Developments in modern hemophilia care

Hassan, S.

Chapter 7

Preventing or eradicating factor VIII antibody formation in patients with hemophilia A; what can we learn from other disorders?

Shermarke Hassan, Karin Fijnvandraat, Johanna G. van der Bom, Samantha C. Gouw

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Abstract

Eradication of factor VIII-specific neutralizing antibodies (also known as inhibitors) by the traditional method of immune tolerance induction (ITI) is costly and unsuccessful in one out of three patients. Furthermore, effective inhibitor prevention strategies are presently lacking. An overview is given in this narrative review of anti-drug antibody prevention or eradication strategies that have been used in disorders beyond hemophilia A with the aim of analyzing what we can learn from these strategies for hemophilia A.

Prevention of anti-drug antibody formation using rituximab, methotrexate and intravenous immunoglobulins in patients with Pompe disease seems effective but carries a high risk of adverse events. Based on studies in patients with rheumatoid arthritis and inflammatory bowel disease, it seems likely that treatment with methotrexate alone would also be able to prevent inhibitor formation in hemophilia A patients. Besides side effects, it is unclear whether immune tolerance to FVIII would persist after cessation of immunomodulatory therapy with methotrexate. A combination of cyclophosphamide and corticosteroids, used to treat antibody-mediated pure red cell aplasia, could be further investigated to eradicate inhibitors in hemophilia A patients who are refractory to ITI.

In summary, insights gained from research on anti-drug antibody formation in other diseases could be helpful in devising alternative treatment strategies for inhibitor development.

Hemophilia A

Hemophilia A is a hereditary X-linked hemorrhagic disorder that is caused by genetic mutations in the *f8* gene. These mutations lead to a deficiency of functional clotting factor VIII (FVIII) which is associated with frequent bleeds, especially in joints and muscles. In the long term, repeated joint bleeds cause bleeding-induced joint damage with concomitant disability and reduced quality of life. The disease can be treated by intravenous administration of the deficient factor with FVIII concentrates. The severity of the disease is based on the plasma concentration of clotting factor and is usually classified as severe (‹ 0.01 IU/ml), moderate (0.01-0.05 IU/ml) or mild (› 0.05–0.40 IU/ml). Compared to mild and moderate patients, patients with severe hemophilia A experience more frequent bleeding episodes. In addition, most bleeds in patients with mild or moderate hemophilia A are due to trauma or surgery whereas the majority of bleeds in severe hemophilia A occur spontaneously (i.e., are non-traumatic bleeds).[1]

Inhibitor formation

A major treatment complication in hemophilia A is the formation of neutralizing antibodies against FVIII, also known as inhibitors (because they inhibit the function of FVIII), which renders subsequent treatment with FVIII ineffective. Inhibitor formation is most common in patients with severe hemophilia A, as roughly one in three of these patients develop clinically relevant inhibitors.[2] There is a strong relationship between the incidence of inhibitor formation and the number of days that a patient is exposed to treatment with FVIII (also referred to as the number of exposure days). In patients with severe hemophilia A, inhibitors develop after a median of 15 exposure days[3] and almost all inhibitors occur within the first 75 exposure days[4, 5]. Due to the relatively severe bleeding phenotype of these patients, they are exposed to FVIII at a very young age, especially if prophylactic treatment with FVIII is initiated. Consequently, most severe hemophilia A patients develop inhibitors at a very young age. The median age at which inhibitors were detected was 1.3 years (IQR: 1.0-2.0) in a European registry of 108 severe hemophilia A patients.[6] In patients with neutralizing antibodies, normal doses of FVIII concentrates are no longer effective as prevention or treatment for bleeding. Therefore, these patients need to be treated with FVIII bypassing agents such as recombinant activated factor VII (rFVIIa) or activated prothrombin complex concentrate (aPCC).[7]

Immune tolerance induction

To eradicate inhibitors in hemophilia A patients, frequent administration of high doses of FVIII over a long period of time is needed. This is commonly known as "immune tolerance induction" (ITI).[8] Well known ITI protocols include the "Bonn" protocol (basic protocol: 100-150 IU/kg FVIII twice daily)[9] and the "van Creveld" protocol (starting dose: 25 IU/kg FVIII every other day, dosage is decreased when FVIII recovery exceeds 30%)[10]. The required duration of ITI to obtain tolerance to factor VIII varies per patient. In a large multicenter randomized clinical trial comparing a high (200 IU/kg/day) and a low dose of ITI (50 IU/kg three times/week), the time until complete recovery was 15.5 months (IQR: 10.8-22.0) in the low-dose group and 10.6 months (IQR: 6.3-20.5) in the high-dose group.[11] Inhibitors are successfully eradicated in roughly two-thirds of patients.[12] The inhibitor relapse rate after successful ITI varies between 2.3-10% in most studies.[13-15] As treatment and prophylaxis with rFVIIa or aPCC is less effective and more expensive than treatment with FVIII[7], morbidity among patients with inhibitors is higher and their quality of life is lower than that of patients without inhibitors.[16, 17] In patients with moderate or mild hemophilia A, the inhibitor titer may spontaneously decrease and become unmeasurable due to the continuing production of endogenous FVIII. However, when treatment with a (wild type) FVIII concentrate is indicated, the inhibitor titer may rise again due to an anamnestic response, reflecting lack of sustained tolerance.[18]

Drawbacks of immune tolerance induction

As of now, ITI is the standard treatment for patients with inhibitors. Although ITI is a safe and relatively successful inhibitor eradication strategy, the long duration and high intensity of treatment is very demanding for the usually young patients and their families and it is very costly. In addition, effective treatment options for inhibitor patients who are refractory to ITI are lacking. New inhibitor prevention/eradication strategies are therefore urgently needed to improve patient outcomes and reduce ITI cost.

Preventing or eradicating anti-drug antibody formation: what can we learn from other research disciplines?

The problem of anti-drug antibody formation is not confined to hemophilia A.[19] Many biopharmaceuticals are immunogenic to a certain degree, ranging from a limited immune reaction to a major clinically relevant antibody response. For example, clinically relevant anti-drug antibody formation is (or used to be) a significant problem in patients using tumor necrosis factor (TNF) inhibitors (used in rheumatic diseases)[20], epoetin (used for anemia in chronic renal failure)[21], interferon beta (used in multiple

sclerosis)[22], alglucosidase alfa (used in Pompe disease)[23] and peglocitase (used to treat gout).[24, 25] Most anti-drug antibody research is disease-specific and knowledge is not shared easily across research disciplines.

Promising new therapies to treat or bypass inhibitor development are also underway (e.g. engineered FVIII-specific regulatory T-cells).[26] However, these novel therapies are still out of reach for the near future. There is a need for alternative treatment strategies that can be implemented today, rather than sometime in the future (i.e. that make use of therapeutics that are currently on the market).

Over the last years, many different anti-drug antibody prevention or eradication strategies (mainly using immunomodulatory agents) have been investigated in patients with disorders other than hemophilia A. This review therefore aims to compile information on the efficacy and safety of these different strategies and to contemplate whether knowledge from these other fields might inspire novel treatment strategies for hemophilia A patients.

Prevention of anti-drug antibody formation

What is already known in hemophilia A

In general, risk factors for inhibitor development can be divided into genetic risk factors such as FVIII genotype, ethnicity, HLA-type and genetic polymorphisms that encode proteins involved in the immune system such as IL-10 and TNF-alfa.[27] In addition, there are treatment-related risk factors such as the intensity of FVIII treatment, the frequency of FVIII exposure, FVIII dose, exposure to FVIII during surgery, prophylactic vs. on-demand treatment and the specific type of FVIII product used.[28] As genetic risk factors for inhibitor formation (such as FVIII genotype) are immutable, strategies to prevent inhibitor formation have focused on influencing treatment-related risk factors.

A single-arm study published in 2009 evaluated the effect of a treatment regimen that aimed to minimize the risk of inhibitor formation.[29] The treatment regimen consisted of early initiation of prophylaxis and minimizing exposure to “danger signals” (due to trauma, surgery, infection, vaccination etc.) during FVIII infusion. Surprisingly, only 1/26 (3.8%) patients on this modified treatment regimen developed inhibitors compared to 14/30 (47%) patients in the control group. These results were not replicated in a follow-up study (the EPIC study) as 8/19 (42.1%) patients on the same protocol developed an inhibitor.[30]

As mentioned earlier, there are several novel therapeutics that could be used for preventing inhibitor formation in high-risk patient groups, some of the therapeutics are currently being investigated in patients with hemophilia A. These novel therapeutics include an anti-tissue factor pathway inhibitor antibody (concizumab), a bispecific antibody against FIXa/FX that mimics the function of FVIII (emicizumab), a rFVIIa product with enhanced half-life due to fusion with albumin (rFVIIa-FP) and a short interfering RNA molecule that inhibits production of antithrombin (fitusiran). As of yet, none of these therapeutics have received market approval by the FDA or EMA.[31]

Several animal studies using FVIII-deficient mice have found that a short course of treatment with rapamycin[32], anti-CD20 therapy[33], anti-CD3 therapy[34] or dexamethasone[35] significantly prevented inhibitor formation, even after cessation of the immunomodulatory agent. As of yet, no human studies have evaluated inhibitor prevention with these immunomodulatory agents in patients with hemophilia A.

Prevention of anti-drug antibody formation: what is known from other diseases

Most evidence on the prevention of anti-drug antibody formation comes from patients with Pompe disease and patients with rheumatoid arthritis or inflammatory bowel disease. The following paragraphs will review the available evidence on the efficacy and safety of anti-drug antibody prevention strategies in these patient groups.

Antibodies against recombinant human acid alpha-glucosidase in patients with Pompe disease

There is very limited experience with immunomodulatory therapy to prevent anti-drug antibody formation in very young pediatric patients. Pompe disease (also known as glycogen storage disease type II) is an autosomal recessive lysosomal storage disease caused by a deficiency of the lysosomal enzyme acid alpha-glucosidase (GAA), which leads to accumulation of glycogen in the lysosome. The clinical phenotype is a spectrum that ranges from the classic infantile phenotype (the most severe form in which progressive cardiac hypertrophy is always present) to the late onset "childhood" and "adult" phenotypes.[36]

The overall incidence of Pompe disease is roughly 1:40,000 for all types[37, 38] and 1:138,000 for patients with the classic infantile phenotype[37]. Overall, anti-drug antibody formation is especially problematic in patients with the classic infantile phenotype and less so in patients with the adult-onset phenotype.[39, 40]

The classic infantile form has a more severe and rapidly progressing clinical course than the late-onset childhood and adult forms and is associated with hypertrophic cardiomyopathy, muscle weakness and respiratory distress. In general, untreated patients with classic infantile Pompe disease die within the first year of life.[41, 42]

Enzyme replacement therapy with recombinant human acid alpha-glucosidase (rhGAA) is the only available treatment option. In patients with classic infantile Pompe disease enzyme, replacement therapy is initiated as soon as patients are diagnosed to prevent further clinical deterioration. Roughly, 66-75% of patients with classic infantile Pompe disease have some residual GAA production (CRIM-positive patients) whereas 25-33% produce no GAA at all (CRIM-negative patients).[23, 43, 44] Being CRIM-negative is strongly associated with a low therapeutic response to enzyme replacement therapy.[23] Several studies have shown that the majority (› 90%) of patients with infantile Pompe disease develop anti-drug antibodies, regardless of CRIM-status.[23, 44, 45] Antibody testing is usually performed using an enzyme-linked immunosorbent assay. High-titer antibodies against rhGAA occur more commonly in CRIM-negative patients[44] and are associated with a poor therapeutic response to enzyme replacement therapy. Compared to patients with low-titer antibodies, patients with high-titer antibodies have worse clinical outcomes in terms of overall survival, ventilator-free survival, left ventricular mass index and the Alberta Infant Motor Scale.[46]

Anti-drug antibody formation in other lysosomal storage disorders

Anti-drug antibody formation also occurs in other lysosomal storage disorders, such as Gaucher disease and Fabry disease. Gaucher disease is an autosomal recessive disorder in which the enzyme glucocerebrosidase is deficient, leading to accumulation of glucocerebroside in the lysosomes of cells (mainly macrophages). The most common clinical manifestations are anemia, thrombocytopenia, hepatosplenomegaly and various manifestations of bone disease.[47] Roughly 15% of patients with Gaucher disease develop IgG-antibodies against glucocerebrosidase replacement therapy.[48] Over time, most patients (90%) are tolerized to glucocerebrosidase.[49] Cases of patients with sustained neutralizing antibody activity that impacts clinical efficacy are extremely rare.[50]

Fabry disease is an X-linked disorder in which the enzyme alpha-galactosidase A is deficient, leading to accumulation of globotriaosylceramide in cells. Clinical manifestations during childhood include neuropathic pain and angiokeratoma. In later life, renal, cardiac and cerebral manifestations of the disease become more prominent.[51] Around 73% of men and 12% of women with Fabry disease develop IgG-anti-

bodies against alpha-galactosidase A replacement therapy. Males with Fabry disease have less residual enzyme activity compared to females (because Fabry disease is X-linked) which leads to higher rates of anti-drug antibody formation in males. Anti-drug antibody formation seems to negatively influence biochemical parameters in the blood and urine.[52, 53] The association between anti-drug antibody formation and clinical outcomes is less clear.[53, 54] This is in part caused by the lack of a uniform assay methodology to detect anti-drug antibodies and the limited effectiveness of enzyme replacement therapy in this progressive disorder.[53] Overall, anti-drug antibody prevention/eradication strategies are very rarely applied in patients with Gaucher disease or Fabry disease. The next paragraph will focus on anti-drug antibody prevention strategies in patients with Pompe disease.

Overview of anti-drug antibody prevention strategies in Pompe disease

Several small studies (mostly case-reports or case-series) have evaluated anti-drug antibody prevention strategies; the mostly CRIM-negative patients with Pompe disease in these studies were treated with immunomodulatory agents at the start of enzyme replacement therapy. Because Pompe disease is a progressive disorder, anti-drug antibody formation (that renders enzyme replacement therapy ineffective) leads to irreversible damage. The prevention of anti-drug antibodies would expectedly lead to better outcomes. The four largest case-series, all published between 2013-2017, included 38 CRIM-negative patients that underwent immunomodulatory therapy to prevent anti-drug antibody formation (table 1).

In 2013, a case-series[55] was published that evaluated immunomodulatory therapy in four CRIM-negative patients with Pompe disease at the start of enzyme replacement therapy (table 1). Three patients received an initial cycle of rituximab and maintenance therapy with rituximab, sirolimus and intravenous immunoglobulins (IVIG). One patient received an initial cycle of rituximab and maintenance therapy with mycophenolate and IVIG. IVIG was given to provide passive immunity during the period of B-cell depletion due to rituximab. In total, 1 patient developed high-titer anti-rhGAA antibodies. This was the patient that received maintenance therapy with mycophenolate. The other 3 patients remained antibody negative until the end of the follow-up. Because these patients received maintenance rituximab every 12 weeks, B-cell recovery (defined as having B-cells within normal range after B-cell depletion) was not observed in these patients. No immunomodulation-related adverse events were reported, with the exception of one patient who experienced multiple viral respiratory tract infections during treatment with immunomodulatory agents.

Because of prolonged B-cell depletion, patients were not vaccinated during the study (except with seasonal influenza vaccine).

That same year, another case-series was published, this study reported on 7 CRIM-negative patients with Pompe disease who received immunomodulatory therapy at the start of enzyme replacement therapy to prevent anti-drug antibody formation (table 1).[56] The treatment regimen used (total duration 5 weeks) consisted of rituximab and methotrexate, in addition, IVIG was administered. In total, 4/7 patients were antibody negative until the end of follow-up. The period between B-cell recovery and antibody measurement might have been too short (3.5 months) to assess the effect of treatment in one patient who was antibody-negative. Furthermore, B-cell recovery was not measured at all in another antibody-negative patient. These patients were compared to a historical cohort of 11 CRIM-negative patients who were treated with enzyme replacement therapy alone. Compared to patients treated with immunomodulatory therapy, all patients treated with enzyme replacement therapy alone developed anti-rhGAA antibodies during follow-up. In addition, these patients had significantly worse clinical outcomes (ventilator-free survival and left-ventricular mass index) than patients who were treated with immunomodulatory therapy. One patient developed a possible immunomodulation-related infection and had to be hospitalized.

In 2016, a retrospective analysis reported on 13 CRIM-negative patients from the UK of whom 8 were treated with rituximab and methotrexate at the start of enzyme replacement therapy to prevent anti-drug antibody formation (table 1).[57] One out of 8 CRIM-negative patients treated with immunomodulatory therapy developed intermediate-titer anti-rhGAA antibodies (peak titer was 1:12800 at 8 months old). The remaining 7 patients remained antibody-negative during follow-up. B-cell recovery after initial treatment with rituximab did not occur in 1 patient that remained antibody-negative during follow-up. Furthermore, it is unclear if the follow-up period after B-cell recovery was long enough to assess the effect of treatment in the other 6 patients who remained antibody-negative during follow-up. Another 5 CRIM-negative patients did not receive immunomodulatory therapy, of these, only 2 were tested for antibodies. Both patients had high-titer anti-rhGAA antibodies (peak titer 1:204,000). Survival was higher among CRIM-negative patients receiving enzyme replacement therapy and immunomodulatory therapy when compared to CRIM-negative patients treated with enzyme replacement therapy alone. Information about adverse events during immunomodulatory therapy was not reported.

Table 1. Overview of largest case-series evaluating immunomodulatory therapy in CRIM-negative patients with classic-infantile Pompe disease.

First author (Year of publication)	N	Median age (range) at start of treatment	Treatment
Elder (2013)[55]	4	7 months (2-8)	Initial rituximab cycle (375 mg/m^2 per week for three weeks or two doses of 750 mg/m2 10-14 days apart). Maintenance rituximab (375 mg/m^2 every 12 weeks). sirolimus (initial dose 0.6-1 mg/m^2 per day) or mycophenolate (300 mg/m^2 per day). Monthly IVIG (initial dose 500–1000 mg/kg).
Banugaria (2013)[56]	7	3.5 months (0.4-6.5)	Rituximab (IV, 375mg/m^2 weekly for 4 weeks). Methotrexate (SC, 0.4 mg/kg, three doses per week for 3 weeks) IVIG (400-500 mg/kg, monthly for 5-6 months).
Broomfield (2016)[57]	8	4.3 months (0-6.7)	Rituximab (intravenous, weekly for 4 weeks, dose not reported). Methotrexate (subcutaneous, 3 days per week for 6 weeks, dose not reported).
Kazi (2017)[58]	19	3.4 months (range 0.1–10.9)	The ITI cycle consisted of rituximab, methotrexate, and IVIG. Exact dosing was not reported but probably similar to the study by Banugaria et al.

A study published in 2017 evaluated immunomodulatory therapy to prevent anti-drug antibody formation at the start of enzyme replacement therapy in a larger cohort of 19 CRIM-negative patients.[58] (table 1) The treatment regimen consisted of rituximab, methotrexate, and IVIG. Eight patients never developed antibodies. There was B-cell recovery after depletion with rituximab in all these patients. Similar to the previous study, it was unclear if the follow-up period after B-cell recovery was long enough to assess the effect of treatment. Seven patients had low antibody titers at the end of follow-up (defined as titers ≤ 1:6,400). The remaining 4 patients developed intermediate to high antibody titers. These patients were compared to a historical cohort of 11 CRIM-negative patients who were treated with enzyme replacement therapy alone. All patients treated with enzyme replacement therapy alone developed anti-rhGAA antibodies during follow-up. In addition, these patients had significantly worse survival

Median follow-up (range)	Negative antibody status	Positive antibody status, median peak titer (range)	Suspected treatment-related infections	Anti-drug antibody assay
27.8 months (11-36)	3/4	1/4 (25%), titer not reported	1/4	Enzyme-linked immunosorbent assay.
16.1 months (10.6-23.2)	4/7	3/7, 1:6,400 (1,600-6,400)	1/7	Enzyme-linked immunosorbent assay and confirmation using radioimmunoprecipitation. Performed by product manufacturer.
Not reported	7/8	1/8, 1:12,800	Not reported	Performed by the product manufacturer, exact methodology not reported.
24.2 months (range 6.0-100.2)	8/19	11/19, 1:6,400 (200-51,200)	4/19	Performed by the product manufacturer, exact methodology not reported.

than patients who were treated with immunomodulatory therapy. Four patients who were treated with immunomodulatory therapy developed a serious bacterial infection.

Taken together, 22 out of 38 (58%) CRIM-negative pediatric patients with classic infantile Pompe disease who were treated with rituximab, methotrexate and IVIG to prevent anti-drug antibody formation did not develop anti-rhGAA antibodies. In comparison, literature has shown that virtually all (› 90%) CRIM-negative pediatric patients with classic infantile Pompe disease develop anti-rhGAA antibodies.[23, 44, 45] In patients with Pompe disease, it seems that concomitant immunomodulatory therapy for a short period of time (5-6 weeks) at the start of enzyme replacement therapy is effective in preventing anti-drug antibody formation in a large proportion of patients.

In addition, long-term follow-up results indicate that these patients maintain tolerance to rhGAA after cessation of immunomodulatory therapy. The treatment duration was very short, causing minimal interruption to the vaccination schedule while the rate of adverse effects (such as opportunistic infections) was minimal.

In total, 6/30 CRIM-negative patients with Pompe disease included in the studies by Elder et al.[55], Banugaria et al.[56] and Kazi et al.[58] developed a serious bacterial or viral infection during treatment with rituximab and methotrexate. Apart from infections, no other serious adverse events were reported in these studies. Information about adverse events was not reported for the 8 patients included in the study by Broomfield et al.[57]

Due to suppression of the immune system, the treatment regimen can reduce the response to pediatric vaccinations and may cause severe complications if a live vaccine is administered. Several studies in which rituximab was administered to patients with Pompe disease withheld vaccination and resumed schedule after normalization of the CD19 count (which was used as a marker for B-cell recovery), this took roughly 3-6 months after ending the treatment regimen.[56, 58]

The reported studies had several limitations; the studies evaluating anti-drug antibody prevention strategies in patients with Pompe disease were very small and consisted of case-series (due to rarity of this disorder). Furthermore, the immunomodulatory treatment protocols varied between patients in some studies; some patients underwent several cycles of the same immunomodulatory treatment protocol and other patients received modified versions of the protocol. The median follow-up time may have been too short to adequately assess long-term tolerance to rhGAA. For example, some patients with short-follow-up time may have been antibody-negative, months after cessation of immunomodulatory therapy, due to the lingering immunosuppressive effect of the treatment on B-cells. Lastly, the antibody assay methodology was not uniform and sometimes not reported at all, complicating comparisons between studies and pooling results. Given the drawbacks mentioned above, the results of these studies should be interpreted with caution.

Prevention of anti-drug antibody formation in Pompe disease: which treatment strategies could be considered in hemophilia A

A short course of treatment with rituximab, methotrexate and IVIG was enough to prevent anti-drug antibody formation and induce immune tolerance in CRIM-negative patients with classic infantile Pompe disease. However, roughly 20% of patients

developed a serious bacterial or viral infection. In hemophilia A, far less patients develop clinically relevant anti-drug antibodies and the consequences of anti-drug antibody formation are not as severe. Hemophilia A patients that develop inhibitors can be treated with ITI which has fewer side effects than treatment with immunomodulatory agents. In addition, patients who are refractory to ITI can still be treated with bypassing therapy. Therefore, in pediatric patients with hemophilia A, the benefits of this treatment protocol do not outweigh the potential risks due to adverse events (mainly infections).

Antibodies against TNF-inhibitors in patients with rheumatoid arthritis or inflammatory bowel disease

Anti-tumor necrosis factor monoclonal antibodies (TNF inhibitors) such as infliximab or adalimumab are often used in rheumatoid arthritis (RA) as a second line agent when treatment with non-biologic disease modifying anti-rheumatic drugs (DMARDS) such as methotrexate fails. TNF inhibitors and DMARDS such as azathioprine are also used to treat patients with severe inflammatory bowel disease (i.e. Crohn's disease or ulcerative colitis).

Large proportions of patients with rheumatoid arthritis or inflammatory bowel disease develop antibodies against TNF inhibitors. However, due to heterogeneity in assay methodology, reported incidence rates of anti-drug antibody formation vary widely. Most studies used enzyme-linked immunosorbent assays (ELISA) or radioimmunoassays (RIA).[59] One meta-analysis that included 2350 patients with a variety of chronic inflammatory diseases using infliximab reported that 20.8% of patients had anti-infliximab antibodies.[20] These antibodies also reduce the efficacy of these drugs.[20] Methotrexate and azathioprine are primarily used to treat disease activity in patients with rheumatoid arthritis or inflammatory bowel disease. The immune response against TNF inhibitors may be mitigated by these drugs when they are used in combination.

Overview of anti-drug antibody prevention strategies in rheumatoid arthritis/inflammatory bowel disease

Several randomized controlled trials (RCTs) have evaluated the effect of TNF inhibitor monotherapy vs. combined therapy consisting of a TNF inhibitor and methotrexate/azathioprine on anti-drug antibody formation in patients with rheumatoid arthritis or inflammatory bowel disease. Most large RCTs report a significant decrease in the incidence of anti-drug antibody formation with concomitant use of methotrexate or azathioprine (table 2).[60-64] Anti-drug antibody formation was assessed using ELISA,

Table 2. An overview of the largest RCTs evaluating the effect of concomitant immunomodulation on anti-drug antibody formation against TNF inhibitors in patients with rheumatoid arthritis or inflammatory bowel disease. All patients were antibody-negative at baseline.

Study author, year of publication, disease	Treatment
Colombel, 2010 (Crohn's disease) [60]	
Infliximab	Intravenous infliximab, 5mg per kg at weeks 0, 2, 6 and then every 8 weeks.
Infliximab + Azathioprine	Intravenous infliximab, 5mg per kg at weeks 0, 2, 6 and then every 8 weeks. Oral azathioprine, 2.5mg per kg daily.
Panaccione, 2014 (ulcerative colitis)[61]	
Infliximab	Intravenous infliximab, 5mg per kg at weeks 0, 2, 6 and 14.
Infliximab + Azathioprine	Intravenous infliximab, 5mg per kg at weeks 0, 2, 6 and 14. Oral azathioprine, 2.5mg per kg daily.
Matsumoto, 2016 (Crohn's disease)[62]	
Adalimumab	Subcutaneous adalimumab, 160mg at week 0, 80mg at week 2 and then 40 mg every other week.
Adalimumab + Azathioprine	Subcutaneous adalimumab, 160mg at week 0, 80mg at week 2 and then 40 mg every other week. Oral azathioprine, maximum 100 mg daily (dose escalation from 25mg or 50 mg daily to 100mg daily during the first 4 weeks).
Emery, 2009 (rheumatoid arthritis)[63]	
Golimumab	Subcutaneous golimumab, 100mg once monthly.
Golimumab + Methotrexate	Subcutaneous golimumab, 50mg or 100mg once monthly. Oral methotrexate, 20mg per week (dose escalation from 10mg per week during the first 8 weeks).
Kremer, 2010 (rheumatoid arthritis)[64]	
Golimumab	Intravenous golimumab, 2mg per kg or 4mg per kg every 12 weeks.
Golimumab + Methotrexate	Intravenous golimumab, 2mg per kg or 4mg per kg every 12 weeks. Oral methotrexate, 15-25mg per week.

* Positive for antibodies according to the study's definition.
IQR: interquartile range, SD: standard deviation.

	Age	Follow-up	antibody-positive*/Total N (%)	Anti-drug antibody assay
		30 weeks		Enzyme-linked immunosorbent assay
	Median: 35.0 years (IQR: not reported)		15/103 (14.6%)	
	Median: 34.0 years (IQR: not reported)		1/116 (0.9%)	
		16 weeks		Assay not reported
	Mean: 38.5 years (SD: 12.7)		7/37 (19%)	
	Mean: 38.0 years (SD: 12.2)		1/31 (3%)	
		26 weeks		Radioimmunoassay
	Mean: 29 (SD: 12)		10/76 (13.2%)	
	Mean: 32 (SD: 12)		3/75 (4.0%)	
		24 weeks		Electrochemiluminescence immunoassay
	Mean: 48.2 (SD: 12.85)		14/104 (13.5%)	
	Mean: 50.6 (SD: 11.58)		6/211 (2.8%)	
		48 weeks		Enzyme-linked immunosorbent assay
	Mean: 49.2 (SD not reported)		17/194 (9%)	
	Mean: 49.6 (SD not reported)		10/299 (3%)	

RIA or electrochemiluminescence immunoassay (ECLIA). All RCTs presented in table 2 only included patients that had not been previously treated with the specific TNF inhibitor that was used. Similarly, a systematic review and meta-analysis that included both observational and interventional studies (n = 2611) estimated that concomitant treatment with DMARDs, mainly methotrexate and azathioprine, significantly prevented the risk of anti-drug antibody formation in patients with a variety of chronic inflammatory diseases using a TNF inhibitor (OR: 0.32, 95%CI: 0.25-0.42).[65]

Thus, strong evidence exists that methotrexate and azathioprine significantly prevent anti-drug antibody formation in antibody-negative patients with rheumatoid arthritis and inflammatory bowel disease. However, there were no methodologically sound comparative studies that assessed whether the immunomodulatory effect persisted after cessation of methotrexate or azathioprine.

Prevention of anti-drug antibody formation in rheumatoid arthritis and inflammatory bowel disease: which treatment strategies could be considered in hemophilia A

It is very likely that methotrexate or azathioprine would be able to prevent anti-drug antibody formation in hemophilia A patients. Compared with the treatment protocol consisting of rituximab, methotrexate and IVIG currently used to treat anti-drug antibody formation in CRIM-negative patients with Pompe disease, using only methotrexate or azathioprine would have a more favorable safety profile. However, whether immune tolerance to FVIII would persist after cessation of immunomodulatory therapy with methotrexate or azathioprine remains unknown.

The patients in the aforementioned studies received doses of methotrexate that were high enough to produce a therapeutic response. It is possible that a lower dose, with a reduced risk of adverse events, could be enough to prevent anti-drug antibody formation. Based on the studies conducted in patients with Pompe disease, these immunomodulatory agents would not have to be administered indefinitely. A short course of methotrexate at treatment initiation (e.g. during the first 10-20 exposure days to FVIII) could be sufficient to prevent anti-drug antibody formation and induce immune tolerance. However, studies evaluating methotrexate in very young pediatric patients are lacking. The mean age of patients included in each study reported in table 2 varied from 29.0-50.6 years old. In contrast, most severe hemophilia A patients that develop an inhibitor do so at the age of 1-2 years old.[6] Nevertheless, this treatment strategy could be a target for further investigation in patients at high risk for persistent inhibitors. In this case, an accurate model to predict the risk of persistent inhibitor development would be needed.

Eradication of anti-drug antibodies

What is already known in hemophilia A

Inhibitor eradication strategies using immunomodulatory agents have been tried since the 1970s.[66, 67] Nowadays, immunomodulatory agents are mostly used as second-line therapy in patients who have already failed ITI.[68] The agents are generally administered as adjunctive therapy in combination with ITI. One of the most well-known examples is the Malmö protocol which consists of extracorporeal immunoadsorption, followed by cyclophosphamide and IVIG in combination with high-dose ITI.[69]

In 2014, a systematic review assessed the effect of immunomodulatory agents (alone or in combination with ITI) on inhibitor eradication success rates.[68] In total, 46 case reports or case-series were included, comprising 208 patients. Complete recovery was defined as having a negative inhibitor titer, having normalized pharmacokinetic parameters was not mandatory. In most cases, immunomodulatory agents were administered concomitantly with ITI. Many patients had previously failed first-line treatment with ITI and had high peak inhibitor titers.

Most patients were treated with either cyclophosphamide (alone or in combination with other drugs) with a complete recovery rate of 40-44%. The second most used immunosuppressive agent was rituximab (alone or in combination with other drugs) which was associated with a complete recovery rate of 40-63%. As most patients failed previous ITI and had a poor prognosis for treatment success, the aforementioned success rates are quite good. However, as case-reports and case-series with positive results are far more likely to be published[70], the published recovery rates are most likely an overestimation of the true recovery rate. In addition, it was unclear if the follow-up time was long enough to accurately assess the relapse rate for most patients.

Overall, current evidence on the effectiveness of ITI in combination with an immunomodulatory agent such as rituximab or cyclophosphamide is inconclusive, because of small studies with methodological limitations.[68, 71] As far as we know, no randomized studies have been performed.

Very few studies have evaluated rituximab monotherapy, this treatment could be useful due to the low costs of treatment when compared to the high cost of ITI.[72] The largest study is a non-comparative trial from 2014 in which the effectiveness of monotherapy with rituximab was studied in 16 inhibitor patients with inhibitor titers › 5

BU (13 patients had failed previous ITI).[73] Only three out of 16 patients had a drop in inhibitor titer below 5 BU during follow-up and persistent tolerance after re-challenge with FVIII. These results suggest that inhibitor eradication with rituximab monotherapy is not as good as ITI. However, the treated group consisted of patients with a poor prognosis who failed ITI.

Eradication of anti-drug antibodies: what is known from other diseases

Anti-drug antibody eradication strategies have been extensively described for patients with antibody-mediated pure red aplasia due to epoetin use and multiple sclerosis patients with antibodies against interferon beta. The following paragraphs will review the available evidence on the efficacy of anti-drug antibody eradication strategies in these two patient groups.

Antibody-mediated pure red cell aplasia due to epoetin use in patients with chronic kidney disease

Antibody-mediated pure red cell aplasia (PRCA) is a rare but severe side-effect of treatment with epoetin (recombinant human erythropoietin) in patients with reduced production of endogenous erythropoietin, which is most often due to severe chronic kidney disease (CKD). PRCA is caused by the formation of antibodies against epoetin, that also cross-react with endogenous erythropoietin. This leads to profound anemia, a very low reticulocyte count and very low levels of erythroid precursors in the bone marrow. Antibody-mediated pure red cell aplasia bears some similarity to inhibitor formation in mild hemophilia A; in both conditions, antibodies against an exogenous protein (FVIII/epoetin) cross-react with the endogenous protein (FVIII/erythropoietin). The most commonly used assays to detect anti-drug antibodies are radioimmunoprecipitation assays (RIPA) or ELISA. Testing for neutralizing antibodies using an assay that measures in-vitro inhibition of epoetin activity by antibodies is available but rarely used.[74]

Overview of anti-drug antibody eradication strategies in antibody-mediated pure red cell aplasia

Around 200 cases of antibody-mediated PRCA occurred between 1998-2004 and almost all were associated with the use of a particular epoetin product (tradename: Eprex). The increased immunogenicity of this product during this time period was likely due to a formulation change.[75]

A retrospective analysis[76] evaluated the long-term outcome (median follow-up: 9 months) of 170 patients with antibody-mediated PRCA due to epoetin use. Out of

170 patients, 19 patients received a renal transplant (with concomitant immunosuppression), 89 non-transplant patients received immunosuppressive treatment to eradicate anti-drug antibodies while 62 patients did not receive any treatment. In total, 44/89 (49%) non-transplant patients that were treated with immunosuppressive agents to eradicate anti-drug antibodies achieved hematological recovery. In comparison, only 1/62 (2%) patients who received no immunomodulatory treatment achieved hematological recovery. Hematological recovery was defined as having ≤ 1 red blood cell transfusion per month, hemoglobin levels ≥ 80 g/L (8 g/dL) and a reticulocyte count › 20 × 10^9/L. The specific type of anti-drug antibody assay(s) used and the immunosuppressive treatment that patients underwent were not accurately reported. However, the authors report that most of the more recently diagnosed patients were treated with prednisone (alone or in combination with IVIG), cyclophosphamide (alone or in combination with prednisone) or cyclosporine. Treatment with epoetin after hematological recovery was rare; nevertheless, 19/34 (56%) patients who were re-challenged with epoetin had good clinical response to epoetin. It was not reported if patients were re-challenged with Eprex or a different epoetin product. Good clinical response to epoetin was defined as having stable hemoglobin level ≥ 80 g/L (8 g/dL) and independence from red blood cells transfusions. The best predictor of good clinical response to epoetin was a negative-antibody status at re-initiation of epoetin therapy.

In a retrospective analysis of 47 patients with PRCA the efficacy of anti-drug antibody eradication strategies was evaluated.[77] Nine patients were not treated with any kind of immunomodulatory therapy, none of these patients recovered during follow-up (median follow-up: 12 months, IQR: 8-13). Eleven patients were treated with multiple different immunosuppressive treatment protocols (the exact type of treatments were not accurately reported), the remaining 26 patients received one type of treatment. Three treatment regimens were most commonly used; 7/8 (87%) patients treated with corticosteroids and cyclophosphamide, 4/6 (67%) patients treated with cyclosporine and 10/18 (56%) patients treated with corticosteroids with/without IVIG achieved hematological recovery (table 3). None of the recovered patients had a relapse during the follow-up period (duration of follow-up was not reported).

Thus, treatment with immunomodulatory therapy alone seems to be effective at eradicating anti-drug antibodies in CKD patients with antibody-mediated PRCA. The highest rate of hematological recovery (87%) was achieved by using a combination of corticosteroids and cyclophosphamide.[77] Around 56% of patients with successful hematological recovery had good clinical response to epoetin.[76] The exact treatment

protocols were not reported and probably varied significantly on a case-by case basis. More importantly, only a small proportion of patients were re-exposed to epoetin, and the level of exposure (intensity, frequency) was not reported. Therefore, the actual success rate of the used immunomodulatory agents is not known.

Eradication of anti-drug antibodies in antibody-mediated pure red cell aplasia, which treatment strategies could be considered in hemophilia A

Overall, the immunomodulatory agents used to treat antibody-mediated PRCA could be considered in hemophilia A patients who are refractory to ITI. However, the reported success rates of the aforementioned anti-drug antibody eradication strategies (which varied from 56%-87%) will expectedly be lower when applied to hemophilia A patients with inhibitors. This is because inhibitors in hemophilia A patients who are refractory to ITI are expected to be more difficult to eradicate. Moreover, a proportion of patients who were initially treated successfully will have an anamnestic response to FVIII after re-exposure (lowering the overall success rate even further). Therefore, these anti-drug antibody eradication strategies might not be as effective in hemophilia A patients with inhibitors who are refractory to ITI.

Table 3. Overview of most commonly used immunomodulatory agents used to treat antibody-mediated pure red cell aplasia.*

Treatment**	Time to recovery	Hematological recovery***
Corticosteroids, oral, starting dose: 1 mg/kg/day. Cyclophosphamide, oral, dose not reported.	Median duration: 3 months (range: 1-7)	7/8 (87%)
Cyclosporine, oral, 200 mg/day.	‹ 3 weeks for all patients	4/6 (67%)
Corticosteroids, oral, starting dose: 0.5-1 mg/kg/day with (n = 14) or without (n = 4) IVIG, 0.4 mg/kg daily for 5 days every 6 weeks.	Median duration: 3 months (range: 1-18)	10/18 (56%)

* Table adapted from Verhelst et al.[77]

** Some patients were treated with multiple different immunosuppressive regimens. Consequently, the total number of treated patients is unknown.

*** Hematological recovery was defined as being transfusion-independent and having a reticulocyte count › 20,000/μL

Alternatively, these treatment options could be used to treat patients in low-resource countries as a first-line treatment strategy if ITI is not available because of the costs. However, because of poor access to antibiotics and medical care in general, a severe treatment-related bacterial infection in a pediatric patient in a low-resource country

would also be more difficult to treat and therefore the benefits of this approach are not expected to outweigh the risks.

Antibodies against interferon beta in patients with multiple sclerosis

Multiple sclerosis is an auto-immune disease that is characterized by demyelination of the spinal cord and brain. This leads to neurological symptoms such as motor and sensory problems, paresthesia and cognitive impairments. Patients with relapsing-remitting multiple sclerosis (RRMS) are often initially treated with interferon beta-1a or interferon beta-1b, these products are associated with relatively high rates of anti-drug antibody formation. One study among 1115 Swedish and Icelandic patients reported an overall prevalence of neutralizing anti-drug antibodies of 32% using a Myxovirus resistance gene-A (MxA) protein assay. It is difficult to estimate the exact prevalence of anti-drug antibody formation in patients using interferon beta because the assay methodologies used to detect anti-drug antibody formation are highly heterogeneous.[78, 79] However, neutralizing antibodies seem to be slightly associated with a reduced therapeutic effect of these products.[80]

Glucocorticoids are used to treat exacerbations of multiple sclerosis in adults because of their anti-inflammatory and immunosuppressive effects.[81] Less often, monthly therapy with glucocorticoids is used with the aim of reducing long-term disability outcomes in patients with relapsing-remitting multiple sclerosis.[82, 83]

Overview of anti-drug antibody eradication strategies in multiple sclerosis patients using interferon beta

Between 2002-2009, 3 comparative trials (327 patients in total) have evaluated the use of monthly pulse therapy with methylprednisolone to prevent or eradicate antibody formation in patients with relapsing-remitting multiple sclerosis. At baseline, some or all patients were positive for antibodies against interferon beta. The results of these three studies are discussed in the following paragraphs and summarized in table 4.

In 2002, an open label RCT reported on 161 patients that were treated with either interferon beta 1b (n = 81) or interferon beta-1b in combination with methylprednisolone (n = 80).[84] The presence of neutralizing anti-drug antibodies was assessed using a Myxovirus resistance gene-A (MxA) protein assay. Antibody status of patients at baseline was not reported. After 12 months 26.8% of patients treated with interferon beta-1b and 12.1% of patients treated with interferon beta-1b + methylprednisolone had one or more samples that were antibody-positive (relative reduction 54.9%,

Table 4. An overview of studies evaluating the effect of concomitant immunomodulation with methylprednisolone on antibody formation against interferon-beta in patients with multiple sclerosis.

Study (year of publication)	Treatment	Study design
Pozzilli (2002)[84]		RCT
Interferon beta	INFB: Subcutaneous interferon beta-1b, 8 MIU every other day.	
Interferon beta + Methylprednisolone	INFB+MP: Subcutaneous interferon beta-1b, 8 MIU every other day. Intravenous methylprednisolone, 1000mg 1 x per month.	
Sorensen (2009)[85]		RCT
Interferon beta	IFNB: Subcutaneous interferon beta-1a, 44 µg 3 x per week	
Interferon beta + Methylprednisolone	INFB+MP: Subcutaneous interferon beta-1a, 44 µg 3 x per week. Oral methylprednisolone, 200mg on 5 consecutive days monthly.	
Hesse (2009)[86]		Non-randomized trial
Control group	-	
Methylprednisolone	MP: Oral methylprednisolone, 500mg on 3 consecutive days monthly.	

* Positive for antibodies according to the study definition.
RCT randomized controlled trial
SD standard deviation

	Age	antibody-positive* / Total N at baseline (%)	Follow-up	Antibody-positive/ Total N at study end (%)	Neutralizing anti-drug antibody assay
			15 months		Myxovirus resistance gene-A protein assay
	Mean: 33.1 years (SD: 8.1)	?/81 (?%)		19/71 (26.8%)	
	Mean: 31.2 years (SD: 6.7)	?/80 (?%)		8/66 (12.1%)	
			96 weeks		Antiviral neutralization bioassay
	Mean: 39.5 years (SD: 7.8)	16/46 (35%)		13/43 (30%)	
	Mean: 37.8 years (SD: 7.4)	12/47 (26%)		9/39 (23%)	
			6 months		Cytopathic effect assay
	Median: 41 years (range: 22–59)	35/35 (100%)		33/35 (94%)	
	Median: 43 years (range: 27–62)	38/38 (100%)		36/38 (95%)	

p=0.05). Although the reduction in antibody formation was significant, the number of disease relapses and the progression of disability during the first year of treatment were similar (table 4).

In 2009, an RCT assessed treatment with either interferon beta-1a (n = 46) or interferon beta-1a in combination with methylprednisolone (n = 47).[85] The presence of neutralizing anti-drug antibodies was assessed using an antiviral neutralization bioassay. Thirty-five percent of patients treated with interferon beta-1a alone and 26% of patients treated with interferon beta-1a + methylprednisolone were already antibody positive at baseline. There was no significant difference in the cumulative incidence of anti-drug antibody formation between groups, 30% of patients on interferon beta-1a and 23% of patients on interferon beta-1a + methylprednisolone were antibody-positive after 96 weeks (table 4).

Lastly, a non-randomized clinical trial evaluated if methylprednisolone could be used to restore interferon beta bioactivity in antibody positive patients with absent in vivo response to interferon beta who had discontinued interferon beta therapy.[86] The presence of neutralizing anti-drug antibodies was assessed using a cytopathic effect assay. Thirty-eight patients were treated with methylprednisolone and 35 patients were not treated. The in vivo response to interferon beta and antibody status were similar after 6 months (table 4).

Overall, evidence from studies in patients with multiple sclerosis suggests that methylprednisolone is not effective for preventing or eradicating antibodies against interferon beta.

Eradication of anti-drug antibodies in multiple sclerosis: which treatment strategies could be considered in hemophilia A

It seems that monthly treatment with methylprednisolone has no added benefit in terms of preventing or eradicating antibodies against interferon beta. In contrast, using oral corticosteroids to treat patients with antibody-mediated PRCA was a moderately successful strategy. (Table 3) The difference in efficacy may be partly explained by the fact that in patients with antibody-mediated PRCA, oral corticosteroids were mostly given in combination with IVIG or cyclophosphamide. Given the aforementioned results, inhibitor eradication in hemophilia A patients using methylprednisolone alone should not be considered as it is not expected to be effective.

Conclusion

Insights gained from clinical research into anti-drug antibody formation in other diseases could be helpful in devising alternative treatment strategies for inhibitor development in hemophilia A. Immune modulatory treatment can be associated with potentially severe side effects. The benefits of this treatment however, may outweigh the potential risks in subgroups of inhibitor patients with poor prognosis.

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