

Infectious Complications of Chronic Lymphocytic Leukemia

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Infectious complications continue to be one of the major causes of morbidity and mortality in patients with chronic lymphocytic leukemia (CLL). The pathogenesis of infections in these patients is multifactorial. Hypogammaglobulinemia is an important predisposing factor for infection in patients with early-stage disease and for those treated with conventional alkylating agents. However, the proportion of patients treated with purine analogs and monoclonal antibodies such as rituximab and alemtuzumab is increasing. As a result of this therapy, these patients often experience profound and sustained T-cell immunodeficiency. Consequently, the spectrum of organisms causing infections in these patients is changing from common bacterial organisms to less common opportunistic pathogens such as *Pneumocystis*, *Listeria*, mycobacteria, herpesviruses, and *Candida*. This review focuses on the pathogenesis and risk factors for infections in patients with CLL, the spectrum of infectious organisms associated with the newer agents, and the management of these infections with an emphasis on prophylaxis and vaccination strategies.

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Chronic lymphocytic leukemia (CLL) remains the most common type of leukemia in the Western world, and infectious complications continue to be one of the principal causes of morbidity and mortality in patients with this disorder.¹⁻³ Alkylating agents such as chlorambucil have been the mainstay of treatment for CLL. In the last two decades there has been a dramatic increase in the armamentarium of antineoplastic agents available to combat this disorder, ranging from the purine analogs such as fludarabine and cladribine to the monoclonal antibodies rituximab and alemtuzumab. When used in combination, impressive complete response rates have been achieved for newly diagnosed CLL patients, with molecular remissions even being described with some of the newer combination regimens.⁴ These advances in our ability to induce complete and possibly long-lasting remissions come at a cost, however, with a spectrum of opportunistic infections due to the immunosuppressive effects of these newer agents.

Pathogenesis of Infection

The pathogenesis of infections in patients with CLL is complex and multifactorial. The CLL patient is rendered susceptible to infections due to disease-related immunosuppression, compounded by the state of often prolonged immunosuppression induced by the agents used to treat this disorder. About 80% of CLL patients will sustain infectious complications at some point during their disease course, and 50% to 60% of patients will die due to infection.⁵ Multiple aspects of the immune system are defective or aberrant in CLL patients, and often multiple deficiencies coexist in the same patient. While humoral immunodeficiency has long been considered the key defect, it is being increasingly recognized that T-cell and natural killer (NK) cell defects, as well as neutrophil dysfunction and defects in the complement system, contribute significantly to the state of immunodeficiency.

Hypogammaglobulinemia

The association between hypogammaglobulinemia and infections in CLL patients has been recognized and validated by studies dating back to the 1960s.⁶ Patients with hypogammaglobulinemia appear to be particularly vulnerable to recurrent bacterial infections, especially with encapsulated organisms. The incidence and severity of hypogammaglobulinemia increases with increased disease duration, and also with ad-

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vanced stage of the disease.^{1,2} Although the pathogenesis of hypogammaglobulinemia is probably secondary to abnormalities of nonclonal CD5⁻ B cells, the ability of large granular lymphocytes from CLL patients to downregulate B-cell immunoglobulin synthesis has also been implicated.⁷ The malignant CLL B cells themselves have likewise been implicated, by virtue of their ability to inhibit autologous CD95⁺ immunoglobulin-producing plasma cells through the interaction with CD95 ligand present on the malignant B cells.⁸ There is no consensus regarding correlation between infections and a deficiency of any particular immunoglobulin subclass. An increased risk of infection has been described with IgG deficiency in some reports, while others correlate infectious complications with IgA deficiency.² Rozman et al reported that a low level of gamma globulin (<700 mg/dL), as well as low initial IgG and IgA levels, correlates with a poorer survival; however, when adjusted for disease stage, only IgA deficiency correlated with poorer survival.⁹ These findings were corroborated by another group that demonstrated a significant correlation between low IgA levels and the risk of infections.¹⁰ Additionally, a small case-control study described selective deficiency of IgG3 and IgG4 subclasses in CLL patients compared to age- and sex-matched controls.¹¹ In an attempt to correlate the systemic humoral defect in CLL patients with mucosal immunity, the serum and mucosal (salivary) immunoglobulin levels were examined in a series of CLL patients and healthy controls.¹² Salivary IgM levels, but not salivary IgG or IgA levels, were decreased in CLL patients compared to healthy controls. Furthermore, although patients with low serum IgA levels had a higher infection rate, there was no correlation between serum IgM or IgG levels or salivary immunoglobulin levels and the infection rate. Additional studies are needed to determine the role of mucosal immunity of CLL patients, and indeed to understand if mucosal B cells are part of the malignant B-cell clone. Interestingly, in another study, no significant differences in immunoglobulin subclass levels, mannan-binding lectin, or responses to *Hemophilus influenzae* serotype b conjugate vaccination responses were found among two groups of patients that differed in the mutational status of the B-cell clone.¹³ In addition, some CLL patients have recurrent infections despite normal immunoglobulin levels, suggesting the possibility of qualitative immunoglobulin defects, which are unfortunately difficult to assess in a systematic manner. These patients often mount a poor secondary immune response with low titer IgG antibodies to common pathogens such as *Escherichia coli* and *Streptococcus pneumoniae* and demonstrate suboptimal responses to vaccination against *H influenzae* and *S pneumoniae*.¹⁴ These findings support the speculation that the inability of the B cells in CLL to mount an immune response to a particular pathogen may be more important than the hypogammaglobulinemia itself.

Defects in Cell-Mediated Immunity

Although studies suggest impaired cell-mediated immunity in CLL patients, it may be difficult to distinguish between

intrinsic defects related to the disease process and secondary defects acquired as a consequence of immunosuppressive therapy.¹⁵ Chiorazzi et al have demonstrated a functional defect in helper T cells in CLL patients.¹⁶ Similarly, other investigators have reported an increase in T-cell suppressor activity and reversal of the CD4/CD8 ratio, which may correlate with the disease stage and degree of hypogammaglobulinemia.¹⁷⁻¹⁹ The decreased capacity of enriched T cells (E-rosette positive) from CLL patients to induce differentiation of normal B lymphocytes compared to T lymphocytes from healthy controls in a pokeweed mitogen-stimulated system has also been demonstrated, suggesting a role of defective cell-mediated immunity in the pathogenesis of hypogammaglobulinemia in this disorder.²⁰ A marked decrease in NK cell activity in CLL patients compared to healthy controls has also been reported.²¹ This appears to correlate with the disease stage, being inducible with interferon in early- but not advanced-stage patients. Similarly, another group has demonstrated defective lymphokine-activated killer (LAK) cell activity, and reduced susceptibility of CLL B cells to autologous, and in most cases allogeneic LAK cells.²² It is not known whether or not these functional defects are reversible, although at least two studies suggest restitution of NK cell activity and antibody-dependent cell-mediated cytotoxicity (ADCC) in CLL patients after interferon treatment.^{23,24}

Complement Defects

The complement system plays a critical role in protection against infection with encapsulated organisms through opsonization and subsequent neutrophil activation. Multiple studies demonstrate abnormal complement activity in CLL patients. Schlesinger et al demonstrated a deficiency in at least one of the complement proteins in 18 of 26 CLL patients compared to age- and sex-matched controls.²⁵ The most common deficiency was of properdin (11/18 patients). These abnormalities correlated with disease stage (100% of patients with advanced-stage disease compared to 40% with early-stage disease) and with the frequency of infectious complications. Similarly, Varga et al found a correlation between low initial complement levels and a decreased overall survival, which was most prominent for patients with advanced disease stage.²⁶ Another group demonstrated reduced expression of complement receptors CR1 and CR2 on B-CLL cells, as well as decreased activation of the alternate complement pathway, but it is unclear if these translate into an increased risk of infection.²⁷

Neutrophil and Phagocytic Defects

Although the absolute neutrophil count is often normal in untreated CLL patients, eventually most will experience neutropenia as a consequence of progressive marrow involvement and myelosuppressive chemotherapy. Although some studies suggest that neutrophil function is normal in CLL patients, other reports have demonstrated enzyme deficien-

cies (myeloperoxidase and lysozyme) in the neutrophils and monocytes of untreated CLL patients, with normal levels being demonstrated in patients in remission.²⁸ A decrease in random migration and fMLP and C5a-induced chemotaxis in neutrophils of CLL patients with active infections compared to healthy controls or CLL patients without infections has also been demonstrated.²⁹

Spectrum of Infections

Therapy With Alkylating Agents

Bacteria account for the majority of infections in patients treated with alkylating agents with or without corticosteroids.³ Although a multitude of pathogens have been reported, infections with *Staphylococcus aureus*, *Streptococcus pneumoniae*, *Hemophilus influenzae*, *Klebsiella pneumoniae*, and *Escherichia coli* are predominant.³⁰ These bacterial infections have a propensity to occur at mucosal sites, especially the respiratory tract. Other common sites of involvement include the urinary tract, skin and soft tissues, and the bloodstream, the latter being more common in patients with severe neutropenia. In contrast to encapsulated organisms, infections caused by *Nocardia*, *Listeria*, or mycobacteria are relatively uncommon in CLL patients treated with conventional cytotoxic agents. Similarly, while fungal and viral infections are not commonly seen, infections with *Candida* and *Aspergillus* may occur in the context of prolonged therapy-related neutropenia, concurrent treatment with corticosteroids or prolonged therapy with broad-spectrum antibiotics. *Pneumocystis carinii* pneumonia (PCP) tends to be relatively rare in patients treated with alkylators. While herpesvirus infections do occur relatively frequently, they tend to be localized rather than disseminated, and despite their morbidity seldom account for mortality in this patient population.³

Therapy With Purine Analogs

Over the last two decades the purine analogs fludarabine, cladribine, and pentostatin have made a major impact on the treatment of CLL, as well as other indolent B-cell lymphoproliferative disorders. The most experience has been with fludarabine, which in large single-institution phase II and multicenter randomized phase III studies has emerged as one of the most active single agents in inducing remission in these patients.^{31,32} In addition, significant improvements in complete response (CR) rates have been observed when fludarabine has been combined with monoclonal antibodies and other agents.^{33,34} These improved outcomes come at the cost of not only an increased incidence but also a changing spectrum of infections, notably an increased incidence of opportunistic infections.³⁵

Fludarabine

The pathogenesis of infections related to fludarabine is multifactorial, related to both qualitative and quantitative T-cell abnormalities induced by treatment. Fludarabine undergoes intracellular phosphorylation to an active metabolite capable of inhibiting DNA and RNA synthesis. Additionally, fludara-

bine also mediates sustained inhibition of cytokine-induced activation of STAT1- and STAT1-dependent gene transcription in normal resting or activated lymphocytes, which can lead to a state of prolonged immunosuppression.³⁶ In an early study, fludarabine was found to induce a marked reduction in all T-cell subpopulations, with a decrease in CD4⁺ cells persisting for as long as 11 months after completion of treatment.³⁷ Keating et al reported single-institution data on 174 CLL patients who received first-line therapy with fludarabine.³² The median CD4⁺ count decreased from a pretreatment value of 1,562 cells/ μ L to 172 cells/ μ L after three cycles and 163 cells/ μ L after six cycles of treatment. A similar but less pronounced drop was observed with the CD8⁺ counts at the same time points. The effect of fludarabine treatment on immunoglobulin levels was variable, with some but not all patients demonstrating improvement in immunoglobulin levels. Other immunologic consequences of fludarabine therapy include monocytopenia and a transient reduction in NK cells.

The spectrum of infections in CLL patients receiving fludarabine includes not only bacterial infections, but also opportunistic infections caused by *Listeria*, *Nocardia*, and mycobacteria.^{38,39} Fungal infections are most often caused by *Candida* and *Aspergillus*, and may be disseminated.⁴⁰ The incidence of *Pneumocystis* infections is greater in patients treated with concurrent fludarabine and corticosteroids.⁴¹ Viral infections are common, especially varicella zoster virus (VZV) infection, with a high incidence of post-herpetic neuralgia.^{42,43} Other viral infections reported include adenovirus, cytomegalovirus (CMV), and reactivation of hepatitis B.

In an attempt to identify risk factors associated with infections, Anaissie et al retrospectively studied 402 patients at a single center who received fludarabine therapy either alone or in conjunction with corticosteroids.⁴¹ Many of these patients had received prior alkylating agents as first-line therapy. Major infections occurred in 58% (144 of 248) previously treated patients compared to 34% (53 of 154) previously untreated patients ($P < .001$). Risk factors for major infection identified by multivariate analysis included advanced stage disease (Rai stages III and IV), a history of previous chemotherapy, and elevated serum creatinine. A baseline granulocyte count of greater than 1,000 cells/ μ L appeared to be protective. Opportunistic infections as listeriosis and PCP were more frequent in previously treated patients who received concurrent fludarabine and corticosteroids. A baseline CD4⁺ count of less than 50 cells/ μ L was predictive of an increased likelihood of developing a cutaneous VZV infection.

Infection risk has also been examined in other series of fludarabine-treated patients. Response to fludarabine appears to inversely correlate with frequency of infections, with some studies revealing fewer infections in patients who attain a CR compared to those who achieve a partial response (PR).³² In another series, the frequency of serious infections leading to hospital admission increased after patients became refractory to fludarabine therapy, being reported as 0.17 infections per month in fludarabine-refractory patients compared to 0.03 infections per month in fludarabine-sensitive

patients ($P < .0005$).⁴³ Morrison et al reported on the infectious complications of patients enrolled on the intergroup trial Cancer and Leukemia Group B (CALGB) 9011, in which 544 previously untreated intermediate/high-risk CLL patients were randomized to therapy with chlorambucil, fludarabine, or both agents.⁴⁴ Patients treated with concurrent fludarabine and chlorambucil had significantly more infections than patients treated with either single agent ($P < .0001$). Comparing the two single-agent arms, there were more infections per month ($P = .055$), more major infections ($P = .008$), and more VZV infections ($P = .004$) in patients receiving fludarabine compared to chlorambucil. Of note, in this study no patients received concurrent corticosteroids, and the incidence of *Pneumocystis* infections was remarkably low ($<1\%$), despite the lack of PCP prophylaxis. *Candida* infections were relatively common, and there were no *Aspergillus* infections; two cases of mycobacterial infections were reported. Sorensen et al reported data on 724 patients treated with second-line fludarabine via the group C mechanism of the National Cancer Institute.⁴⁵ Grade IV hematologic toxicity was observed in 43% of patients and was associated with infection in 22% of patients. In another large three-arm French series of previously untreated Binet stage B or C patients randomized to therapy with fludarabine versus CAP (cyclophosphamide, vincristine, prednisone) or ChOP (intravenous vincristine and doxorubicin, and oral prednisone and cyclophosphamide), no opportunistic infections were noted in the 341 patients who received 6 months of fludarabine therapy.⁴⁶

While these studies differ considerably in trial design and patient and disease characteristics (refractory versus untreated), several generalizations related to fludarabine therapy in CLL patients and infectious complications may be made. Infectious complications are more commonly observed in those patients treated with fludarabine than with conventional alkylator-based therapy. Previously treated patients with advanced Rai stage disease and an elevated serum creatinine appear to be at the greatest risk for infection. Patients remain at an increased risk for infection for up to 1 year after completing treatment, with this risk lowest in patients who attain a CR. Once patients become refractory to fludarabine, their risk for major infections increases significantly. Opportunistic infections, especially PCP and listeriosis, are more common in previously treated patients, especially those receiving concurrent corticosteroids. Routine PCP prophylaxis for single-agent fludarabine therapy in the first-line setting does not appear to be warranted, based on the intergroup trial experience.

Cladribine

Although cladribine has not been as extensively investigated in CLL patients as fludarabine, and the two agents have not been compared in randomized studies, it appears that efficacy is equivalent, and similar rates and types of infectious complications are seen. Cladribine has also been investigated in hairy cell leukemia and other lymphoproliferative diseases, and has been shown to cause a prolonged and severe

decline in the CD4⁺ levels, which return to normal levels at a median of 40 months after therapy.⁴⁷ Juliusson et al studied lymphocyte subsets in blood and marrow of 68 patients treated with cladribine.⁴⁸ They demonstrated that although the CD8⁺ counts returned to within the normal range at a median of 3 months after completing treatment, the CD4⁺ lymphocytes required 1 to 2 years to return to normal. The incidence and spectrum of infections is similar to that observed with fludarabine therapy.^{49,50} As with fludarabine, risk factors for infection include a history of prior chemotherapy, advanced age, and a history of an infection within the 6 months preceding cladribine therapy.⁵¹ Prior treatment with other purine analogs appears to significantly enhance the risk of infectious complications. In one study, 28 patients with fludarabine-refractory disease who were subsequently treated with cladribine experienced significant myelosuppression with a high incidence of infections (65%), fever of unknown origin (21%), and pneumonia or bacterial sepsis (25%).⁵² Of the ten deaths occurring within 60 days of starting cladribine; eight could be attributed to infections. Robak et al reported on a cohort of 378 CLL patients treated with cladribine with or without prednisone, half of whom were previously treated.⁵³ Infections and fever of unknown origin were observed in 49% of pretreated and 38% of previously untreated patients ($P = .03$). HSV and VZV infections occurred in 25% of untreated and 20% of previously treated patients, respectively, while fungal pulmonary infections were seen in 7% of untreated patients. No cases of PCP or listeriosis were observed; however, it is not known if PCP prophylaxis was routinely administered. The same group conducted a large randomized study comparing cladribine to chlorambucil (both with concurrent prednisone) in 229 previously untreated CLL patients.⁵⁴ An increase in neutropenic fever and fever of unknown origin was noted in the patients receiving cladribine compared to those treated with chlorambucil (23 v 11%, respectively, $P < .02$), as were HSV and VZV infections (21 v 11%, respectively).

Pentostatin

Pentostatin is a potent inhibitor of the enzyme adenosine deaminase (ADA), which is present in high concentrations in lymphoid tissues. Inhibition of this enzyme leads to a clinical scenario similar to severe combined immunodeficiency (SCID), with the immune defects persisting for several months after discontinuing the drug.⁵⁵ In a phase II CALGB study of 39 CLL patients (33% previously untreated, 67% treated with one prior regimen), 52% of patients developed infectious complications, most of which occurred in pretreated patients with advanced stage disease.⁵⁶ The spectrum of infections was similar to that seen with the other purine analogs, and included streptococcal and pseudomonal infections, HSV and VZV, disseminated candidiasis, and PCP. In another multi-institutional Eastern Cooperative Oncology Group study, 39 previously untreated CLL patients received a combination of pentostatin, chlorambucil, and prednisone.⁵⁷ While the overall response rate was 87%, with a time to treatment failure of 32 months, the toxicity was con-

siderable. Grade 3 infections occurred in 31% of patients, including bacterial, fungal and *Pneumocystis* pneumonias, sepsis, and VZV infections. A third of patients had to discontinue therapy secondary to toxicity. Further studies using pentostatin in combination with cyclophosphamide with or without rituximab are ongoing.

Therapy With Monoclonal Antibodies

Over the last decade, considerable interest has emerged in incorporating the monoclonal antibodies rituximab and alemtuzumab into treatment regimens for CLL and other lymphoproliferative disorders. While alemtuzumab has shown considerable promise as a single agent in these patients, the responses to single agent rituximab have been considerably lower in patients with CLL or small lymphocytic lymphoma compared to other indolent lymphomas (12% v 58%, respectively).⁵⁸ The use of dose-escalated rituximab has been examined in several schedules.^{59,60} While infectious complications have been uncommon in these trials, rituximab has more commonly been studied in combination with agents as the purine analogs.

Alemtuzumab

The monoclonal antibody alemtuzumab is directed against the phosphatidylinositolglycan (PIG)-anchored CD52 glycoprotein, which is expressed in high density on most (>95%) normal and malignant B and T lymphocytes, NK cells, and monocytes, but not on CD34⁺ hematopoietic progenitor cells.⁶¹ Since the 1990s, considerable experience has been gained with the use of alemtuzumab in the therapy of lymphoid malignancies, particularly CLL.⁶² This agent appears to be promising in multiple high-risk subsets of CLL patients, such as those with extensive marrow involvement or with *p53* mutations.⁶³ Although acute infusional events commonly occur with this agent, a major toxicity concern is the lymphocytopenia induced by alemtuzumab, whether administered by an intravenous (IV) or subcutaneous (SC) route, rendering patients susceptible to opportunistic infections.

Immune reconstitution following alemtuzumab therapy has also been extensively studied.⁶⁴⁻⁶⁶ A profound reduction in peripheral blood CD4⁺ cells has been noted, with a nadir 4 weeks after therapy, followed by slow but steady recovery.⁶⁴ This finding was confirmed in a study by Rai et al, in which a profound reduction in B cells, CD4⁺ and CD8⁺ T cells, and NK cells was observed.⁶⁵ The CD4⁺ and CD8⁺ cells nadired by the fourth week of treatment, but there was evidence of some T-cell recovery (although not to baseline levels) by the end of the 16 week treatment period, with continued recovery at follow-up 4 weeks after completing treatment. Lundin et al reported on patients treated with an 18-week course of thrice-weekly SC alemtuzumab.⁶⁶ Immunologic parameters pretreatment were compared to post-treatment levels, with a significant reduction in the CD4⁺ cells (median, 1,524 v 43 cells/ μ L, respectively) and CD8⁺ cells (median, 1,167 v 20 cells/ μ L, respectively). There was a similar profound depletion of the B cells, NK cells, and monocytes. The median time to reach a peripheral blood

CD4⁺ and CD8⁺ counts of greater than 100 cells/ μ L was 4 months, with median counts remaining less than 25% of baseline values for all lymphocytes subsets until 9 months post-therapy. No correlation was observed between the cumulative dose of alemtuzumab and the duration or severity of immunosuppression. CD52⁻ T-cell subsets arose during therapy, and comprised greater than 80% of all CD4⁺ and CD8⁺ cells in the blood at the end of therapy. The emergence of CD52⁻ T cells with a paroxysmal nocturnal hemoglobinuria (PNH) phenotype has also been reported by other investigators, suggesting that alemtuzumab selects for cells deficient in CD52, allowing for the emergence of a PNH-like clone.^{67,68}

The increased risk of infection with alemtuzumab therapy is a consequence of the prolonged and profound lymphocytopenia. Patients are at risk for opportunistic infections beginning 3 to 4 weeks after the start of alemtuzumab therapy (which corresponds to the nadir CD4 and CD8 counts) and lasting at least 12 to 16 weeks following completion of therapy. The spectrum of infections includes bacterial septicemias and pneumonias, as well as opportunistic infections with CMV, *Aspergillus*, and PCP. In an early report, 29 patients with relapsed or alkylator-refractory CLL were treated with IV alemtuzumab thrice weekly for a period of 3 weeks.⁶⁹ Grade 4 neutropenia occurred in 10% of patients. Infectious complications were significant and included localized HSV reactivation in 38%, PCP pneumonia in 7%, bacterial pneumonia in 14%, and septicemia in 10%. Routine antimicrobial prophylaxis was not administered on this study. Similarly Rai et al reported on 24 fludarabine-refractory patients treated with IV thrice-weekly alemtuzumab for a maximum of 16 weeks.⁶⁵ Antimicrobial prophylaxis was at the discretion of the treating physician. Opportunistic infections developed in 10 patients (42%), and were more common in nonresponders (8/16) compared to responding patients (2/8). Infections included two cases of PCP (neither patient had received prophylaxis), as well as candidiasis, disseminated herpes zoster, and invasive aspergillosis. In a subsequent multicenter study of IV alemtuzumab in 93 fludarabine-refractory CLL patients, grade 3 or 4 infections were reported in 25 patients (27%).⁶⁴ On this trial, prophylaxis with trimethoprim/sulfamethoxazole (TMP/SMX) and famciclovir was mandated, beginning on day 8 and continuing for at least 2 months after completion of treatment. Only 3/31 (10%) responding patients developed grade 3 or 4 infections compared to 36% of nonresponders. Septicemia occurred in 14 patients (15%). Eleven patients (12%) developed opportunistic infections, which included *Aspergillus* pneumonia (n = 1), invasive aspergillosis (n = 1), rhinocerebral zygomycosis (n = 1), systemic candidiasis (n = 1), cryptococcal pneumonia (n = 1), *Listeria* meningitis (n = 1), and PCP pneumonia (n = 1). Seven cases of CMV reactivation were noted, with three cases of grade 2 and four cases of grade 3 infection. Opportunistic infections were more common in the more heavily pretreated patients. Nine deaths occurred during treatment or within a 30-day follow-up period, of which five were related to infectious complications. The infectious complications on this study appeared less common than in other

studies in which prophylaxis was not routinely administered. In another series of 34 relapsed/refractory CLL patients treated with alemtuzumab, five (15%) developed CMV viremia at a median of 28 days from the first treatment dose.⁷⁰ All patients were febrile but none had other clinical evidence of CMV infection; all five patients responded to ganciclovir with prompt resolution of fever and viremia. In the first-line setting, Lundin et al treated 41 symptomatic CLL patients with SC alemtuzumab thrice weekly for 18 weeks.⁷¹ Antimicrobial prophylaxis was administered during and continued for 8 weeks following treatment, and consisted of valacyclovir 500 mg orally twice daily, fluconazole 50 mg every day, and TMP/SMX thrice weekly. Treatment was well tolerated with no episodes of febrile neutropenia or major (grade >1) bacterial infections. CMV reactivation occurred in four patients (10%) and resolved either spontaneously or promptly following intravenous ganciclovir. Only one patient developed PCP pneumonia; this patient was allergic to TMP/SMX and had not received prophylaxis. Similarly, SC alemtuzumab was studied in 16 heavily pretreated (median of three regimens, including alkylators and fludarabine) patients.⁷² All patients received PCP prophylaxis, and no cases of PCP were observed. Asymptomatic CMV reactivation occurred in two patients and resolved without treatment. One patient died from a polymicrobial infection.

In summary, these data suggest a high frequency of bacterial and opportunistic infections in patients treated with alemtuzumab, whether administered by an IV or SC route. As with the purine analogs, the infections are more common in heavily pretreated patients and in nonresponders. CMV reactivation appears to be particularly frequent, although it usually responds promptly to ganciclovir treatment. Strong consideration should be given to antimicrobial prophylaxis with TMP/SMX, acyclovir, or famciclovir and fluconazole during alemtuzumab therapy. Prophylaxis should be continued for at least 8 to 12 weeks following completion of treatment.

Combination Chemoimmunotherapy

In an effort to improve CR and disease-free survival rates, and based on in vitro evidence of synergy, combinations of rituximab and purine analogs have been investigated. In a randomized phase 2 CALGB study in which rituximab was administered either sequentially or concurrently with fludarabine in 104 previously-untreated CLL patients, grade 3 or 4 infections occurred in 23% and 20% of patients, respectively.⁷³ Opportunistic infections were seen in eight patients on the concurrent arm (16%) and 14 (26%) on the sequential arm. Most were localized viral infections; PCP pneumonia occurred in only two patients. Based on these findings, the authors concluded that routine prophylaxis for herpesvirus infections was justified, but routine PCP prophylaxis was not warranted. Chemoimmunotherapy with fludarabine, cyclophosphamide, and rituximab (FCR) in both previously untreated and relapsed/refractory patients has recently been reported.^{4,33} When used as initial therapy in 224 CLL patients, although combined grade 3 and 4 neutropenia

was observed in 52% of patients, only 2.6% of treatment courses were associated with major infections, and there was a low incidence of PCP ($n = 3$), *Aspergillus* ($n = 2$), and CMV ($n = 1$) infections.³³ Reactivation of herpes infection (herpes simplex = 8, herpes zoster = 3) was seen in 11 patients (5%). In this study, approximately half the patients received prophylactic TMP/SMX and two thirds received prophylactic valacyclovir. There was no difference in the incidence of major or minor infections in those who received TMP/SMX prophylaxis; however, VZV reactivation occurred in none of the 154 patients who received prophylactic valacyclovir compared to three of 70 patients who did not receive treatment. The use of FCR in 177 patients with relapsed CLL has also been recently reported.⁴ Grade 3 and 4 neutropenia occurred in 62% of courses. Major infections (sepsis, pneumonia, or infections necessitating hospitalization) occurred in 16% of treated patients, in 5% of assessable courses. One patient developed CMV pneumonia. Herpes simplex and zoster infections were associated with 1% of assessable treatment courses. The incidence of major infections was not higher in fludarabine-refractory patients compared to fludarabine-sensitive patients.

Currently alemtuzumab is also being studied in different combination regimens. Faderl et al reported on the combination of alemtuzumab and rituximab therapy in 48 patients with relapsed, refractory B-cell malignancies (the majority of which were CLL).⁷⁴ All patients received prophylactic TMP/SMX and valacyclovir. Infections occurred in 25 patients (52%) and CMV antigenemia was observed in 13 patients (24%); of these only seven (13%) were symptomatic and needed therapy. The authors concluded that this combination was well tolerated, with a toxicity profile comparable to single agent alemtuzumab. In a preliminary report, Rai et al described 56 CLL patients treated on CALGB 19901 with a sequential regimen of fludarabine (monthly for four cycles) followed by 6 weeks of IV alemtuzumab for those patients who had stable or responding disease.⁷⁵ Prophylactic TMP/SMX and acyclovir were mandated during and for 6 months following alemtuzumab therapy. Grade 3 or 4 major infections were observed in 12 of 36 patients who received alemtuzumab. Infections with CMV occurred in eight patients during or within 4 months of completion of alemtuzumab therapy. The outcome was fatal in one patient. Another patient had persistent CMV infection, while resolution of the infection was partial/complete in the remaining six. In response to the CMV event, the authors initiated vigorous CMV surveillance by weekly qualitative polymerase chain reaction (PCR)-based testing to enable early recognition and treatment. This same study was extended to a second cohort of patients who received 6 weeks of SC (instead of IV) alemtuzumab therapy following fludarabine induction.⁷⁶ All patients underwent weekly PCR assays for CMV surveillance. Of the three patients who converted to CMV seropositivity, all recovered with ganciclovir therapy. Additional studies combining alemtuzumab and purine analogs are ongoing, and will be of interest, not only for efficacy, but also with respect to toxicity, especially infectious complications.

Strategies for Prevention of Infection

Given the high rate of infectious complications inherent to the disease process itself and the various treatment modalities that render further immunosuppression, identification of effective prophylactic strategies for this patient population is critical. These approaches include the use of prophylactic immunoglobulin for high-risk patients, the use of prophylactic antimicrobial agents, and vaccination against common pathogens.

Immunoglobulin Therapy

In a multicenter double-blind study, 84 CLL patients at high risk for infections (history of hypogammaglobulinemia, infections, or both) were randomized to receive IV immunoglobulin G (IVIG) (400 mg/kg) every 3 weeks or placebo.⁷⁷ While moderately severe bacterial infections were reduced by 50% in patients receiving IVIG, there was no subsequent reduction in the incidence of nonbacterial infections or mortality in these patients. A subset of these patients continued IVIG in the same schedule in a crossover double-blind study.⁷⁸ Serious infections were less common in the months in which the patients received IVIG ($P = .001$). Another group treated 15 patients with CLL (history of hypogammaglobulinemia, recurrent infections or both) with a fixed dose of IVIG at 10 g every 3 weeks, and compared the infection-related events in these patients over a 169 patient-month treatment period to the 168 patient-months preceding the IVIG treatment.⁷⁹ The number of infection-related hospital admissions and fevers was significantly lower during the IVIG treatment period. In another study with crossover design, a significant reduction in the incidence of infectious complications was observed during IVIG prophylaxis (300 mg/kg every 4 weeks) compared to those patients being observed.⁸⁰ However, interestingly the restoration of serum IgG levels in 17 of 25 treated patients did not parallel a decrease in the infectious events. Another study compared two different IVIG dose regimens (250 mg/kg v 500 mg/kg every 4 weeks) for infectious events in 34 high-risk CLL patients.⁸¹ There was no significant difference in the incidence of infections, although both groups had significantly fewer infections compared to historical placebo-treated controls. Despite the encouraging results from these trials, several caveats are of importance. IVIG therapy does not replace IgA and IgM, both of which have been implicated, at least in some studies, in the increased incidence of infection. Additionally, in a cost-effectiveness analysis, it was determined that IVIG therapy resulted in a gain of 0.8 quality-adjusted days per patient per year of therapy at a cost of \$6 million per quality-adjusted life year gained, thus implying that IVIG is not cost-effective in preventing infections in CLL patients.⁸² Furthermore, IVIG has never been prospectively compared to prophylactic antibiotics, which may be more cost-effective and efficacious for these patients. Additionally, in the era of purine analogs and alemtuzumab therapy, most of the serious and opportunistic

infections are secondary to T-cell defects, which are not likely to be altered by IVIG therapy.

Vaccination

Although CLL patients are vulnerable to infections with encapsulated organisms, vaccination strategies have often been disappointing in these patients, especially in those with advanced refractory disease, because of impaired primary and secondary immune responses.¹⁴ Serologic responses to pneumococcal polysaccharide and *Hemophilus influenzae* type b vaccines were evaluated in 25 CLL patients, with a very modest increase in post-vaccination protective antibody levels.⁸³ The patients who developed a response were those with early stage disease and higher baseline immunoglobulin levels. Similarly, influenza vaccine appeared to be more effective in rendering an immune response in patients whose baseline IgG level was greater than 700 mg/dL. In another study, 31 CLL patients and 25 controls were studied for serologic responses to vaccination against pneumococcal polysaccharide, *Hemophilus influenzae* type b conjugate and tetanus toxoid antigens.⁸⁴ While the control group demonstrated a robust antibody response to all antigens, the patient group demonstrated responsiveness only to the *Hemophilus* conjugated vaccine, with 54% patients developing post-vaccination antibodies at the protective level. The authors concluded that plain polysaccharide antigens appear to be ineffective, while conjugated vaccines may generate responses even in hypogammaglobulinemic patients with advanced stage disease. Other studies have demonstrated improved in vivo antibody production to *Hemophilus* type b and tetanus-toxoid conjugated antigens by achieving histamine type-2 receptor blockade by ranitidine.⁸⁵ These studies have only evaluated the aspect of seroconversion, whereas data is scant about the more important clinical endpoints of protection from infection, prevention of overt infection and death. Further novel strategies are needed to enhance the immunogenic potential of vaccines or to augment the host response to vaccines in this patient population.

Antimicrobial Prophylaxis

No prospective randomized studies have validated the role of prophylactic antibiotics in CLL patients. Much of these data come from several larger studies, many of which leave the decision to initiate antibacterial prophylaxis to the treating physician. Nevertheless, some general conclusions can be drawn. Antimicrobial prophylaxis is probably neither needed nor cost-effective in early-stage CLL patients with normal or minimally decreased immunoglobulin levels, and is probably not required during conventional alkylator therapy. The data regarding purine analogs is more controversial. In CALGB 9011, the incidence of PCP was remarkably low (<1%) despite the lack of prophylaxis in patients treated with single agent fludarabine.⁴⁴ In Anaissie's study, the incidence of infections was highest in fludarabine-treated patients who had received prior chemotherapy, in patients with an advanced Rai stage disease, and in those patients with an elevated serum creatinine.⁴¹ One may consider antibacterial prophylaxis

laxis in these very high-risk patients with either amoxicillin/clavulanic acid or TMP/SMX; the latter also offers the advantage of PCP prophylaxis. If prophylaxis is initiated, it should be continued for at least 2 months after completion of treatment. PCP and *Listeria* infections are common only in patients who are receiving concurrent fludarabine and corticosteroids. These patients should be considered for prophylaxis with TMP/SMX. Antiviral prophylaxis might benefit patients with a low initial CD4⁺ count (<50/ μ L).

With single-agent alemtuzumab therapy, early studies did not incorporate routine antimicrobial prophylaxis, and there was a high incidence of bacterial infections as well as opportunistic infections.^{65,69} Most of the subsequent studies have routinely included PCP prophylaxis with TMP/SMX and viral prophylaxis with famciclovir. The incidence of PCP pneumonia has been low, but CMV reactivation continues to be a problem and needs to be considered in those patients on alemtuzumab who develop fever. Weekly surveillance for CMV infection by qualitative PCR-based assays should be considered during the treatment period.⁷⁶ One should also consider TMP/SMX, famciclovir and fluconazole prophylaxis in alemtuzumab-treated patients, especially in heavily pretreated patients who have also received purine analogs in the past.

Conclusions

CLL patients are susceptible to infectious complications secondary to immune defects associated with the primary disease process, and also due to immunosuppression secondary to treatment. In patients with early-stage disease, infection risk is mainly related to hypogammaglobulinemia. T-cell defects become increasingly common in patients treated with purine analogs and alemtuzumab. While patients are at risk for infections with common bacteria at any disease stage or with any treatment modality, the spectrum of infections has changed dramatically over the past two decades with the emergence of a multitude of opportunistic infectious agents. Heavily pretreated patients appear to be at the greatest risk for serious infections, and should be considered for appropriate antimicrobial prophylaxis. As more trials incorporate combinations of purine analogs and monoclonal antibodies, it will be important not only to analyze traditional outcomes like response rates, progression-free and overall survival, but also to determine the impact these treatments may have on the incidence and spectrum of infectious complications.

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