

# Rituximab-Associated Infections

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After more than 10 years of use, rituximab has proven to be remarkably safe. However, accumulated evidence now suggests that under some circumstances it may significantly increase the risk of infections. This risk is difficult to quantify because of confounding factors (namely, concomitant use of immunosuppressive or chemotherapeutic agents and underlying conditions), as well as under-reporting. Increased number of infections has been documented in patients treated with maintenance rituximab for low-grade lymphoma and in patients with concomitant severe immunodeficiency, whether caused by human immunodeficiency virus (HIV) infection or immunosuppressive agents like fludarabine. From the practical standpoint, the most important infection is hepatitis B reactivation, which may be delayed and result in fulminant liver failure and death. Special care should be placed on screening for hepatitis B virus (HBV) and preemptive antiviral treatment. Some investigators have reported an increase in *Pneumocystis* pneumonia. Finally, there is increasing evidence of a possible association with progressive multifocal leukoencephalopathy (PML), a lethal encephalitis caused by the polyomavirus JC. This review enumerates the described infectious complications, summarizes the possible underlying mechanisms of the increased risk, and makes recommendations regarding prevention, diagnosis and management.

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**R**ituximab (Rituxan, Genentech, Inc, South San Francisco, CA; Mabthera, Roche, Switzerland) causes B-cell death after targeting the surface protein CD20 by a variety of mechanisms. It is effective in a variety of hematologic malignancies, both as a single agent and in combination with chemotherapy. The efficacy of rituximab to deplete B cells prompted its consideration as therapy in autoimmune diseases where these cells are considered to have a significant role, including rheumatoid arthritis (RA) and systemic lupus erythematosus (SLE). Randomized controlled trials in RA showed efficacy,<sup>1-4</sup> and rituximab is currently approved by the US Food and Drug Administration (FDA) for RA refractory to tumor necrosis factor (TNF)-inhibitors. Several trials have shown significant efficacy in mixed cryoglobulinemia.<sup>5,6</sup> Many case series suggest it is also effective in SLE,<sup>7</sup> but the results of randomized controlled trials have been disappointing.<sup>8</sup> There is accumulat-

ing evidence of efficacy of rituximab in immune thrombocytopenic purpura (ITP), and many case series and case reports of its use as immunosuppressive agent in a variety of autoimmune conditions, including vasculitis, polymyositis, and pemphigus. Randomized trials in multiple sclerosis are ongoing; early results seem very promising.<sup>9</sup> Finally, rituximab has been incorporated in solid organ transplantation regimens with the aim of preventing hyper-acute rejection in patients sensitized to human leukocyte antigen (HLA) or in cases of ABO incompatibility.<sup>10</sup>

Rituximab administration results in profound depletion of normal B cells for several months, but immunoglobulin levels remain unaltered in most patients. This is thought to be due to the fact that long-lived plasma cells do not express CD20. The lack of effect on immunoglobulin levels suggested that rituximab administration could have minimal effect on the occurrence of infections. Continued use of this agent has brought to light a modest increase in infectious risk that underlines the complexities of the immune response. Infectious complications possibly related to rituximab have been reported from each of the clinical disciplines where it is commonly used (see Table 1). Many confounding factors are present, and determining the specific effect of rituximab on the risk of infection remains elusive.

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**Table 1. Rituximab Infectious Complications**

	Evidence	Comments
<b>Established increased infectious complications</b>		
Overall infections	Meta-analyses in hematologic malignancies <sup>11,12</sup> Randomized trials in RA <sup>1</sup>	Increased severe infections (grade 3 or 4) when used as maintenance therapy in follicular lymphoma Mild infections in RA
Hepatitis B reactivation	Case series <sup>13-15</sup> Case reports <sup>16-18</sup>	Reports only in hematologic malignancies
PML	Case series, <sup>19</sup> case reports <sup>20-22</sup>	Most cases in hematologic malignancies, but a few in RA, SLE, and immune cytopenia
<b>Possibly increased infectious complications</b>		
<i>Pneumocystis jirovecii</i> pneumonia	Retrospective series compared to historical controls <sup>23,24</sup> Case series <sup>25-27</sup> Case reports <sup>28-31</sup>	Cases in hematological malignancies, RA, autoimmune diseases, solid organ transplant
Enterovirus encephalitis	Case reports <sup>32-35</sup>	Known complication of other B-cell immunodeficiencies
Parvovirus B19	Case reports <sup>36-39</sup>	Good response to IVIG
Cytomegalovirus	Case reports <sup>20,28,40</sup>	CMV disease is very uncommon except in HIV or following allogeneic transplant; there are several reports in hematologic malignancies treated with combination chemotherapy
West Nile virus	Case reports <sup>41,42</sup>	Increased severity and negative serology may be anticipated because of effect of rituximab on B cells
Babesiosis	Case-control study <sup>43</sup>	Most patients with persistent babesiosis had received rituximab
Mycobacterial disease	Case reports <sup>44</sup>	Severe <i>Mycobacterium avium</i> and <i>M kansasii</i> , no other reports

Abbreviations: PML, progressive multifocal leukoencephalopathy; RA, rheumatoid arthritis; SLE, systemic lupus erythematosus; IVIG, intravenous immunoglobulin; CMV, cytomegalovirus; HIV, human immunodeficiency virus.

## INFECTIONS ASSOCIATED WITH RITUXIMAB WHEN USED AS AN ANTINEOPLASTIC AGENT

### Overall Infectious Complications

The pivotal trial of rituximab in low-grade lymphoma administered four doses of 375 mg/m<sup>2</sup> at weekly intervals to 166 patients with relapsed low-grade or follicular lymphoma, and showed profound depletion of B cells (usually to undetectable levels) that persisted for 6 to 12 months. Mean serum levels of IgG and IgA remained within the normal range and mean IgM levels decreased slightly below normal. Only 23 of 166 patients had significant reduction of their immunoglobulin levels. There were 68 infectious episodes, 61 of which were grade 1-2 and the other seven grade 3.<sup>45</sup> The incidence of infections was not higher than

expected. Subsequent studies, including several randomized controlled trials, have shown similar low-grade findings. For example, when used as a single agent as first-line therapy in chronic lymphocytic leukemia (CLL) or small lymphocytic lymphoma, only three grade 3 infections were seen in 44 patients.<sup>46</sup> Some studies of combination therapy showed a significant number of infections: 20% when used with fludarabine in previously untreated patients with CLL, including several opportunistic infections,<sup>28</sup> and up to one-third when used in combination with fludarabine and cyclophosphamide (FCR) in patients with more advanced, refractory, or previously treated CLL.<sup>47,48</sup> The frequency of infection did not seem higher than with fludarabine alone. In a randomized trial the addition of rituximab to combination chemotherapy with

fludarabine, cyclophosphamide, mitoxantrone (FCM) did not result in increased infections, despite a higher frequency of lymphocytopenia.<sup>49</sup> In the most important randomized controlled trial of rituximab in diffuse large B-cell lymphoma, the addition of rituximab to CHOP chemotherapy (cyclophosphamide, doxorubicin, vincristine, and prednisone) did not result in increased incidence of infection, which was high in both arms (65% all grades, 12% and 20% grade 3 and 4, respectively).<sup>50</sup> Of note, the long-term follow-up of the participants in this trial did show a trend for increased late infections in rituximab recipients.<sup>51</sup> A phase II randomized study of rituximab added to cyclophosphamide and fludarabine for the initial treatment of mantle cell lymphoma did not show increased infections in the rituximab arm (78 patients) with a median follow-up of 38 months.<sup>52</sup> Confirming the results of individual trials, a meta-analysis of rituximab use in indolent or mantle cell lymphoma,<sup>53</sup> as well as a systematic review of all randomized controlled trials published until mid 2006,<sup>54</sup> failed to demonstrate any increase in infections. In contrast, infections seemed to be more common in some of the trials for the treatment of human immunodeficiency virus (HIV)-associated lymphoma,<sup>55</sup> and in one of them there was a significant increase in the infection-related mortality.<sup>56</sup> The increased mortality occurred primarily in the patients with lowest CD4 count and was caused by non-opportunistic infections. A more recent phase II trial of CHOP-rituximab in HIV-infected patients that excluded patients with more advanced AIDS did not report increased infections.<sup>57</sup> This would support the idea that rituximab may be an additive risk factor mainly in cases of advanced immune compromise. Increased infections have also been shown by two meta-analyses of the trials of maintenance rituximab for follicular lymphoma.<sup>11,12,58</sup> Vidal et al compiled the data for infections in 1,056 patients from five trials reported in four papers and two abstracts<sup>59-62</sup> and determined the risk of infection is approximately double than the control (confidence interval [CI], 1.21-3.27) when rituximab is used as maintenance. Aksoy et al included five randomized trials and four phase II studies in their meta-analysis, and their estimated risk ratio for infection was 2.82 (CI, 1.28-6.23). This meta-analysis disclosed the increased risk of neutropenia associated with maintenance rituximab (risk ratio = 2.4; 95% CI, 1.5-3.9;  $P < .001$ ),<sup>12</sup> but it did not address the role of neutropenia in infectious episodes. Hypogammaglobulinemia was not assessed in either of the two meta-analyses. Besides the data from these randomized trials and meta-analyses, a single-institution case series suggested a high frequency of non-neutropenic infections in patients treated with rituximab.<sup>63</sup> These investigators observed 40 episodes of non-neutropenic, non-opportunistic infections in 19 of 97 patients treated with rituximab between 2002 and 2005, with a clear

association with hypogammaglobulinemia (15 of the 19 patients with infections had hypogammaglobulinemia, the other four were not tested). The main risk factor for infection was the use of fludarabine.

When rituximab is used as part of the regimen for autologous hematopoietic stem cell transplantation (HCT) or as maintenance after HCT, persistent hypogammaglobulinemia seems to be common.<sup>64-66</sup> The frequency of reported infections varies widely: Nishio et al reported that one of 14 patients developed repeated bacterial infections and required intravenous immunoglobulin (IVIG),<sup>65</sup> while Brugger et al documented infections in 25 of 30 patients, including seven pneumonias, one case of tuberculosis, and one case of zoster requiring hospitalization.<sup>67</sup> Goldberg et al reported four opportunistic viral infections (two cases of progressive multifocal leukoencephalopathy [PML] and two cases of cytomegalovirus [CMV] disease) in 62 patients who received peritransplant rituximab, and contrasted this with no similar cases seen in their 276 patients previously transplanted without rituximab.<sup>20</sup>

## Hepatitis B

Although not a significant problem in the randomized trials, hepatitis B virus (HBV) may reactivate following chemotherapy for hematologic malignancies and cause acute hepatitis and fulminant liver failure.<sup>68,69</sup> These complications may be more frequent when rituximab is part of the regimen.<sup>13,17,70-73</sup> Targhetta retrospectively analyzed 394 lymphoma patients who had isolated anti-HBc antibody (anti-HBc) as the only evidence of HBV infection, and found the frequency of hepatitis B was three times higher in those who received rituximab as part of their chemotherapy (2.7% *v* 0.8%).<sup>15</sup> In a retrospective study, eight of 10 HBsAg-positive lymphoma patients who did not receive lamivudine prophylaxis developed a flare of hepatitis B, as did four of 95 patients who were HBsAg-negative.<sup>74</sup> In a prospective study of 46 patients who were HBsAg-negative but anti-HBc-positive, Yeo et al identified five cases of HBV reactivation with hepatitis in 21 patients who received rituximab plus CHOP (R-CHOP) and no cases in the 25 who received CHOP.<sup>13</sup> Hepatitis may occur at any time following the initiation of chemotherapy, but it seems to happen more commonly after the immunosuppressive effect of the chemotherapeutic regimen subsides,<sup>13,68</sup> which may explain some delayed cases in patients who received rituximab.<sup>13,75</sup>

Liver failure and death have been reported in up to 50% of patients with lymphoma who experience hepatitis B flare. Consequently, screening of all patients should be implemented, and preventive measures adopted.<sup>76</sup> Screening for evidence of hepatitis B should include, at a minimum, measurement of HBsAg, anti-

**Table 2. Prevention and Management of Hepatitis B Reactivation in Patients Receiving Rituximab-Containing Regimens for Hematologic Malignancies**

	Interpretation	Action
HBsAg <sup>-</sup> plus anti-HBs <sup>-</sup> plus anti-HBc <sup>-</sup> HbsAg <sup>+</sup>	Hepatitis B-naïve  Active HBV replication; probably carrier, but it could be acute hepatitis B	HBV immunization if feasible  Obtain baseline HBV DNA level Obtain HBeAg and anti-HBe Start anti-HBV treatment with lamivudine* Monitor with HBV DNA levels and ALT Monitor ALT
HBsAg <sup>-</sup> plus anti-HBs <sup>+</sup> plus anti-HBc <sup>+</sup>	Past hepatitis B	Measure HBV DNA level with sensitive assay if ALT is or becomes abnormal
HBsAg <sup>-</sup> plus Anti-HBs <sup>-</sup> plus anti-HBc <sup>+</sup>	Possible occult HBV infection or False positive anti-HBc	Measure HBV DNA level with sensitive assay HBV immunization if feasible†
HbsAg <sup>-</sup> plus HBsAb <sup>+</sup> plus HbcAb <sup>-</sup>	Vaccination to hepatitis B or Occult hepatitis B infection	Measure HBV DNA level with sensitive assay‡ Start anti-HBV treatment with lamivudine

Recommended serological markers to be obtained in all patients before receiving rituximab-including chemotherapy or immunosuppression: hepatitis B virus (HBV) surface antigen and antibody (HBsAg and anti-HBs), and HBV anticore antibody (anti-HBc).

\*Some experts recommend combination therapy in HIV-infected individuals

†Depending on the setting and the need for starting chemotherapy, immunizing against hepatitis B may be the first option. In case of past hepatitis B, there will be an anamnestic antibody response and positive anti-HBs in 2 weeks.

‡In individuals without a reported/documented vaccination.

HBs, anti-HBc, and frequently hepatitis B DNA by quantitative polymerase chain reaction (PCR). Extensive recommendations regarding prevention and management of hepatitis B in patients with hematologic malignancies have been proposed, and the reader should consult these for an in-depth discussion of the different possible strategies.<sup>76,77</sup> A summary of interpretation of the results and recommendations is presented in Table 2. The most important points to consider are as follows: (1) a negative HBsAg does not rule out active HBV replication in the liver; (2) in many patients, the only evidence of hepatitis B is a positive anti-HBc, and the risk of hepatitis B reactivation of these patients is between 19%<sup>13</sup> and 33%<sup>14</sup>; and (3) the best management approach is not known. The two main concerns regarding patients receiving rituximab are: (1) they may not show an antibody response to HBV vaccination, making this intervention less useful than in other patient populations; and (2) they may be at risk for delayed reactivation of HBV following completion of chemotherapy, due to the long half-life of rituximab.

The cornerstone of preventing hepatitis B reactivation in patients known to be at risk is the administration of antivirals, typically lamivudine (combination therapy with tenofovir and lamivudine, as part of an effective antiretroviral regimen, has been recommended to prevent reactivation in HIV-infected pa-

tients undergoing chemotherapy for lymphoma<sup>78</sup>). It is not clear how long to continue lamivudine treatment, but a minimum of 6 to 12 months after completing treatment is often recommended.<sup>13,77</sup> If the chemotherapy included rituximab, we would recommend prophylactic lamivudine and monthly monitoring of ALT for at least 1 year, as immune reconstitution of the B-cell compartment may take up to a year.

HBV reactivation following rituximab monotherapy (as opposed to combination chemotherapy) for lymphoma has been less commonly reported,<sup>79</sup> but in a retrospective analysis from a single institution it was also frequent.<sup>14</sup> There were no reported cases of hepatitis B reactivation in the trials of rituximab for RA.

### Progressive Multifocal Leukoencephalopathy

PML is a viral demyelinating disease of the brain, originally described in 1958 in two patients with CLL and one with Hodgkin disease.<sup>80</sup> It is caused by lytic infection of oligodendrocytes by the polyomavirus JC. Except in the setting of HIV infection, PML is a rare disease. However, dozens of cases have been reported following rituximab administration for hematologic malignancies, SLE, RA, autoimmune pancytopenia, and ITP,<sup>19</sup> prompting a MedWatch alert by FDA, modification of the prescribing information (including a “black box warning”), and a letter from the manufacturer to

all physicians who may prescribe rituximab. Typically, PML presents subacutely with cognitive impairment (confusion or disorientation), motor weakness or poor coordination, speech problems, and/or vision changes. Magnetic resonance imaging (MRI) of the brain shows multiple subcortical areas of demyelination without edema or gadolinium enhancement.<sup>81</sup> The diagnosis is made by the combination of the clinical picture with the MRI plus the demonstration of JC virus infection in the brain (either by brain biopsy or by quantitative PCR of JC in the cerebrospinal fluid [CSF]). Most cases are diagnosed by PCR, but the sensitivity of this test may be as low as 75% and brain biopsy may be required. Most patients with rituximab-related PML were receiving this agent as part of the treatment regimen for hematologic malignancy, and had received a median of four previous or concomitant chemotherapy treatments.<sup>19</sup> The patients had received a median of six doses of rituximab (range, 1–28) and the diagnosis of PML was established between 1 and 90 months after the first dose (median, 16 months).<sup>19</sup> Given that the disease is known to occur in these diseases, the true effect of rituximab (if any) remains to be quantified.<sup>82</sup> In contrast, RA was not known to be associated with PML. The description of the third case of PML in RA, which was also the first patient who had not previously received TNF-inhibitors, made the FDA issue a new clinical alert stating that patients with RA who receive rituximab may have an increased risk of PML, and recommending consideration of PML in any patient being treated with rituximab who presents with new-onset neurologic manifestations. Diagnostic tests include MRI and lumbar puncture. In the case of patients with hematologic malignancies, the differential diagnosis often includes CNS or meningeal involvement with the tumor, drug or radiation toxicity (in case intrathecal chemotherapy or cranial irradiation has been used), and opportunistic infection.

There is no satisfactory treatment for PML, which is almost universally fatal in a few months. In HIV-infected people, improvement of immune function with antiretrovirals is the only approach with some degree of effectiveness. In the cases of PML associated with the monoclonal antibody natalizumab (an anti-integrin used in the treatment of multiple sclerosis), discontinuation of the monoclonal and of any concurrent immunosuppression, together with plasma exchange and immunoabsorption, resulted in a patient surviving the infection with major neurologic sequelae.<sup>83</sup>

### Meningoencephalitis Caused by Enterovirus

Enteroviral meningoencephalitis has been described in children with agammaglobulinemia, and there have been several case reports of this infection in patients receiving rituximab.<sup>32–34,84</sup> The initial presentation may be similar to PML, but the CSF often shows mild pleo-

cytosis and increased protein, and the MRI, when abnormal, often shows enhancement.<sup>32,33</sup> The diagnosis is made by PCR of the CSF. IVIG had no effect in one of the reported cases,<sup>33</sup> resulted in short-lived improvement in another,<sup>34</sup> and, combined with the antiviral agent pleconaril, effected marked clinical improvement of several months' duration in the other two.<sup>35</sup>

### Parvovirus B19

Several cases of parvovirus B19 causing pure red cell aplasia in patients receiving rituximab have been described.<sup>36–39,85</sup> Cases have presented with persistent anemia of unknown etiology, sometimes following a febrile illness with a rash.<sup>36</sup> The total immunoglobulin level may be within normal limits. Serology against B19 is characteristically negative, and the diagnosis is usually made by PCR. The presence of large pronormoblasts with nuclear inclusion bodies in the bone marrow biopsy may suggest the diagnosis.<sup>38</sup> The reported cases responded to high-dose IVIG.

### *Pneumocystis jirovecii* Pneumonia

*Pneumocystis jirovecii* is now known to be a fungus, and a well-established cause of interstitial pneumonia in immunocompromised patients. *Pneumocystis jirovecii* pneumonia (PCP) is generally considered a disease associated with defects in T-cell-mediated immunity. The correlation of PCP with decreased CD4 T cells in patients with AIDS allows the timely initiation of anti-*Pneumocystis* prophylaxis when the CD4 count is below 200/ $\mu$ L. In non-HIV-infected patients, corticosteroids are the main risk factor, but fludarabine, pentostatin, and cyclophosphamide have also been associated with increased risk.

There have been many reports of PCP following the administration of rituximab for a variety of indications: treatment of aggressive B-cell lymphoma as part of CHOP-14 or CHOEP-14 (cyclophosphamide, doxorubicin, vincristine, etoposide, and prednisone) regimens,<sup>25–27</sup> together with CHOP as R-CHOP,<sup>23,24</sup> and as monotherapy or combined with other agents for treatment of RA,<sup>31</sup> autoimmune hemolytic anemia,<sup>86,87</sup> pure red cell aplasia,<sup>88</sup> pemphigus,<sup>89</sup> and treatment of acute rejection of kidney transplant.<sup>29,30</sup> Interestingly, it has been known for some time that B cells may play a significant role in the protection against *Pneumocystis*. Transgenic B-cell-deficient mice are susceptible to *Pneumocystis carinii* (formerly species *murina*).<sup>90</sup> B cells are necessary for clearance of pneumocystis in the mouse model, but do this even in the absence of specific antibody.<sup>91</sup> In a mouse model in which B cells did not express major histocompatibility complex (MHC) class II antigens (and so were unable to act as antigen-presenting cells [APCs]), PCP could not be controlled.<sup>92</sup> Supporting the evidence from the animal model, PCP has been documented in patients with

agammaglobulinemia, common variable immunodeficiency, and Good's syndrome, all of which are B-cell immunodeficiencies.

In summary, although far from conclusive, there is suggestive evidence that the use of rituximab may increase the risk of PCP. The weakest part of the evidence is that many of the diagnoses of PCP were based on detection of beta-d-glucan in serum or PCR in respiratory specimens, tests that had not been used in the historical controls.<sup>23,24</sup> The need for PCP prophylaxis in patients receiving rituximab for hematologic malignancies remains to be determined.

### Other Infections

Babesiosis, a zoonosis caused by the parasite *Babesia microti*, has been particularly difficult to eradicate in patients who have received rituximab.<sup>43</sup> In the reported case-control study, eight of 14 patients who had persistent parasitemia 1 month after receiving standard treatment had received rituximab.<sup>43</sup> As eradication of the parasite is typically associated with seroconversion and this does not take place in rituximab recipients, there is biological plausibility supporting a more severe course of the disease. A similar pathophysiology may apply to West Nile virus infection, with case reports of severe disease without serological response.<sup>41,42</sup> Cases of herpes simplex and varicella zoster reactivation have been reported, but these are known complications of lymphoma and its treatment. More concerning are several cases of CMV disease.<sup>20,28</sup> Although CMV reactivation or infection is common in a variety of immunosuppressive regimens, CMV disease is uncommon outside of HIV infection and allogeneic stem cell transplantation. A case series of 46 patients who received autologous stem cell transplantation for lymphoma with or without rituximab found three of 17 rituximab-treated patients and none of 29 non-rituximab-treated patients developed CMV complications (two pneumonitis and one asymptomatic reactivation).<sup>40</sup> Preliminary data from the solid organ transplant literature do not support increased risk of CMV with rituximab treatment,<sup>93</sup> and the association is questionable at this time.

### RITUXIMAB IN AUTOIMMUNE DISEASES: RA, SLE, CRYOGLOBULINEMIA, ITP, AND OTHER AUTOIMMUNE DISEASES

The standard dosing of rituximab in RA is 1,000 mg twice, administered 2 weeks apart. It may be given with weekly methotrexate, but the overall immunosuppression of these patients is generally lower than those receiving chemotherapy for hematologic malignancies. Perhaps for this reason, infections have been even less of an issue in trials for these conditions than in oncology. In the large randomized trials in RA,<sup>1-4</sup> serious infections were described in only 1% to 3% of patients.

In the largest of these studies, involving 520 patients, there were only seven serious infections in the rituximab group, compared with three in the placebo group. There were no cases of tuberculosis or opportunistic infections over the 24 weeks of the study.<sup>1</sup> A meta-analysis of three randomized controlled trials did not find any increase in infections.<sup>94</sup> Whether this is a reflection of the decreased amount of antibody used or the underlying state of immune competence remains unknown. Although rituximab has been very safe in RA trials, a number of cases of severe or opportunistic infections have been reported, including PCP<sup>31</sup> and PML.<sup>19</sup> The true incidence of these complications and the contribution of rituximab remain unknown.

A systematic review of rituximab in ITP revealed that it resulted in platelet response in 62.5% of adults.<sup>95</sup> The overall mortality was 2.9%, but most deaths did not seem to be related to the agent. The lack of controlled studies makes it impossible to make a statement about infectious risk. A large series in children with refractory ITP did not document any infection in 49 children during a median follow-up of 39.5 months, and showed an overall response rate of 69%.<sup>96</sup> At least one case of PML has occurred in a patient who received rituximab for ITP.<sup>19</sup> Rituximab has not been as successful in other autoimmune cytopenias: in a report of eight patients with autoimmune neutropenia and three patients with pure red cell aplasia, only two patients with neutropenia responded, and there were two deaths caused by infection (PCP and bronchiectasis).<sup>88</sup> Rituximab is also frequently used to treat type II mixed cryoglobulinemia (usually associated with hepatitis C virus [HCV]).<sup>5,6</sup> There were no infections in the largest series, but the level of HCV viremia increased significantly.<sup>5</sup> Severe infections were reported in two of seven patients with cryoglobulinemia after renal transplant.<sup>97</sup> There are multiple case series reporting the use of rituximab to treat a variety of refractory autoimmune diseases,<sup>98</sup> including vasculitis,<sup>99</sup> and pemphigus.<sup>100,101</sup> Scattered reports of severe or opportunistic infections in these patients are particularly difficult to interpret, because very frequently intense immunosuppression is being administered at the time rituximab is added, and no comparison is possible.

### INFECTIONS ASSOCIATED WITH RITUXIMAB USE IN SOLID ORGAN TRANSPLANTATION

Rituximab was used originally in solid organ transplantation to prevent acute rejection in cases of HLA-sensitization<sup>102</sup> or ABO-incompatible transplants,<sup>103,104</sup> or to treat acute humoral rejection.<sup>105</sup> Several case series of rituximab use in highly sensitized patients have been published showing either no infections during relatively short follow-up periods<sup>106</sup> or "no more infections than expected."<sup>105,107</sup> However, a retrospective series of 34 patients documented increased num-

ber of infections when rituximab was added to a rejection-prevention regimen that included plasmapheresis, IVIG infusion, and administration of rabbit anti-thymocyte globulin (ATG).<sup>108</sup> Thirteen patients experienced 21 infectious complications, mainly bacterial skin and soft tissue infections and bacteremia. The largest analysis of infectious complications of rituximab in solid organ transplant reviewed 77 kidney transplant recipients who received rituximab therapy (2–8 courses [median, 4] of 375 mg/m<sup>2</sup> each) for various reasons between 2004 and 2008 and compared them to 902 control patients.<sup>109</sup> Forty-six infections occurred in 35 patients (45%): 28 bacterial, 5 viral, and 13 fungal (including four cases of *Pneumocystis* infection). Seven patients died of infection. Risk factors for infection included lower total lymphocyte counts and CD4 lymphocyte counts and higher doses of tacrolimus and corticosteroids. The most important predictive factor for infectious disease-related death was the combined use of rituximab and rabbit ATG.

Besides these series, there is a growing series of case reports of infections in solid organ transplant recipients after receiving rituximab, including pneumocystis,<sup>29,30</sup> bacterial fasciitis, *Cryptococcus*, and disseminated herpes.<sup>110</sup>

### POTENTIAL MECHANISMS OF INCREASED RISK OF INFECTIONS ASSOCIATED WITH RITUXIMAB

Of the multiple associations described in the preceding sections, the one better supported by evidence is the increased risk of infection with maintenance rituximab detected by meta-analyses.<sup>11,12,58</sup> These seem to be non-opportunistic infections, and they could be explained by hypogammaglobulinemia and neutropenia, which are known to occur more frequently with more frequent administration of rituximab. Hypogammaglobulinemia is uncommon after rituximab therapy, but it was associated with infection (present in 15 of 19 patients) in a retrospective case series of non-neutropenic infections following rituximab.<sup>63</sup> A minority of patients do develop severe hypogammaglobulinemia. This has been described mainly with maintenance adjuvant rituximab in autologous stem cell transplantation,<sup>64,66</sup> repeated courses of rituximab for hematologic malignancies,<sup>111,112</sup> and repeated administration in autoimmune cytopenias.<sup>113,114</sup> Not surprisingly, infections may occur in this setting, and can be prevented by administration of IVIG.

Depletion of B cells would be expected to result in poor antibody responses to new antigens. Several studies have documented that patients receiving rituximab exhibit decreased to absent humoral responses to new antigens,<sup>115,116</sup> more so than to recall antigens.<sup>117–119</sup> A few patients who contracted West Nile fever while receiving rituximab did not develop an immunoglobu-

lin response, supporting the clinical relevance of these observations.<sup>41,42</sup> Similar pathophysiology may underlie the severity of babesiosis<sup>43</sup> and parvovirus B19 infections<sup>38</sup> in patients treated with rituximab.

Besides their role in antibody synthesis, B cells may act as APCs and may be important cofactors of effective immune responses. B cells were shown to be essential in a model of murine pneumocystosis.<sup>92</sup> If this were also true in humans, it is possible that the increasing number of reports of PCP in patients who have received rituximab represents a real phenomenon and not just an artifact of reporting bias. The potential importance of B cells for the control of mycobacterial infections<sup>120</sup> was suggested as a possible explanation in the two cases of severe non-tuberculous mycobacterial disease reported by Lutt et al.<sup>44</sup> However, to date there has been no sign of increased incidence of tuberculosis in patients treated with rituximab. There is some evidence that the B-cell depletion induced by rituximab may result in abnormal activation of CD4<sup>+</sup> T cells in response to antigen.

Finally, some data suggest rituximab may interfere with T-cell function.<sup>121</sup> The most compelling human evidence probably comes from studies of rituximab in ITP, where abnormalities in T-cell cytokine profiles and normalization of number and activity of T-regulatory cells have been described.<sup>122,123</sup>

### SUMMARY AND RECOMMENDATIONS

Rituximab has proven remarkably safe over years of use in hundreds of thousands of patients. The more important infectious risks seem to be reactivation of hepatitis B and increased infections with repeated administration. In addition, data derived from the experience in oncology and solid organ transplantation support the notion that the administration of rituximab to patients with pre-existing immune defects (advanced HIV infection) or concomitant intense immunosuppression may result in severe and opportunistic infections. It is possible that rituximab increases the risk of PCP, but the evidence is inconclusive. Finally, rare infections like PML and enterovirus meningoencephalitis have been described in patients receiving rituximab. Based on these facts, the following tentative recommendations can be made. Thorough screening for occult hepatitis B infection should be performed before starting treatment for hematologic malignancies with rituximab, and prolonged HBV suppression with lamivudine should be considered. Regarding late infections possibly related to persistent hypogammaglobulinemia, there is no evidence on which to base firm recommendations. We advise measuring immune globulin levels and white blood cell count in patients who experience significant infectious episodes following rituximab administration. In case of repeated infections in the presence of significant hypogammaglobulinemia,

it may be reasonable to consider IVIG to maintain an IgG level >400 mg/dL. Late neutropenia associated with rituximab seems to respond well to granulocyte colony-stimulating factor. PCP prophylaxis has been recommended for patients receiving R-CHOP-14 or R-CHOEP-14. Finally, a high level of suspicion for infection is advised when rituximab is administered in the setting of defects of cell-mediated immunity. Many infections have been described months after the last dose of rituximab. PML should be considered in every patient with new cognitive or neurologic defects, and brain MRI and lumbar puncture with quantitative PCR for JC virus performed.

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