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Infectious complications of chronic lymphocytic leukaemia: pathogenesis, spectrum of infection, preventive approaches

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Infectious complications continue to be a major cause of morbidity and mortality in patients with chronic lymphocytic leukaemia (CLL). The pathogenesis of infections in these patients is multifactorial, related to inherent immune defects and therapy-related immunosuppression. Hypogammaglobulinaemia is an important predisposing factor for infection in all patients. The use of the purine analogues such as fludarabine, and monoclonal antibodies such as rituximab and alemtuzumab, has introduced a new spectrum of infectious complications caused by pathogens such as *Pneumocystis*, *Listeria*, mycobacteria, herpesviruses *Candida* and *Aspergillus*, related to the cellular immune suppression induced by these agents. This review focusses on the pathogenesis and risk factors for infections in patients with CLL, the spectrum of infectious complications and preventive approaches to infection in these patients, using antimicrobial and immunoglobulin prophylaxis and vaccination strategies.

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Infections have been known to have a major impact on the course of patients with chronic lymphocytic leukaemia (CLL) over many decades. This disease is unique in that not only are underlying abnormalities in immune function related to the primary disease process present, but also further perturbations in immune function occur, related to the therapies rendered for the primary disease process. The spectrum of infections diagnosed in CLL patients has evolved, in relation to the introduction of new effective therapeutic agents as the purine analogues and monoclonal antibodies. Approaches for prevention of infection include antimicrobial prophylaxis, as well as immunoglobulin

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replacement and vaccination. We discuss the pathogenesis of infection in patients with CLL, as well as the spectrum of infectious complications and approaches to infection prevention.

Pathogenesis of infection in patients with chronic lymphocytic leukaemia

Patients with CLL are at risk for infection for a variety of aetiologies. These patients have inherent immune defects in humoral, as well as cell-mediated, immunity, which are related to the primary disease process. These defects include hypogammaglobulinaemia, abnormalities in T-cell subsets and defects in complement activity and neutrophil/monocyte function [1,2]. In addition, specific immunodeficiencies related to therapies rendered to these patients result in additional immunosuppression.

Hypogammaglobulinaemia is the most recognised inherent immune defect in patients with CLL. It is related to defective functioning not only of T cells, but also of the non-clonal CD5-negative B cells. The immunoglobulin subtypes IgG3 and IgG4 are predominantly affected, resulting in lower levels of these immunoglobulins. Hypogammaglobulinaemia occurs in virtually all CLL patients during their disease course, and tends to become more pronounced with prolonged disease duration and advanced stage disease. Generally there is no reversal in this defect, even in the setting of clinical response to a given treatment intervention. An association between hypogammaglobulinaemia and the frequency of infections and survival in these patients has been demonstrated. However, no consistent correlation between a specific immunoglobulin class deficiency and infection risk has emerged [1,3,4]. The respiratory tract is the most common site of infection in these patients, which may be related to deficiencies in serum IgA and IgG4, as well as defects in mucosal immune function [4].

Several recent studies have examined the impact of immunoglobulin V_H mutational status on humoral immunity and infections [5,6]. Sinisalo et al. reported no significant differences in levels of immunoglobulins or mannan-binding lectin, immune response to *Hemophilus influenzae* B vaccination and rate of infections among patients with mutated, versus unmutated, immunoglobulin V_H genes status [5]. In a retrospective review of 231 CLL patients, those with unmutated immunoglobulin V_H genes ($n = 52$) had a shorter time to first infection (31 vs. 62 months, $p < 0.001$) and higher infection-related mortality ($p < 0.001$) than patients with mutated immunoglobulin V_H genes [6]. Immunoglobulin levels were comparable among these patients. Those patients with abnormalities in p53, as well as CD38-positivity cells had a shorter time to first infection ($p < 0.001$). ZAP-70 levels had no impact occurrence. Lastly, no correlation between any of these parameters and recurrent infections was found.

Despite the common occurrence of mucosal infections as respiratory tract infections in CLL patients, the integrity of mucosal immune function, the relationship between systemic and mucosal immune dysfunction and the characterisation of mucosal B cells are not well delineated. It is not clear if mucosal immunity is regulated independently of systemic immunity in these patients, and if mucosal B cells are part of the malignant B-cell clone. In a preliminary report in which serum and mucosal (salivary) immunoglobulin levels were measured in CLL patients, salivary immunoglobulin M (IgM) levels were profoundly decreased in hypogammaglobulinaemic CLL patients, but no differences were found in salivary immunoglobulin G (IgG) or immunoglobulin A (IgA) levels among CLL patients and controls [4]. In addition, no correlation was found between salivary immunoglobulin levels and infection occurrence.

Although it is known that CLL patients also have inherent defects in various parameters of cell-mediated immunity and complement activity, the impact of these defects on infection risk and the reversibility of these defects with therapeutic response are less clear than the associations demonstrated with hypogammaglobulinaemia. Similar to B-cell defects, the T-cell defects are more pronounced with advanced-stage disease. This may be related to secretion of transforming growth factor beta (TGF-b), which inhibits B-cell proliferation, by B-CLL cells, as well as release of circulating IL-2 receptor, which binds endogenous IL-2 resulting in down-regulation of T-cell function. Furthermore, the CD8-positive T cells secrete interleukin (IL)-4, which induces bcl-2 protein expression, possibly contributing to the CLL pathogenesis and disease progression.

Decreased levels of at least one component of the complement system are found in most advanced-stage CLL patients, and also in many with early-stage disease [1]. The most common deficiency

described has been that of properdin. Defects in complement activation and binding and reduced expression of complement receptors CR1 and CR2 on B-CLL cells have also been found.

Quantitative and qualitative neutrophil and monocyte defects have also been reported in CLL patients. The absolute neutrophil count, which is normal to slightly decreased in untreated patients, may decline further with increased marrow involvement with CLL and secondary to therapy-related myelosuppression. Defects in neutrophil phagocytic and bactericidal activity, including a decrease in C5a-induced chemotaxis, have also been demonstrated [1]. Lastly, deficiencies in b-glucuronidase, lysozyme and myeloperoxidase levels in monocytes have been reported.

Spectrum of infectious complications in patients with CLL

There has been an evolution in the spectrum of infections that are more commonplace in patients with CLL. For the most part, this has been related to the introduction of newer therapies that have their own unique impact on immune function, as the purine analogues and monoclonal antibodies. We discuss this spectrum as follows by type of therapy administered.

Alkylator-based therapies

For many years, the standard therapy for patients with CLL was chlorambucil, given alone or in combination with corticosteroids. As in previously untreated patients, the majority of infections occurring in alkylator-treated patients are bacterial, caused by common organisms such as *Staphylococcus aureus*, *Streptococcus pneumoniae*, *Haemophilus influenzae, Escherichia coli*, *Klebsiella pneumoniae* and *Pseudomonas aeruginosa* [1]. Recurrent infections are a common phenomenon in these patients, as is a mucosal origin of infection, especially the respiratory and urinary tracts. Fungal and viral infections, including those caused by opportunistic organisms, occur much less often in patients receiving alkylator-based therapy. When seen, this is generally in heavily pre-treated patients with advanced-stage disease and therapy-related neutropenia.

Fludarabine-based regimens

With the introduction of the purine analogues, specifically fludarabine, for initial therapy of CLL patients over the past two decades, based upon improved treatment outcome, a different spectrum of infectious complications has emerged [7–13]. The purine analogues result in quantitative and qualitative T-cell abnormalities underlying the pathogenesis of these infections. Inhibition of cytokine-induced activation of STAT-1- and STAT-1-dependent gene transcription results in a decline in peripheral blood T-cell counts, which is seen early in the treatment course and persists for up to 2 years after discontinuation of therapy [14]. A greater impact is seen on CD4-positive T cells, than on CD8-positive or NK cells. Fludarabine also leads to decreases in B-cell and monocyte counts and has a variable impact on immunoglobulin levels. Besides the occurrence of bacterial infections common to CLL patients, a variety of opportunistic infections, caused by organisms such as *Listeria, Mycobacterium*, *Nocardia,* systemic fungi such as *Candida*, *Aspergillus*, and *Cryptococcus*, *Pneumocystis*, and herpesviruses such as cytomegalovirus (CMV), varicella zoster and herpes simplex, have been reported [8–12]. The addition of corticosteroids to fludarabine increases the risk of these opportunistic infections, and therefore is generally avoided [8,11,13].

Risk factors for infection in fludarabine-treated patients have been identified from various large treatment series. Infections are most common in the first several cycles of therapy and relatively uncommon after discontinuation of therapy in responding patients [8]. Therapeutic response may be a risk factor, although treatment response may not correlate with immune reconstitution [9,10]. Other demographic characteristics identified as infection risk factors have included advanced-disease stage, receipt of prior CLL therapy, elevated lactic dehydrogenase (LDH) and renal insufficiency [8,9,13,15–17]. In a retrospective review of 187 CLL patients, risk factors for major infections identified in multivariate analysis included the number of prior regimens (risk ratio [RR], 1.8) and haemoglobin <12 g dl^{-1} (RR, 0.6) [16]. Age and renal function had no impact on infection risk in this analysis; the number of prior regimens was the only risk factor for herpes zoster infections. A low baseline IgG level was identified as

a risk factor for infection in the North American intergroup trial in which 544 patients received initial CLL therapy with fludarabine, chlorambucil or both agents (F+C) ($p = 0.02$) [12]. Advanced age and a decreased creatinine clearance were risk factors only in F+C-treated patients ($p = 0.004$ and 0.03, respectively). No association between infection and response to therapy or advanced-stage disease was found in this series.

Infection risk and the spectrum of infection in patients treated with fludarabine, as compared to conventional alkylator-based regimens, have been examined in several series [12,18]. In a large trial in which advanced-stage CLL patients were randomised to initial therapy with single agent fludarabine or one of two anthracycline-based regimens, no opportunistic infections occurred in the fludarabine-treated patients [18]. The infectious complications among patients randomised to chlorambucil, fludarabine or F+C on the intergroup trial have also been reported [12]. Patients receiving F+C had more infections than patients receiving either single agent ($p < 0.0001$). Comparing the patients treated with either single agent, those receiving fludarabine had more infections per month of follow-up, major infections and herpesvirus infections compared to patients treated with chlorambucil ($p = 0.055$, 0.008, 0.004, respectively). In total, only three *Pneumocystis* infections occurred, and no *Aspergillus* infections were seen. Lastly, in a meta-analysis of single agent fludarabine or alkylator-based therapy trials, grade 3/4 infections were more common with fludarabine therapy [19].

Based upon the initial treatment success with fludarabine, the combination of fludarabine plus cyclophosphamide (FC) has been studied in several CLL trials [20–23]. Infection rates of <10% were reported in both arms of the randomised intergroup trial of initial CLL therapy with FC plus growth factor support and antiviral prophylaxis, or single agent fludarabine [21]. All patients received *Pneumocystis* prophylaxis. The German CLL Study Group studied FC as first-line therapy in a phase 2 trial, as well as a randomised study, comparing it to single agent fludarabine in younger CLL patients [22,23]. In the latter study, the occurrence of severe and opportunistic infections was comparable (fludarabine 33%, FC 40%) [24]. However, dose reductions due to myelosuppression were more common with FC, which may have affected the infection rate. The addition of the bcl-2 antisense agent oblimersen to the FC regimen does not appear to increase the risk of opportunistic infections [25]. In one series of previously treated patients, 57% of FC-treated patients had infections or fever of unknown origin, including 26% caused by herpesviruses, and 7% by fungal pathogens [26]. The incidence of these infections in the first three cycles of therapy was 74%. Lastly, Epstein–Barr virus (EBV)-associated lymphoproliferative disorders have been reported in patients who received FC followed by autologous stem cell transplant (SCT) [27].

Rituximab-based regimens

Rituximab has been studied as a single agent as well as part of combination regimens for therapy of CLL. This agent causes a transient reduction in the B-cell count, which occurs early in the treatment course. Infections, including opportunistic infections, are uncommon when rituximab is used as a single agent [28]. In the CALGB study of concurrent versus sequential fludarabine plus rituximab (FR) in previously untreated CLL patients, the grade 3/4 infection rate was 20% [29]. Opportunistic infections, most of which were localised herpesvirus infections, occurred in eight patients receiving concurrent FR (16%), and 14 patients receiving sequential therapy (26%). Only two cases of *Pneumocystis* pneumonia occurred among these patients.

The fludarabine, cyclophosphamide and rituximab (FCR) regimen has been studied as initial therapy and as therapy for relapsed/refractory CLL [30,31]. In general, antiviral and *Pneumocystis* prophylaxis were used with this regimen. In 224 treatment-naive patients, although a third of patients had at least one infection and 10% had fever of unknown origin, only 2.6% had major infections [30]. Although 5% of patients had reactivation of herpes simplex or zoster, none occurred in patients on antiviral prophylaxis. A small number of opportunistic infections were seen (*Pneumocystis, Aspergillus, Candida glabrata* and CMV). In a large series of relapsed/refractory patients receiving FCR therapy, major infections occurred in 16%, including herpesvirus infections complicating 1% of treatment courses and one case of CMV pneumonitis [31]. Therapy was terminated early in 6% of patients due to infections. Incidence of major infection was comparable in fludarabine-sensitive and fludarabine-refractory patients.

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Other purine analogues

As with fludarabine, treatment with 2-chlorodeoxyadenosine (2-CDA) and deoxycoformycin (pentostatin) results in quantitative abnormalities in T-cell subsets that persist after discontinuation of therapy for as long as 1–2 years. Risk factors for infection with these agents are similar to those found with fludarabine. Prior therapy was a risk factor for infection in a series of patients age <55 years treated with 2-CDA (45% vs. 26%, $p < 0.05$) [32]. A 16% infection rate was found in another series of elderly patients treated with 2-CDA reported by these same authors [33]. In a phase II trial of 2-CDA therapy in patients with fludarabine-refractory CLL, grades 3–5 infections occurred in 43% of patients [34]. Bacterial infections were most common, although cases of herpesvirus infection, toxoplasmosis and candidal esophagitis were also reported. Additional opportunistic infections such as disseminated herpes zoster and pulmonary aspergillosis have also been reported in refractory CLL patients treated with 2-CDA [35]. In a series of 378 CLL patients treated with 2-CDA alone or with prednisone, infections/fever of unknown origin and herpesvirus infections occurred more commonly in previously treated than in treatment-naive patients (49% vs. 38%, $p = 0.03$, and 25% vs. 20%, respectively) [36]. In another trial in which patients were randomised to initial therapy with 2-CDA or chlorambucil plus prednisone, febrile neutropenia/fever of unknown origin were more common with 2-CDA therapy (56% vs. 40%, $p = 0.02$), as were herpesvirus infections (21% vs. 11%) [37]. In a phase II CALGB trial of pentostatin therapy in patients both previously treated and treatment-naive, infections occurred in over 50% of patients, especially heavily pretreated patients, and were seen early in therapy [38]. Opportunistic infections (e.g., herpesviruses, *Candida* and *Pneumocystis*) occurred in 26% of patients. In an ECOG study of pentostatin, chlorambucil and prednisone initial CLL therapy, grade 3 infections were seen in 31% of patients, including herpes zoster infection in 20% [39]. The regimen of pentostatin, cyclophosphamide and rituximab (PCR) has been used in previously treated CLL patients, with grade 3/4 infections seen in 28% of patients [40]. As first-line therapy, this regimen resulted in a 10% incidence of grade 3/4 infections [41]. *Pneumocystis* and antiviral prophylaxis are generally used with this regimen.

Alemtuzumab

In initial trials of therapy with the anti-CD52 antibody alemtuzumab for CLL, therapy-related immune defects noted were neutropenia and reductions in B, T and NK cells, that develop early in treatment, persist for up to a year after therapy is discontinued and are unrelated to the route of administration [42–48]. This agent is usually given with *Pneumocystis*, antifungal and antiviral prophylaxis. CMV reactivation is a major issue, occurring in 10–25% of patients. Other opportunistic infections reported include cases of toxoplasmosis, adenoviral infection and acanthamoebiasis [49]. In the pivotal trial of this agent for fludarabine-refractory patients, 27% of patients had grade 3/4 infections, caused by pathogens such as *Aspergillus, Zygomycetes, Candida*, *Listeria*, *Pneumocystis* and CMV [45]. Infections were less common in responding, than in non-responding, patients (10% vs. 36%, $p < 0.01$). In a more recent trial in which patients were randomised to initial therapy with alemtuzumab or chlorambucil, although treatment outcome was improved with alemtuzumab, CMV events were more common [50]. Alemtuzumab has also been used in combination with agents such as rituximab or fludarabine in phase II studies. In these trials, infectious complications have been common, especially CMV reactivation and other opportunistic infections such as aspergillosis [51,52].

The incidence of symptomatic CMV infection with alemtuzumab therapy ranges from 10% to25%, with a peak onset 4–6 weeks after initiation of therapy [53]. It has been suggested that CMV infections are more common in previously treated patients and are uncommon after discontinuation of therapy. More recently, the use of alemtuzumab consolidation has been examined in a variety of clinical trials, all of which have CMV reactivation as a common complication [54–57]. In a trial in which 41 patients responding to induction therapy received alemtuzumab consolidation 5 months later, 15 (37%) had infectious complications, including nine cases of CMV reactivation and three cases of EBV-positive large cell lymphoma [54]. In a smaller trial of fludarabine induction followed by alemtuzumab consolidation 5 months later, one-third of patients had CMV reactivation [58]. Another trial of fludarabine or FC induction therapy followed by randomisation to alemtuzumab consolidation

versus observation 2 months later was terminated early as seven of 11 patients receiving alemtuzumab consolidation developed grade 3/4 infections, including four cases of CMV viraemia [55]. The use of alemtuzumab consolidation has also been examined in two CALGB trials [56,57,59]. In a trial of fludarabine induction with alemtuzumab consolidation for patients with responsive or stable disease 2 months later, grade 3 or 4 infections were relatively common in the first 57 patients, including eight cases of CMV reactivation [56,57]. Subsequently, weekly polymerase chain reaction (PCR) testing was implemented, and only three of the next 18 patients were having CMV reactivation with no disease. Preliminary data on infectious complications in the subsequent CALGB trial in which FR induction was followed by alemtuzumab consolidation 4 months later have been reported [60]. Seven grade 5 infectious toxicities occurred in responding patients, including infections with EBV, *Listeria, Legionella*, CMV and *Pneumocystis*. Comparative infectious complications from three serial CALGB CLL trials have also been reported, contrasting infectious complications seen with single agent fludarabine (CALGB 9011), concurrent or sequential FR (CALGB 9712) and fludarabine induction followed by alemtuzumab consolidation (FA) (CALGB 19901) [59]. Infections were more common with FA compared to either single agent fludarabine (38% vs. 23%, $p = 0.01$) or FR (38% vs. 20%, $p = 0.0007$). *Pneumocystis* infections were distinctly uncommon on all three of these clinical trial regimens. However, with regard to CMV infections, none was seen among 188 patients receiving single agent fludarabine, one occurred among 104 patients treated with FR, but 12 of 59 patients had CMV infection during alemtuzumab consolidation following FR induction therapy. Questions have been raised with regard to these differential results with alemtuzumab consolidation and infectious complications, but have no clear answers at the present time. These include the impact of the cumulative dose of either fludarabine or alemtuzumab, the effect of induction rituximab therapy on immune function and the timing of alemtuzumab consolidation following induction therapy, which has varied among these studies. Hopefully, prospective assessment of immune function at various treatment intervals in future treatment trials may provide some answers to these questions.

Preventive approaches

Antimicrobial prophylaxis

There are no standard guidelines for antimicrobial prophylaxis in CLL patients, and in general, most recommendations for these agents are derived from clinical trials and anecdotal reports. Herpesvirus infections, most of which were localised grade 1/2 infections, have been found to be more common in patients receiving single agent fludarabine, as compared to chlorambucil in a retrospective review [12]. Some advocate the use of antiviral prophylaxis in CLL patients with a low CD4 count and in elderly patients [61]. The addition of prednisone to fludarabine appears to increase the risk of opportunistic infections by pathogens as *Listeria* and *Pneumocystis*, thus prophylaxis is used with this infrequently used regimen [11]. As the addition of rituximab to fludarabine does not appear to increase infectious complications, no routine prophylaxis is recommended for use with this regimen [62]. However, when cyclophosphamide is added to fludarabine-based therapy (FC, FCR), antiviral and *Pneumocystis* prophylaxis are used. *Pneumocystis*, antiviral, and sometimes antifungal, prophylaxis is recommended with the use of alemtuzumab. As the purine analogue and alemtuzumab-related immune defects persist after discontinuation of therapy, antimicrobial prophylaxis is often continued for up to 6 months after discontinuation of therapy.

With the significant risk for CMV reactivation with the use of alemtuzumab, guidelines for preemptive therapy of CMV infections were developed [53,63]. Weekly PCR testing for CMV antigen is recommended when feasible for patients receiving this agent [64]. CMV prophylaxis with ganciclovir, continued for 2 months after discontinuation of therapy, has been used by some in patients receiving alemtuzumab [65]. In patients with symptomatic CMV infection, a 14- to 21-day course of ganciclovir, or alternatively valganciclovir, therapy is recommended until symptoms resolve and there is one negative PCR test, or two consecutive negative PCR tests have been obtained. In these guidelines, it is stated that alemtuzumab may be continued, unless patients are persistently symptomatic. For patients with asymptomatic CMV infection, a 1- to 2-week course of ganciclovir is recommended, with

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continuation of alemtuzumab therapy. These surveillance and preemptive approaches have been found to decrease the rate of symptomatic CMV reactivation.

Prophylactic immunoglobulin

In a large randomised trial of prophylactic intravenous immunoglobulin (IVIG) in CLL patients with hypogammaglobulinaemia or prior infections, although minor/moderate infections were decreased, the use of IVIG resulted in no decrease in major infections or mortality and was not cost-effective [66]. The use of low-dose IVIG prophylaxis has also been examined, with a reduction in infection rates in some studies [67]. However, increases in baseline immunoglobulin levels may not correlate with this reduction in infection. Importantly, these IVIG infusions do not replete IgM or IgA, low levels of which are implicated risk factors for infection. Cost-effectiveness remains an issue with these lower doses, especially in comparison to prophylactic antimicrobial therapy. Although there are no discrete guidelines for use of IVIG, this adjunctive therapy is used by some in patients with prior serious or recurrent bacterial infections.

Vaccinations

A variety of immunisations, including pneumococcal, influenza, haemophilus, tetanus, typhoid, diphtheria and mumps, have been examined in small series of CLL patients [68–70]. Because of impaired antibody production and defects in antigen presentation, responses to immunisation responses have been suboptimal. It is also not known if response to vaccination is different in patients with active disease or on therapy, as compared to those in remission. Although there are no formal vaccination guidelines for these patients, pneumococcal and seasonal influenza vaccines are generally used. As herpes zoster vaccines have not been studied in immunocompromised patients, efficacy as well as side-effect profile is unknown in this population and they should not be administered to CLL patients. Response to immunisation might be better with protein and conjugated vaccines and vaccine adjuvants than with polysaccharide vaccines [70].

In summary, infections continue to have a major impact on the course of CLL patients, regardless of treatment status. With therapeutic advances in this disease over the past several decades, not only should traditional disease outcome parameters (e.g., response rate, remission duration and survival) be assessed, but also the impact of these agents on immune function and infectious complications is of importance. Further delineation of mucosal immune function in these patients may be of interest. It will also be useful to identify subsets of patient at increased risk of infection, so that prophylactic and therapeutic interventions may be best used to reduce the incidence and impact of infectious complications in these patients.

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