration of RBCs by macrophages and could also have other immune modulating effects[26].

Rituximab can be highly effective treatment for AIHA complicating CLL[60–62]. The mechanism of the beneficial effect of rituximab in the treatment of autoimmune cytopenia in CLL is not well understood. Although it is known that rituximab is cytotoxic to the normal B cells responsible for synthesis of anti-RBC antibodies, this does not explain the rapid responses to therapy that have been observed. The optimal dose and duration of rituximab therapy is not yet established and could be lower than standard doses used to treat CLL because the pathogenic non malignant B cells are likely to be more sensitive to rituximab

than CLL cells. Rituximab is typically used in our institution at the standard dose (375 mg/m^2 weekly for 4 weeks) together with high dose oral prednisone as detailed above. Our practice is to decrease the prednisone dose rapidly after initial control of hemolysis and we have found that prednisone therapy can usually be stopped within 4 −8 weeks of the last dose of rituximab in many patients. Patients are then monitored monthly and retreated with rituximab when there is evidence of recurrent hemolysis. There is no established role for maintenance rituximab but scheduled repeat doses could be used empirically in patients requiring maintenance corticosteroids and those with multiple relapses of hemolysis. The role of newer anti-CD20 monoclonal antibodies such as ofatumumab, which may be more effective at B cell suppression, in the management of AIHA has not yet been determined.

Transfusion of packed RBC can be required for the management of acute hemolysis and can be done safely in most patients with an appropriate cross-match[11,49]. Use of a blood heating coil is advised in the rare patients who have an IgM or cold reactive autoantibody. Accelerated hemolysis of the transfused RBC is common and can often be decreased by administration of IVIG prior to transfusion.

Refractory AIHA can be difficult to manage with no consistently effective treatment options. Splenectomy can decrease hemolysis in AIHA but is less effective than in the management of ITP12،63،64. An alternative therapeutic approach is chemoimmunotherapy with regimens such as rituximab, cyclophosphamide and dexamethasone (RCD)[65] or rituximab cyclophospamide vincristine and prednisone (R-CVP)66. Single agent alemtuzumab has also been successful in controlling refractory AIHA in patients with CLL67.

ITP—The initial treatment options used for management of AIHA can also be effective in the management of ITP. Most patients are initially treated with a corticosteroid which is the only treatment required in about one third of patients[49]. About 40% of patients will have a sustained respond to initial therapy and therapy responses are usually longer than patients with AIHA with a median duration of close to 2 years in one study[49].

Corticosteroid sparing agents can also be used for responding patients who require long term immunosuppression using the same agents described above for AIHA. Rituximab is also effective therapy for ITP complicating CLL[60،68،69]. IVIG usually results in rapid increases in platelet counts but these are often of short duration. In non-splenectomized patients, anti-D immunoglobulin can also be effective therapy12. Splenectomy can be used in refractory or corticosteroid dependent ITP and is more likely to be effective than in the management of AIHA complicating CLL[17]. Patients with refractory or relapsed primary ITP can be responsive to oral high dose dexamethasone (40 mg/d for 4 days)[70] and in our experience, this therapy has been effective in some patients with ITP complicating CLL. Patients with refractory ITP can also be treated with combination chemoimmunotherapy as described above for AIHA.

The goal of treatment of refractory ITP is to maintain a “safe” platelet count of 20 – 50 × 10^9/L[37]. Patients with refractory ITP first need to be evaluated for BM function in order to determine the role of BM failure in their failure to respond to therapy. Those patients with markedly decreased thrombopoiesis secondary to progressive CLL could benefit from appropriate treatment for their CLL. An additional diagnostic consideration in these patients is CMV reactivation which is relatively common in patients with advanced stage and previously treated CLL. CMV infection of hematopoietic cells can increase immune destruction of platelets and megakaryocytes[37].

PRBCA—PRBCA is generally responsive to corticosteroid therapy but patients usually required prolonged high dose therapy or combination therapy to maintain remission[26,71]. Response should be monitored by measuring the absolute reticulocyte count which usually increases within 2–3 weeks of initiation of therapy while substantial improvements in the hemoglobin level can take up to a month. Most patients will require long term low dose maintenance therapy with corticosteroids or cyclosporin which needs to be individualized for each patient[49]. There are case reports of successful treatment of patients with CLL and PRBCA with single agent rituximab [72–75] but the response rate is lower than for treatment of CLL patients with AIHA or ITP. This is possibly because rituximab is likely to be effective only in the approximately 50% of patients who have a humoral mediated autoimmune process. There are also some case reports of successful treatment of PRBC in CLL patients using single agent alemtuzumab therapy[76,77].

AIG—There is limited experience with management of this complication. Immunosuppression is rarely effective and patients should be supported with appropriate treatment of infections. Spontaneous recovery is possible especially in therapy associated AIG[49].

Treatment of “complex” autoimmune cytopenia

There is no recognized standard therapy for patients with both autoimmune cytopenia and progressive CLL. Although these patients are a small minority of the CLL patients population, they can comprise a larger proportion of patients treated in academic referral centers49. Monotherapy with purine analogues and alkylating agents, which are known to increase the risk of autoimmune complications in CLL patients, should be avoided36,46. Despite the data suggesting that combination therapy including purine analogue such as fludarabine and cyclophosphamide36, fludarabine, cyclophosphamide, and rituximab (FCR)[78] and pentostatin, cyclophosphamide and rituximab (PCR)79 do not increase the risk of autoimmune cytopenia, there is no data to support the use of these regimens in patients with active autoimmune cytopenia. Because of this concern, alternative immunochemotherapy regimens that do not include purine analogue such RCD65 and R-CVP66 are frequently used to treat “complex” cytopenia in patients with CLL.

A single institution review of the use of the RCD protocol for the treatment of “complex” or corticosteroid resistant “simple” autoimmune cytopenia was reported recently[65]. All 20 patients responded to treatment with median response duration of 22 months and 9 patients responded to re-treatment with a median response duration of 13 months[65]. Concomitant ITP in 3 patients also responded well to this treatment[65]. Although the duration of response of both autoimmune cytopenia and CLL to treatment was short, median survival from initiation of treatment with RCD was 70 months[65]. Therapy was well tolerated with no hospitalizations and no treatment modifications were required[65]. Despite these clearly beneficial results, the treatment of “complex” autoimmune cytopenia in patients with CLL clearly requires more clinical trial testing and refinement. Testing whether purine analogue containing regimens can be safely used to treat “complex” autoimmune cytopenia will require the conduct of carefully designed clinical trials which will need to be multi-institutional to accrue adequate numbers of patients. Regimens that could be tested for their ability improve the duration of response for both autoimmune cytopenia and progressive CLL include high dose methylprednisolone and rituximab[80] and maintenance rituximab therapy.

Treatment of Therapy Related Autoimmune Cytopenia

Treatment decisions are difficult for patients who develop autoimmune cytopenia during or within 6–12 months of treatment for progressive CLL because of the lack of compelling data

about the risks of recurrence of autoimmune cytopenia with the reinstitution of purine analogue based regimens. Patients who have already had an acceptable response to treatment of their CLL, can be treated for their autoimmune cytopenia using the same modalities used for "simple" autoimmune cytopenia. However, patients with inadequate CLL responses and those with relapsing CLL will clearly need additional conventional treatment for their underlying CLL.

While there is general agreement that monotherapy with either purine analogues or alkylating agents should not be used in CLL patients with treatment related autoimmune cytopenia[41,46], use of combination therapy including purine analogues is controversial. In phase III clinical trials randomizing previously untreated patients to therapy with either fludarabine or fludarabine and cyclosphamide for progressive CLL, there were significantly less AIHA complications with the use of the fludarabine and cyclophosphamide combination in the British trial36 but not in the German81 or American[82] trials. A report from a phase II study where fludarabine, cyclophosphamide and rituximab (FCR) was used as first line therapy for progressive CLL suggested that this combination did not increase the risk of AIHA compared to historical data[78]. However, these data do not support re-treatment of patients with treatment-related autoimmune cytopenia with purine based combination therapy. The safest current treatments for these patients are combination therapies such as RCD, R-CVP or high dose methylprednisolone and rituximab even though these approaches could result in suboptimal durations of response.

Newer Treatment Options

Several new approaches have potential for improving the treatment of patients with CLL complicating autoimmune cytopenia. These include applications of novel treatments already under investigation for idiopathic autoimmune cytopenia (see below) and for progressive CLL in patients who do not have autoimmune cytopenia.

Patients with CLL and autoimmune cytopenias could benefit from measures to improve BM function. Lack of adequate amounts of folic acid could potentially limit BM responses to increased blood cell sequestration especially in AIHA, thus folic acid supplementation (1 mg/d by mouth) until resolution of hemolysis could be of benefit. Idiopathic ITP is associated with an inappropriately low level of thrombopoietin which is associated with decreased thrombopoiesis [37] and a similar defect could be present in CLL patients with ITP. Patients with CLL complicated by ITP could thus potentially respond to treatment with thrombopoietin receptor (TPO-R) agonists such as romiplostim (parental TPO-R peptide agonist) or eltrombopag (oral small molecule TPO-R agonist37. An additional novel approach to the treatment of ITP using SYK inhibitors83 could potentially also be applied to patients with ITP complicating CLL and may even be active against CLL cells84.

Improved therapy of CLL with novel regimens could also be of benefit in the treatment of autoimmune complications. Newer and potentially more efficacious anti-CD20 antibodies such as ofatumumab will need to be tested in patients with CLL and autoimmune cytopenia. Appropriate patients with relapsed/refractory CLL complicated by autoimmune cytopenia could be considered candidates for reduced intensity allogeneic stem cell transplantation which has been reported to achieve durable responses for patients with both CLL and autoimmune cytopenia[49]

Supportive care

The major complication and cause of death following treatment of autoimmune cytopenia in patients with CLL is serious infection11,12,[49]. Infection risk can be reduced by limiting the intensity and duration of immunosuppressive therapy, careful monitoring of the patient, and

by the use of appropriate anti-microbial prophylaxis. We recommend the use of prophylactic antimicrobials against Pneumocystis pneumonia for all patients on long term higher dose (≥ 20 mg /day) prednisone therapy. In addition, patients need to be educated about the risk of infection and treated early and aggressively for infections. An additional concern is that long term immunosuppression could increase the risk of development of second malignancies[12]. Patients should thus be monitored for second malignancies and educated to avoid excessive ultraviolet light and other carcinogens, especially smoking.

Practice Points

1. Autoimmune cytopenia needs to be considered in the differential diagnosis of patients with CLL who develop anemia and/or thrombocytopenia.
2. Patients diagnosed with apparently idiopathic autoimmune cytopenias need to be investigated for an underlying chronic B cell lymphoproliferative disorder.
3. Patients being considered for treatment of progressive CLL because of cytopenia need a BM examination to determine the etiology of their cytopenia.
4. Those patients with autoimmune cytopenia that do not have extensive BM involvement by CLL do not have advanced stage CLL.
4. Purine analogue and alkylator monotherapy can cause autoimmune cytopenia and should not be routinely used to treat patients with CLL complicated by autoimmune cytopenia.
5. The choice of treatment for patients with CLL complicated by autoimmune cytopenia depends on whether their CLL is progressive or not.
6. Autoimmune cytopenia complicating CLL is usually responsive to treatment but the duration of response is often short. Patients thus need careful long term follow up and some may benefit from maintenance therapy.

Research Agenda

1. Etiology of autoimmune cytopenia in CLL is not well defined and determining the mechanism could be very helpful in designing and implementing treatment strategies. Areas of research interest are the mechanisms by which CLL cells disrupt Treg function and the role of the CLL cells in antigen presentation.
2. There is no reliable method of detecting and quantifying pathogenic anti-platelet antibodies in patients with ITP. Development and validation of such a test could be valuable for diagnosis and monitoring the effects of treatment.
3. Multi-institutional trials will be required to test and evaluate for the most efficacious approaches from the current and novel treatments for autoimmune cytopenia complicating CLL.

Summary

Cytopenia, which is the most important autoimmune complication of CLL, can occur at any stage of the disease and can cause severe morbidity and mortality. Autoimmune cytopenia needs for be distinguished from cytopenia caused by progressive CLL and non CLL-related causes, and this will usually require at least a BM examination. The prognostic implications and management of cytopenia in CLL depends on its etiology. Autoimmune cytopenia clearly does not have the same independent poor prognostic significance as cytopenia caused by BM failure. Appropriate management requires early and accurate diagnosis of the autoimmune cytopenia and therapy should be adapted to the severity of both the cytopenia

and clinical stage of the CLL. Appropriate therapy can be highly effective but is rarely curative of the autoimmune cytopenia. Thus all patients require careful long term follow up and early intervention for relapse. Management of autoimmune complications of CLL could be improved by a better understanding of the pathogenesis of immune dysregulation, improvement of diagnostic tests (e.g. reliable assay for anti-platelet antibodies), and the development and testing of new therapies using existing and novel drugs.

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