B-Cell Targeted Therapies in Autoimmune Cytopenias and Thrombosis

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Abstract Ever since the advent of Rituximab and subsequently the emergence of other compounds targeting B cells, a cornucopia of medical applications have been found for this family of compounds. After their establishment as standard of care in many conditions such as rituximab in lymphoma and rheumatoid arthritis, they have been progressively found to aid in the treatment of many other conditions. This area constituted a fertile area of research in the past 12 years. Physicians have investigated the B-cell depleting agents use in cases of autoimmune hematologic cytopenias such as immune thrombocytopenia, Evans syndrome, cold and warm autoimmune hemolytic anemia, and other thrombophilic disorders such as the antiphospholipid syndrome and thrombocytopenic purpura. This chapter presents a historical perspective reviewing the various studies that have been published in this field. In addition, it offers a current assessment of the evidence regarding the use of B-cell depleting agents in the aforementioned conditions.

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1 Introduction

Ever since the advent of Rituximab and subsequently the emergence of other compounds targeting B cells, a cornucopia of medical applications have been found for this family of compounds. After their establishment as standard of care in many conditions such as rituximab in lymphoma, they have been progressively found to aid in the treatment of many other conditions. This area constituted a fertile area of research in the past 12 years (Zimmer et al. 2004). Physicians have investigated the B-cell depleting agents use in cases of autoimmune hematologic cytopenias such as ITP (immune thrombocytopenia), Evans syndrome, cold and warm autoimmune hemolytic anemia, and other thrombophilic disorders such as the antiphospholipid syndrome and TTP (thrombocytopenic purpura).

2 B-Cell Depleting Agents in Primary ITP

2.1 ITP at a Glance

Immune thrombocytopenia is an autoimmune disease of platelet destruction and subsequent thrombocytopenia. The disease has been witnessed to many changes all the way from understanding its pathophysiology to the evolution in its management options. Initially called idiopathic thrombocytopenic purpura and thus the acronym ITP, it went on to be named immune thrombocytopenic purpura, and more recently immune thrombocytopenia (Stasi et al. 1995). Changes in nomenclature emerged due to advances in understanding the disease. It was primarily thought of as a disease of peripheral platelet destruction of unknown origin. ITP's definition progressively evolved to include peripheral platelet destruction due to autoantibodies in addition to an inadequate production by megakaryocytes through disturbed level of erythropoietin (Neunert et al. 2011; Nugent et al. 2009). ITP is classified into primary and secondary. No test has been yet developed that can accurately pinpoint the diagnosis. The primary form is usually diagnosed by exclusion of a secondary form which is usually part of another disease process such as: antiphospholipid syndrome, systemic lupus erythematosus, a hematologic or non-hematologic malignancy, common variable immune deficiency or might be due to hepatitis C or Helicobacter pylori, cytomegalovirus, varicella zoster or human immunodeficiency virus infection or to a vaccination side effect (Cines et al. 2009). Secondary ITP accounts for around 20 % of the total number of patients diagnosed with immune thrombocytopenia (Cines et al. 2009). Of relevance to this chapter is the primary form since this is where B-cell depleting agents come into play.

2.2 Standard of Care and Available Treatments

The management of ITP varies between children and adults. This stems from the fact that children have a more acute disease with a higher tendency for spontaneous remission as opposed to adults (Neunert et al. 2011; Stasi et al. 1995).

2.2.1 Therapy in Children

Initial therapy in an emergency setting for symptomatic patients mainly consists of glucocorticoids, IVIG, or alternatively anti-D in select cases (non-splenectomized, Rh-positive patients). High dose dexamethasone can also be considered in patients who do not respond to the above-mentioned treatments or as an alternative to splenectomy in chronic ITP patients. Splenectomy is exclusively a second-line therapy however, should be delayed until 12 months after the diagnosis since patients might spontaneously remit within this period (Sailer et al. 2006).

2.2.2 Therapy in Adults

Treatment is usually administered in this population when patients are bleeding or are at increased risk of bleeding, such as premenopausal women or when they have coexistent risk factors, lifestyle, preference and pros versus cons of treatment (Stasi et al. 1995; Cohen et al. 2000; Daou et al. 2008). It has been proposed that a platelet count of $30 \times 10^9\ L^{-1}$ can be adopted as a threshold for treatment as it was shown to improve mortality in newly diagnosed ITP patients (Li et al. 2005; Neylon et al. 2003; Neunert et al. 2011). Glucocorticoids, IVIG, and anti-D have all been proposed as treatment options for ITP and are given alone or in combination based on the patients' tolerability of every treatment or the need for a quicker response in some cases (Nugent et al. 2009; Thota et al. 2012; Godeau et al. 1999; Newman et al. 2001; Zimmer et al. 2004). High dose dexamethasone has also been reported to be highly effective however, due to paucity of head to head studies with other treatments, it is not yet considered a standard of care (Cheng et al. 2003). Splenectomy, rituximab, and TPO agonists are all considered adequate second-line therapies (Provan et al. 2010; Arnold 2013; Neunert et al. 2011). Splenectomy is the option physicians have the most experience with not to mention the fact that it has the most studies to support it since it was the first and only second-line treatment option for a long time (Ghanima et al. 2012).

2.3 Rituximab in ITP

The first study exploring B depleting agents in ITP was reported in 2000. It was a retrospective one that included patients who had already been treated with steroids. It showed a 30 % response rate to rituximab when given at 375 mg/m^2 weekly for 4 weeks (as per the lymphoma protocol) (Saleh et al. 2000). The first prospective trial using rituximab in ITP was reported in 2001 (Stasi et al. 2001). This study included 25 patients who had chronic ITP and had failed prior treatments. All of the patients received the 375 mg/m^2 dose. The results from that trial showed an encouraging 52 % response rates in otherwise treatment refractory patients. With the minimal adverse event profile of this drug, it was found to be a safe alternative to more traditional treatments. A large systematic review exploring the efficacy of rituximab as a second-line treatment in patients older than 15 years of age with roughly half of them having undergone splenectomy found that treatment with rituximab gave a complete response rate (CR) of 46.3 % (95 % CI: 29.5–57.7) and a partial response rate (PR) of 24.0 % (95 % CI: 15.2–32.7). In that study PR and CR were defined as exceeding 50×10^9 cells/L and 150×10^9 cells/L, respectively (Arnold et al. 2007). This is different than the most recent ASH guidelines that define a CR as one that leads to a platelet count $\geq 100 \times 10^9$ cells/L without bleeding and a response as one that leads platelet count $\geq 30 \times 10^9$ cells/L accompanied with a greater than twofold increase of platelet count from baseline with absence of bleeding (Neunert et al. 2011). The median time to response and response duration in the above-mentioned review were 5.5 weeks and 10.5 months, respectively. A recent meta-analysis confirmed rituximab's efficacy in ITP revealing an overall response and complete response of 59.7 and 45.7 %, respectively (Barcellini and Zanella 2011). Previous studies have shown that the response rate does not vary whether rituximab is used before or after splenectomy or used after a previous trial of rituximab (Auger et al. 2012). It has also been shown that the 100 mg/m^2 dose can be as effective as the much higher ones used in the lymphoma protocol giving an overall response rate of 71 % (Stasi 2010). A recent systematic review recently published that included 18 observational studies with a total number 323 children with ITP concluded that CR for these patients was 39 % (CI: 30–49 %). The response rate was, however, 68 % (CI: 58–77 %). This is very similar to the response rates observed in adults; however, the definitions of CR ($\geq 100 \times 10^9$ cells/L) and response ($\geq 30 \times 10^9$ cells/L) differed from their adult counterparts (Liang et al. 2012). A multicenter prospective trial of chronic ITP patients, who are candidates for splenectomy and were treated with rituximab, has shown promising results. At 1 year, 40 % of patients had responded (95 % CI: 28–52 %). This group, however, decreased over time with only 6.7 % of the patients experiencing sustained response after a single course of rituximab using the lymphoma protocol. This proved that rituximab could be used to delay or prevent splenectomy (Godeau et al. 2008) A recent large meta-analysis exploring rituximab as an option prior to splenectomy demonstrated a 57 % overall response rate after rituximab treatment with younger patients responding the most (Auger et al. 2012).

Another study using rituximab earlier in the course of treatment of patients with immune thrombocytopenia evaluated 103 patients that were treatment naïve. It consisted of randomizing patients to either dexamethasone alone or to dexamethasone with concomitant rituximab (lymphoma protocol). Results showed a significantly better sustained response by 27 % in patients receiving the steroid and rituximab regiment; 63 % versus 36 % in the steroid only arm. The occurrence of side effects nonetheless (grade 3 and 4) was much more pronounced however in the combined treatment group (Zaja et al. 2010). A newer trial demonstrated that there was no significant difference in the composite outcome of reaching a platelet count less than 50×10^9 cells/L, significant bleeding or need for rescue treatment, between a group of untreated ITP patients receiving their standard treatment and another receiving additional adjuvant rituximab (Arnold et al. 2012). One prohibitive factor in the use of rituximab has been the elevated cost. On average, a splenectomy would cost $20,000 whereas thrombopoietin and rituximab would each cost $2,500–$4,500 per month, and $10,000–$50,000, respectively (Ghanima et al. 2012).

2.4 Proposed Mechanism of Action of Rituximab in ITP

Three mechanisms of action of rituximab have been proposed. The first involves a decrease in macrophage phagocytosis and peripheral destruction of platelet coated with autoantibodies, when the latter are bound by rituximab. This would explain the early response (after 4 weeks) which occurs in the majority of cases after rituximab treatment. It is thought to be mediated by an inhibition of the Fc receptor portion of macrophages. The second mechanism involves B-cell depletion and accounts for the late response by decreasing the number of autoantibody producing B cells. The third mechanism is probably through T cell modulation effect of the drug since in some cases no correlation is observed between the disease severity and the antibody level (Stasi 2010).

2.5 Other B-Cell Depleting Agents in ITP

It seems that rituximab dominates the biologics field in ITP. A recent noteworthy trial has combined low rituximab with alemtuzumab yielded an impressive 100 % overall response rate and a complete response rate of 56 % (Gomez-Almaguer et al. 2010).

3 B-Cell Depleting Agents in TTP

3.1 TTP at a Glance

Thrombotic cytopenic purpura is a rare hematological disease that has an estimated annual incidence of 11.3 cases/1,000,000 people. It has been classically defined as patients presenting with the pentad of thrombocytopenia, uremia, microangiopathic hemolytic anemia, fever, and neurologic symptoms. This continues to be the classic teaching even though these symptoms are present all together in a minority of patients: 5 % of 64 patients from the Oklahoma registry of TTP patients (George 2010). Although TTP shares the same histological appearance with the hemolytic uremic syndrome, it is important to differentiate it from that latter since the management differs radically (Claus et al. 2010). It is considered to be an ancient disease that was first described as early as 1923 (Moschcowitz 2003). It has been proposed that it is due to aggregation of single von Willebrand factors units into a large multimer due to the absence of a metalloproteinase that usually breaks it down in order to keep the system in check (Galbusera et al. 1999). Later on, this metalloproteinase was discovered to be ADAMTS 13 (Zheng et al. 2001). The direct result of this abnormally large multimer is platelet aggregation, injury to RBCs, thrombosis, and subsequent end organ damage. ADAMTS 13's deficiency was considered the culprit of the disease up until recently when some TTP patients were discovered not to have a deficiency in this metalloproteinase (Kremer Hovinga et al. 2010; Vesely et al. 2003). TTP has been classified as either congenital or acquired. Patients having the congenital form of the disease (Upshaw–Schulman Syndrome) constitute 5 % of people with TTP whereas the people with the acquired type make up the remaining 95 %. The acquired type is further subdivided into an autoimmune idiopathic subtype that makes up 70–80 % of those patients versus a secondary subtype that constitutes the rest (Rizzo et al. 2012). Some of the secondary causes that have been described are infections such as HIV, tumors, autoimmune diseases, pregnancy, and stem cell transplant recipients (Kremer Hovinga et al. 2010).

The diagnosis of the disease continues to be mainly a clinical one due to the absence of a specific lab test that could pinpoint the diagnosis. ADAMTS 13 as mentioned earlier maybe negative in a subset of patients due mainly to the variability in the testing methods however, when present, it offers supporting evidence of diagnosis (Shah and Sarode 2013; George 2010).

3.2 Standard of Care and Available Treatments

Plasma exchange is the sinequanone of treatment in TTP since untreated, this disease is usually fatal (Ghanima et al. 2012). It has been shown to be superior to plasma infusion and can reduce mortality to 20 % or less (Rock et al. 1991).

Nonetheless, plasma exchange is not without risk. With a death rate that can reach 3 % not to mention fatal arrests, hypotension, catheter-related complications, and venous thrombosis, plasmapheresis should be used with caution (Cohen et al. 2000; McMinn et al. 2003). In plasma exchange non-responders glucocorticoids and other immunosuppressive agents such as vincristine, cyclosporine, cyclophosphamide, and even splenectomy have been tried with varying degrees of success. In addition, a large numbers of initial responders (>30 %) relapsed at a later time (George 2000, 2010; Sadler et al. 2004).

3.3 Rituximab in TTP

The investigation of rituximab as a potential treatment for TTP started in 2002 after two women with refractory TTP were reported to respond after addition of rituximab to their plasma exchange therapy (Chemnitz et al. 2002). Many studies later on observed the same effect of rituximab in treating refractory TTP patients in conjunction with plasmapheresis. A recent review reviewing six studies each of which included five or more patients reported clinical remission in 97 % from a total of 67 patients treated with rituximab given as per lymphoma protocol (Stasi 2010). This result should be interpreted with caution, since due to the rarity of the disease, many of the studies included represented small case series with no adequate controls (Sallah et al. 2004; Reddy et al. 2005; Heidel et al. 2007; Ling et al. 2009). Another review that included 118 patients with either refractory of relapsing TTP treated with rituximab came to a conclusion that 85 % of patients achieved remission and considered rituximab to be a safe and efficient treatment option in this subset of patients (Caramazza et al. 2010). Rituximab has also been shown to be a good first-line treatment in conjunction with plasma exchange. It has been shown to decrease hospital stay by a mean of 7 days in non-ICU admitted patients. Moreover, it markedly decreased relapse to 10 % versus 56 % in controls (Scully et al. 2011). Rituximab has successfully been used as a preemptive maintenance therapy in patients with recurrent disease and has also been shown to be equally effective in patients with long-standing and recently diagnosed TTP (Herbei and Venugopal 2006; Stasi 2010).

It has been postulated that rituximab acts not only by depleting antibodies against ADAMTS 113 but also by decreasing cytokine production. This stems from the fact that patients with normal ADAMTS 13 levels still respond to the biologic therapy (Kameda et al. 2007; Reddy et al. 2005).

The regular lymphoma dose of Rituximab has been used in most studies however, some have used lower or more numerous dosing regiments with success (Newman et al. 2001; Kivity and Agmon-Levin 2011). Furthermore, some authors advise for performing plasma exchange 24 h after rituximab infusion whereas others recommend doing it after 72 h (Boctor and Smith 2006).

3.4 Other B-Cell Depleting Agents in TTP

No other B-cell depleting agent has been investigated in TTP, but given the success of rituximab, we should be expecting increasing interest in this field in the near future.

4 B-Cell Depleting Agents in Evan's Syndrome

4.1 Definition and Available Therapy

Evan's syndrome is defined as autoimmune hemolytic anemia coexisting with ITP. Patients usually suffer from intermittent exacerbations and remissions in their lifetime. Its diagnosis is usually confirmed by the direct antiglobulin test which is usually positive (Norton and Roberts 2006). Corticosteroids were historically found to be the cornerstone of treatment. This poses a challenge to the treating physician especially when considering corticosteroids side effects on the long term owing to the chronicity of the disease. Research tackling second-line treatments has been scarce in this field and relied mostly on immunosuppressant such as danazol, mycophenolate mofetil, cyclosporine, or splenectomy (Norton and Roberts 2006). We have recently witnessed an emergence of studies employing rituximab as a second-line or even first-line treatment (Barcellini and Zanella 2011).

4.2 Rituximab in Evan's Syndrome

Early reports have shown encouraging results. A response rate of 83 % was first reported which subsequently increased to 94 % in new reports (Norton and Roberts 2006; Barcellini and Zanella 2011). The problem with those numbers however is that they are based on case reports and case series with no adequate controls not to mention the eventual publication bias that predominates such studies. Most of the studies deal with relapsed patients with Evan's syndrome but dosing has been highly variable between different patients. A recent retrospective study looked at the charts of 11 patients having received rituximab, seven for refractory ITP, three for relapsing hemolytic anemia, and one for refractory ITP and hemolytic anemia. An encouraging 82 % response rate was achieved with a 64 % long-term response rate after a 1 year mean follow-up (Michel et al. 2009).

Table 1 The updated (Sapporo) classification criteria for antiphospholipid antibody syndrome

Clinical criteria	Vascular thrombosis	≥1 clinical episodes of arterial, venous or small-vessel thrombosis in any tissue or organ
	Pregnancy morbidity (one of the following)	≥1 fetal death (at or beyond the 10th week of gestation)
		≥1 premature birth before the 34th week of gestation because of eclampsia, severe preeclampsia or placental insufficiency
		≥3 consecutive (pre) embryonic losses (before the 10th week of gestation)
Laboratory criteria	Lupus anticoagulant positivity on ≥2 occasions at least 12 weeks apart	
	Anticardiolipin antibody (IgG and/or IgM) in medium or high titer (i.e., >40, or above the 99th percentile), on two or more occasions at least 12 weeks apart	
	Anti-β_2-glycoprotein-1-antibody (IgG and/or IgM) in medium or high titer (i.e., above the 99th percentile) on two or more occasions at least 12 weeks apart	
Definite APS is present if at least one of the clinical criteria and one of the laboratory criteria are met		

5 B-Cell Depleting Agents in Antiphospholipid Syndrome

5.1 APS at a Glance

The antiphospholipid syndrome (APS) is an acquired autoimmune disease characterized by a hypercoagulable state that leads to arterial and/or venous thrombosis, recurrent pregnancy loss, and persistently positive antiphospholipid (aPL) antibodies, namely anticardiolipin (aCL), lupus anticoagulant (LA), and anti-β_2-glycoprotein I antibodies (anti-β2GPI). The latest classification criteria for diagnosing APS are the 2006 updated Sapporo criteria that require the presence of at least one clinical manifestation and one positive laboratory criteria (Table 1) (Miyakis et al. 2006).

In a small subset of APS patients, the disease can have an accelerated progression resulting in multiorgan failure, called "catastrophic" APS (CAPS). CAPS is characterized by multiple organ involvement with histopathologic evidence of small-vessel thrombosis developing over a very short period of time, in the presence of laboratory confirmation of aPL antibodies (Asherson et al. 2003).

In 2003, Hughes and Khamashta described another group of patients who present with clinical manifestations highly suggestive of APS but with persistently negative LA, aCL, and anti-β2GPI antibodies. This group was collectively referred to as seronegative APS (SNAPS) (Hughes and Khamashta 2003; Nayfe et al. 2013).

Taking into consideration the diverse clinical manifestations of APS, it is suggested that more than one pathological process may be involved. Despite this fact, the current therapeutic approaches are mostly restricted to anticoagulation therapy, which does not happen to benefit all patients (Pierangeli et al. 1995, 1999). Moreover, recurrent thrombotic events can occur in up to 30 % of APS patients (Gharavi et al. 1999), and 2–3 % might experience bleeding complications (Pierangeli et al. 1996). The best treatment for these APS patients who are

intolerant or resistant to long-term anticoagulation remains unclear. New pathogenic mechanisms in APS are under investigation by ongoing research, including aPL-induced activation of platelets, endothelial cells, monocytes, complement and coagulation cascade, leading to the discovery of potential targets and therapies for APS (Comarmond and Cacoub 2012). New data indicates a link between high titers of aPL antibodies and elevated circulating CD5+ B cells, suggesting that APS may be responsive to B cell targeted therapies (Youinou and Renaudineau 2004).

5.2 Pathogenesis of APS

The pathogenic mechanisms behind the clinical symptoms of APS are not fully understood, and many factors contribute in the aPL-induced manifestations of the disease (Comarmond and Cacoub 2012).

5.2.1 aPL Antibodies

aPL antibodies promote thrombus formation in both the venous and arterial circulation, and their thrombogenic properties have been shown in several in vitro and in vivo animal studies to be responsible for the pathogenesis of APS (Domenico Sebastiani et al. 2003; Dagenais et al. 1992; Zhou et al. 2011). Nonetheless, how these antibodies are produced and the exact mechanism by which they mediate thrombosis is not fully elucidated. aPLs comprise a heterogeneous family of autoantibodies; yet, similar HLA class alleles are identified to be consistent with APS patients, namely HLA-DR4, -DR7, and -DRw53 (Doring et al. 2010). aPL antibodies bind to their target cells (i.e., monocytes, platelets, endothelial cells, and trophoblasts) through a mediator plasma apolipoprotein called β2GPI, the main autoantigen for aPL antibodies. Consequently, up-regulation of tissue factor expression on monocytes and endothelial cells takes place leading to thrombosis and fetal loss through a series of signal transduction events (Romay-Penabad et al. 2007). Since aPLs play a central role in the pathogenesis of APS, special interest has been put on B cells, as they are the source of these pathogenic autoantibodies. Over the past 10 years, the role of autoreactive B cells in APS and the breakdown of B-cell tolerance have been extensively studied (Rand et al. 2008; Edwards et al. 1997). In addition to antibody production, B cells have other pathogenic mechanisms in APS, such as modifying their B-cell receptor specificity and acting as antigen presenting cells for self-antigens; besides differentiating into B effector cells (Be-1 and Be-2) which regulate helper T cells and their functions (Wallace 1994; Khattri et al. 2012).

5.3 *Immunomodulatory Approach: B-Cell Targeted Therapies*

Because accumulated data support the pathogenic role of B cells in the development and progression of APS, B-cell targeting therapies have been investigated in both human and murine APS.

5.3.1 B-Cell Depletion in APS

To date, literature review reveals only a limited number of case reports and series published regarding the use of rituximab in the treatment of APS. No randomized clinical trials were retrieved. Rituximab is a chimeric (murine/human) monoclonal antibody that targets CD20 on peripheral B cells, depleting them from the circulation and consequently decreasing disease activity (Willems et al. 2006; Youinou et al. 2009). Rituximab is FDA approved for the treatment of rheumatoid arthritis, B-cell non-Hodgkin's lymphoma (Higashida et al. 2005) and recently for anti-neutrophilic cytoplasmic antibody (ANCA)-associated vasculitis (Cohen Tervaert 2011). In addition to these indications, rituximab is being used as off-label treatment in a number of inflammatory and systemic autoimmune diseases (Butterly et al. 2010).

No reports on other B-cell directed therapies in APS patients were found.

Rituximab was used in the treatment of 27 reported cases of APS (primary, secondary, and APS with concomitant malignancies), including 18 females and 9 male patients, whose age ranged from 3 months to 69 years (Khattri et al. 2012). Of the 27 APS patients, four suffered from concomitant lymphomas; two had non-Hodgkin's lymphoma for which rituximab was administered as part of the R-CHOP chemotherapy regimen (Veneri et al. 2005; Erre et al. 2008), and two received rituximab for marginal zone lymphoma (Manner et al. 2008; Harner et al. 2004), one of which had Sjogren's syndrome also (Harner et al. 2004). Five patients had SLE with secondary APS, where rituximab was used after failure of treatment with anticoagulation and/or immunosuppression for lupus (Cianciulli et al. 2008; Weide et al. 2003; Tomietto et al. 2004; Ahn et al. 2005; Anandacoomarasamy et al. 2006). One patient had Evans syndrome (Ruckert et al. 2008). The rest of the patients had primary APS (Iglesias-Jimenez et al. 2010; Nageswara Rao et al. 2009; Tsagalis et al. 2010; Adamson et al. 2008; van Wissen et al. 2008; Chalam et al. 2007; Ames et al. 2007; Rubenstein et al. 2006; Trappe et al. 2006; Binstadt et al. 2003; Erdozain et al. 2004; Asherson et al. 2008; Sciascia et al. 2011). Table 2 summarizes the clinical and serological manifestations of the reported APS patients along with their outcomes after treatment with rituximab (Khattri et al. 2012). The rituximab dosing regimen used in the majority of the cases was 375 mg/m^2 body surface area, given weekly for 4 weeks. Four patients received rituximab 1,000 mg given 15 days apart. The reported patients were not treatment naïve prior to rituximab administration; most received anticoagulation unless

otherwise contraindicated, eight patients were treated with cyclophosphamide earlier (Ahn et al. 2005; Anandacoomarasamy et al. 2006; Iglesias-Jimenez et al. 2010; Ames et al. 2007; Rubenstein et al. 2006; Binstadt et al. 2003; Asherson et al. 2008), and all but one case (Tsagalis et al. 2010) used corticosteroids. The treatment regimen contained other immunosuppressants too, including azathiprine, mycophenolate mofetil, dapsone, and cyclosporine (Khattri et al. 2012). Improvement in the serological markers of APS was noticed in the majority of the cases, where decrease or normalization of LA, aCL, and anti-β2GPI antibodies was seen. Furthermore, multiple systemic clinical manifestations associated with APS improved after starting rituximab regardless whether the patient suffered from primary or secondary APS. There was a uniformly good response to rituximab in primary APS and in APS associated with SLE (Weide et al. 2003; Cianciulli et al. 2008; Tomietto et al. 2004; Ahn et al. 2005; Anandacoomarasamy et al. 2006; Ruckert et al. 2008; Tsagalis et al. 2010; Adamson et al. 2008; Chalam et al. 2007; Ames et al. 2007; Rubenstein et al. 2006; Trappe et al. 2006; Binstadt et al. 2003; Erdozain et al. 2004; Sciascia et al. 2011; Vianna et al. 1994; Danowski et al. 2009) or lymphoma (Erre et al. 2008; Veneri et al. 2005; Manner et al. 2008; Harner et al. 2004) in the case reports reviewed. Moreover, six out of seven CAPS patients benefited from rituximab treatment, knowing the severity and frequent fatality faced in this entity in spite of standard treatment with anticoagulants and immunosuppressant agents (Manner et al. 2008; Iglesias-Jimenez et al. 2010; Nageswara Rao et al. 2009; van Wissen et al. 2008; Rubenstein et al. 2006; Asherson et al. 2008).

In the BIOGEAS registry, a multicenter, national registry in Spain, rituximab was shown to have beneficial therapeutic effects in APS, with 92 % response rate in 12 APS patients (Ramos-Casals et al. 2008).

Despite the promising data from case reports and the BIOGEAS registry on the beneficial effect of rituximab in APS patients, the literature is still limited on this topic as there are no clinical trial data available yet. Moreover, the above-mentioned published case reports have several limitations including their small number, besides the fact that in all the cases, other immunosuppressants were used including steroids and cyclophosphamide, which creates confusion on whether it was rituximab-induced B-cell depletion by itself or the combination of immunosuppressants used that caused improvement in APS patients. Also, it is worth mentioning that the treated population was diverse including patients with primary or secondary APS or malignancy (Khattri et al. 2012). Finally, in a pilot open-label phase II trial aimed primarily to evaluate the safety of rituximab in aPL-positive patients with non-criteria manifestations of APS, and secondarily to evaluate the effect on the aPL profile and efficacy of treatment, it was suggested that rituximab may be effective in controlling some but not all non-criteria manifestations of APS with a safety profile in aPL-positive patients consistent with that of rituximab (Erkan et al. 2013).

Table 2 Review of the clinical and serological manifestations and outcomes in APS patients treated with rituximab

Clinical manifestations	Serological manifestations	Serological outcome	Clinical outcome
Venous thrombosis (Weide et al. 2003; Ahn et al. 2005; Anandacoomarasamy et al. 2006; Ruckert et al. 2008; Erre et al. 2008; Veneri et al. 2005; Manner et al. 2008; Harner et al. 2004; Iglesias-Jimenez et al. 2010; Nageswara Rao et al. 2009; Tsagalis et al. 2010; Adamson et al. 2008; van Wissen et al. 2008; Chalam et al. 2007; Ames et al. 2007; Rubenstein et al. 2006; Trappe et al. 2006)	aCL, LA, anti-β2GPI	Normalization of aCL, LA, anti-β2GPI	No new thrombotic events
Arterial thrombosis (van Wissen et al. 2008; Asherson et al. 2008; Ruckert et al. 2008)	aCL, LA	LA normalized	Minor self-limiting relapses (Asherson et al. 2008), clinical improvement in others
Hematological (thrombocytopenia, AIHA) (Ahn et al. 2005; Anandacoomarasamy et al. 2006; Ruckert et al. 2008; Erre et al. 2008; Manner et al. 2008; Iglesias-Jimenez et al. 2010; Tsagalis et al. 2010; van Wissen et al. 2008; Chalam et al. 2007; Ames et al. 2007; Rubenstein et al. 2006; Trappe et al. 2006; Erdozain et al. 2004; Asherson et al. 2008; Sciascia et al. 2011)	aCL, LA, anti-β2GPI	LA and anti-β 2GPI normalized, aCL decreased	No new bleeding episodes, thrombocytopenia improved
Neurological (seizures, chorea, cerebral vasculitis, CVA) (Tsagalis et al. 2010; Erdozain et al. 2004; Sciascia et al. 2011; Binstadt et al. 2003; Nageswara Rao et al. 2009; Weide et al. 2003; Tomietto et al. 2004)	aCL, LA, anti-β2GPI	aCL, LA, anti-β2GPI decreased	Seizures resolved

(continued)

Table 2 (continued)

Clinical manifestations	Serological manifestations	Serological outcome	Clinical outcome
Pulmonary (ARDS) (Asherson et al. 2008)	aCL		Restoration of lung function and discontinuation of respiratory support
Renal (acute renal failure) (Tsagalis et al. 2010; Asherson et al. 2008)	aCL	aCL normalized	Improvement in serum creatinine
Gastrointestinal (ischemic bowel, mesenteric and celiac artery occlusion) (van Wissen et al. 2008; Asherson et al. 2008)	aCL, LA		One patient died of sepsis (Asherson et al. 2008), the second had no further thromboembolic events (van Wissen et al. 2008)
Cardiovascular (right atrial thrombus, MI) (Rubenstein et al. 2006; Anandacoo-marasamy et al. 2006; Cianciulli et al. 2008)	aCL, LA		No further intra-cardiac thrombi formation
Adrenal (adrenal hemorrhage) (Nageswara Rao et al. 2009)	LA		Decrease in size of hemorrhage
Cutaneous (vasculitis, livedo, necrosis) (Ruckert et al. 2008; Iglesias-Jimenez et al. 2010; Asherson et al. 2008; Binstadt et al. 2003; Anandacoo-marasamy et al. 2006)	aCL, LA, anti-β2GPI	aCL and anti-β 2GPI normalized	Clinical improvement in skin involvement
Pregnancy loss (Tsagalis et al. 2010)	aCL	aCL normalized	No further pregnancy losses
CAPS cases (Manner et al. 2008; Iglesias-Jimenez et al. 2010; van Wissen et al. 2008; Rubenstein et al. 2006; Asherson et al. 2008; Nageswara Rao et al. 2009)	aCL, LA, anti-β2GPI	aCL, LA, anti-β2GPI normalized	All patients improved except for one death due to complications (sepsis, subdural hematoma) (Asherson et al. 2008) and one had minor self-limiting episodes associated with thrombocytopenia (Asherson et al. 2008)
Primary APS (Ruckert et al. 2008; Tsagalis et al. 2010; Chalam et al. 2007; Ames et al. 2007; Rubenstein et al. 2006; Trappe et al. 2006; Erdozain et al. 2004; Sciascia et al. 2011; Binstadt et al. 2003; Adamson et al. 2008)	aCL, LA, anti-β2GPI	aCL, LA, anti-β2GPI normalized	Improvement in thrombocytopenia, no further thrombotic events

(continued)

Table 2 (continued)

Clinical manifestations	Serological manifestations	Serological outcome	Clinical outcome
APS associated with SLE (Weide et al. 2003; Tomietto et al. 2004; Ahn et al. 2005; Cianciulli et al. 2008; Anandacoomarasamy et al. 2006)	aCL, LA, anti-β2GPI	aCL, LA, anti-β2GPI normalized	No new thrombotic events, seizures resolved
APS associated with lymphoma (Erre et al. 2008; Manner et al. 2008; Veneri et al. 2005; Harner et al. 2004)	aCL, LA, anti-β2GPI	aCL, LA, anti-β2GPI normalized	No new thrombotic events

aCL anticardiolipin antibody, *β2GPI* β2 glycoprotein 1, *LA* lupus anticoagulant, *CVA* cerebrovascular accident, *MI* myocardial infarction, *AIHA* autoimmune hemolytic anemia, *ARDS* acute respiratory distress syndrome

5.4 Conclusion

Rituximab, a chimeric anti-CD20 monoclonal antibody, is FDA approved for the treatment of rheumatoid arthritis, B-cell non-Hodgkin's lymphoma and ANCA-associated vasculitis. The off-label use of B-cell depleting agents in several systemic autoimmune diseases has been studied. Data on the use of rituximab in the treatment of APS is limited to case reports, the BIOGEAS registry and a pilot open-label phase II trial, and suggests a beneficial role in the therapeutic approach of APS. However, well-designed randomized clinical trials are needed to evaluate the use of rituximab, alone or in combination with other immunosuppressants, in improving the clinical and serological manifestations of the disease.

6 B-Cell Depleting Agents in Autoimmune Hemolytic Anemia

6.1 AIHA at a Glance

Autoimmune hemolytic anemia (AIHA) is an uncommon disorder characterized by autoantibodies directed against self red blood cells (RBCs) (Gehrs and Friedberg 2002). Consequently, the normal 100–120 days lifetime of the RBCs is reduced to just a few days in serious cases (Sawitsky and Ozaeta 1970). AIHA can be idiopathic or secondary to infections, other autoimmune conditions or lymphoproliferative disorders, and depending on the thermal range of the autoantibodies involved, the disease can be classified into warm, cold (which includes cold agglutinin disease and paroxysmal nocturnal hemoglobinuria) or mixed (Gehrs

and Friedberg 2002). Whether warm- or cold- type secondary AIHA, each can result from its own more common secondary causes. For instance, secondary warm-type AIHA mostly results from lymphoproliferative disorders (e.g., chronic lymphocytic leukemia, lymphoma) and other autoimmune disorders, including SLE, RA, scleroderma, and ulcerative colitis. Less commonly, it can be caused by neoplasms other than lymphoid and infection. Similarly, secondary cold-type AIHA is primarily caused by lymphoproliferative disorders, but also occurs secondary to infection, especially by mycoplasma, viral pneumonia, infectious mononucleosis, and other respiratory infections, and infrequently due to concomitant autoimmune disorders (Sokol et al. 1981).

6.2 Treatment of AIHA

6.2.1 Standard Treatment

In warm AIHA, the first-line therapy has been the administration of corticosteroids, where the response rate reaches 70–85 %, of which only one third remain in long-term remission after drug discontinuation, 50 % necessitate maintenance doses, and around 20–30 % require second-line therapies including immunosuppressants and splenectomy (Wahl et al. 2008). Splenectomy is probably the most effective second-line treatment with a response rate of 50 %, especially in relapsing patients on corticosteroids or those requiring the equivalent of 10–15 mg prednisone per day to maintain adequate hemoglobin levels. We have to bear in mind the surgical and infective complications, particularly gram-negative sepsis in patients above 65 (Gehrs and Friedberg 2002; Valent and Lechner 2008; Newland et al. 2005). Moreover, patients who are unresponsive to or do not fit for splenectomy, have limited options including cytotoxic or immunosuppressive medications such as azathioprine, cyclophosphamide, or cyclosporine, with a response rate of 40–60 % and associated side effects (52). On the other hand, cold-type AIHA has failed to demonstrate a convincing response to standard therapy, particularly cold hemaglutinin disease (Barcellini and Zanella 2011).

6.2.2 Rituximab: Treatment of Cases Refractory to Standard Therapy

In a recent review carried by Garvey et al. collecting data from studies using rituximab in the treatment of AIHA, rituximab (375 mg/m^2 given weekly for 4 weeks) was found to be effective in treating both warm AIHA and cold hemaglutinin disease, with a median response rate of 60 % and lasting responses for more than 3 years (Garvey 2008). Furthermore, in three more recent studies (Dierickx et al. 2009; Bussone et al. 2009; Penalver et al. 2010), the response rate was higher and ranged from 77 to 93 % with a disease-free survival at 1 and 2 years in 72 and 56 % of cases, respectively (Dierickx et al. 2009). In several case series,

rituximab has been shown to be effective in both patient groups with idiopathic and secondary AIHA, even in those associated with autoimmune and lymphoproliferative disorders, and bone marrow transplant (Shanafelt et al. 2003; Penalver et al. 2010; Quartier et al. 2001; Zecca et al. 2003; Narat et al. 2005; Berentsen et al. 2006; Schollkopf et al. 2006; D'Arena et al. 2007; Gupta et al. 2002; Trape et al. 2003; D'Arena et al. 2006). Moreover, rituximab proved to be effective whether used as a monotherapy or in combination with corticosteroids, immunosuppressants and interferon (Zecca et al. 2003; Narat et al. 2005; Berentsen et al. 2006; D'Arena et al. 2007; Gupta et al. 2002), and irrespective or prior therapy (Penalver et al. 2010; Shanafelt et al. 2003; Quartier et al. 2001; Zecca et al. 2003; Narat et al. 2005; Berentsen et al. 2006; Schollkopf et al. 2006; Gupta et al. 2002; Trape et al. 2003; D'Arena et al. 2006). Time to maximum response varied between the studies from quick response to weeks or even months, where in two recent studies, the median time to response was 3 and 6 weeks, respectively (Bussone et al. 2009; Penalver et al. 2010). It is noteworthy that re-treatment with rituximab is effective with both warm AIHA and cold hemaglutinin disease (Rao et al. 2008; Zecca et al. 2003; Berentsen et al. 2006; Gupta et al. 2002), with re-treatment benefiting some patients more than once (Zecca et al. 2003; Berentsen et al. 2006; Penalver et al. 2010). When comparing rituximab to the next best therapeutic regimen which includes alkylating agents with or without corticosteroids, rituximab was the only treatment able to induce a complete response in cold hemaglutinin disease with a response of 60 % (10 % complete response and 50 % partial response), compared with 16 % (all partial responses) (Berentsen et al. 2006; Berentsen et al. 2004; Barcellini and Zanella 2011). Regarding rituximab safety when given to patients with AIHA, the drug was well tolerated and no adverse events were reported in most cases, besides mild to moderate infusion-related side effects (e.g., fever, chills, hypotension, and upper airway edema) (Rao et al. 2008; Zecca et al. 2003; Schollkopf et al. 2006; Gupta et al. 2002; Trape et al. 2003). Some patients (around 7 %) experienced possible rituximab-related infections (Quartier et al. 2001; Zecca et al. 2003; Narat et al. 2005; Trape et al. 2003; Schollkopf et al. 2006), and few (roughly 2 %) had grade 4 neutropenia (Berentsen et al. 2006; Gupta et al. 2002). Moreover, low dose rituximab (100 mg $\times$ 4 weeks) was tried in patients unresponsive to standard therapy in an attempt to reduce side effects and costs, and it was found effective as a monotherapy (Provan et al. 2007) and in combination with alemtuzumab (Gomez-Almaguer et al. 2010). In a study conducted by Barcellini et al, low dose rituximab along with standard oral prednisone was able to induce an overall response rate of 86 % (complete 67 % and partial 19 %) in 21 patients with warm and cold AIHA; response was sustained (Hb $>$10 g/dl) in 13/14 patients at 6 months and in 11/11 evaluable patients at 1 year (BARCELLINI et al. 2010).

6.3 Conclusion

Rituximab proved to be an effective therapeutic alternative to standard therapy of splenectomy and/or chemotherapy in patients with primary or secondary warm AIHA with a significant response rate and sustained remissions. Furthermore, rituximab induced durable responses in patients with cold hemaglutinin disease, once a disease with very limited therapeutic options (Barcellini and Zanella 2011).

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