Treatment of Acquired Hemophilia by the Bonn-Malmö Protocol: Documentation of an In-Vivo Immunomodulating Concept

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Abstract

Acquired hemophilia (AH) is an extremely rare condition in which auto-antibodies (inhibitors) against clotting Factor VIII induce acute and life-threatening hemorrhagic diathesis due to abnormal blood clotting. The mortality rate of AH is as high as 16% and current treatment options are associated with adverse side effects. We investigated a therapeutic approach for AH called the modified Bonn Malmö–Protocol (MBMP). The aims of MBMP include suppression of bleeding, permanent elimination of inhibitors, and development of immune tolerance, thereby avoiding long-term reliance on coagulation products. The protocol included: 1) immunoadsorption for inhibitor elimination, 2) Factor VIII substitution, 3) intravenous immunoglobulin, and 4) immunosuppression. Thirty-five high-titer patients with critical bleeding that underwent MBMP were evaluated. Bleeding was rapidly controlled during 1 or 2 apheresis sessions and no subsequent bleeding episodes occurred. Inhibitor levels decreased to undetectable levels within a median of 3 d (95% CI, 2-4 d), factor substitution was stopped within a median of 12 d (95% CI, 11-17 d), and treatment was completed within a median of 14 d (95% CI, 12-17 d). Long-term follow-up (7 mo-7 y) showed an overall response rate of 88% for complete remission (CR). When cancer patients were excluded, the CR rate was 97%.
Introduction

The development of auto-antibodies (inhibitors) against blood clotting factors in non-hemophilic patients is a rare, but clinically serious condition that can lead to life-threatening hemorrhage due to failure of the blood clotting system. Several clinical studies have reported mortality rates of 16% to 22% for patients with acquired hemophilia (AH), depending on age, inhibitor titer levels, and individual response to treatment (1,2). AH is an autoimmune disorder, since the antibody is directed against a self-protein, and the clinical symptomatology can be transmitted by passive inter-individual antibody transfer (3).

The estimated incidence of AH is 0.2 to 1 cases per million people per year. The immunological background of the disease is still unknown, which is at least in part due to its low frequency. In about half of the cases, other disorders coincide with AH, such as systemic lupus erythematosus, rheumatoid arthritis, Sjögren syndrome, viral infection, cancer, and pregnancy (5). Cross-reactivity to a variety of coagulation factors due to immunization against an external antigen has been discussed as a possible underlying mechanism (2).

The most common inhibitors in AH are directed against Factor VIII (FVIII), although inhibitors of other coagulation factors, including Factor V (6) and Factor IX (7), have also been described. FVIII inhibitors are primarily oligoclonal or polyclonal IgG1 or IgG4 immunoglobulins. The main antigenic region is the A2 and/or C2 domain of the FVIII molecule. The bound inhibitors block the binding of FVIII to phospholipids, to the von-Willebrand factor and to cofactors of the FVIII molecule. The majority of FVIII inhibitors have complex type II kinetics, with a very rapid and non-linear inactivation of FVIII. A result of this type of kinetics is that it is extremely difficult to saturate the inhibitors by adding antigens. Therefore, FVIII substitution therapy is often unsuccessful in the presence of high-titer inhibitors (3,8). High-dose FVIII substitution, which acts by eliminating the inhibitor in hemophilia A patients, was unsuccessful in AH, particularly in high-titer patients (over 5 Bethesda units (BU/ml)) (9,10).

Various immunosuppressive treatment regimens, which are beneficial in other autoimmune diseases, often fail in AH (2). AH has been treated with FVIII and cyclophosphamide for about 30 years. In a randomized trial of 14 patients, various immunosuppressive regimens with prednisolone and/or cyclophosphamide reduced the inhibitor titers to undetectable levels in 68% of patients with low-titer inhibitors (under 5 BU/ml) (11,12).

The response to chemotherapy, especially in high titer patients, may require several months and appears to be not convincing. A recently published meta-analysis of 245 inhibitor patients by Delgado et al. focused on the response to various treatments and prognostic factors (1). Patients in whom the inhibitor could not be eliminated had a mortality rate of 42%, providing a strong argument for the necessity of rapid and long-term inhibitor elimination as part of AH treatment. The meta-analysis showed an overall mortality rate of up to 16% for various treatment regimens, and a high rate of complications, which were mainly associated with infections related to chemotherapy-induced neutropenia. Indeed, today's leading cause of mortality in AH is not the bleeding itself, but the complications that occur as a result of extended bleeding and the treatment of bleeding, such as surgery in cases of compartment syndrome, transfusion-related reactions, and infections due to prolonged chemotherapy, particularly in elderly patients (13).

Patient age is another important risk factor in outcome. In Delgado's meta-analysis, 50% of the patients suffering from AH were older than 65 years. This group showed a mortality rate of
18%, an observation that has also been described by other authors (14). Mortality in these patients is mainly caused by infections, which are a common complication of long-term immunosuppression. Rapid elimination of the inhibitor might therefore prevent these complications.

In most patients with AH, intravenous administration of immunoglobulin (ivIg) leads to a temporary displacement of inhibitors. Two possible mechanisms that have been proposed include: 1) inhibitor neutralization due to anti-idiotypic antibodies present in the pooled immunoglobulin preparation, and 2) attenuation of inhibitor synthesis. However, not all patients respond to ivIg, and inhibitor titers rarely decrease to undetectable levels in responders (15).

Despite the low estimated incidence of the disease, it is a devastating disorder and the costs of treatment are often immense. Therefore, there is considerable interest in improving and optimizing the existing treatment regimens. The primary objectives for treating patients with AH should be the safe and rapid elimination of the inhibitor and the development of long-term immune tolerance induction (ITI). However, in most studies, AH patients are treated with numerous treatment regimens and variable factor substitutions, making it impossible to determine the immunomodulatory potency of each treatment modality on inhibitor eradication.

Our modified Bonn-Malmö Protocol (MBMP) involves the combination of four therapeutic steps: 1) immunoadsorptive inhibitor removal, 2) high-dose FVIII administration, 3) ivIg, and 4) immunosuppressive medication. Therefore, MBMP incorporates elements of the previously reported Bonn Protocol (9,16), which emphasizes ITI, and the Malmö Protocol, which focuses on immunoadsorption and immunosuppression (17). The present study reports on the largest patient population with AH (n=35) treated with one consistent protocol at a single center.
Methods

Patients
Between 1993 and 2004, a series of 35 AH cases were treated and documented in our center. Patients included in the study were characterized by high-titer inhibitor to FVIII (>5BU) (9,10) and the occurrence of at least one acute bleeding episode. The novel treatment protocol was approved by the Ethical Committee of the Medical Faculty at the University of Bonn. All patients gave informed consent in writing.

From 1993 to 1995, the diagnosis was confirmed by an inhibitor assay using the Bethesda method. From 1995 onwards, the inhibitor analysis was performed with the Bethesda assay as modified by Njimegen (18). Differential diagnosis in relation to the lupus erythematosus-associated inhibitor was established with the dilute Russell viper venom test, lupus activated partial thromboplastin time, the plasma dilution test, and determinations of the Factors II, V, VII, IX, X and XI. The FVIII levels were determined by two methods: the one stage clotting assay and the chromogenic FVIII assay. At the beginning of the MBMP treatment, FVIII was < 1% of the normal range (70%-140%) in all patients.

Recombinant FVIIa (rFVIIa; Novo-Seven®, Novo Nordisk A/S, Bagsvaerd, Denmark) was substituted in 5 patients diagnosed after 1995 (Tab.1: Pat.10,30,31,32,35), to achieve an immediate reduction in bleeding diathesis during the patient’s transfer to our hospital.

Complete remission was defined as normal Factor VIII activity (70%-140%) without factor substitution and undetectable inhibitor titer levels during a minimum follow-up period of 12 months.

Partial remission was defined as attaining FVIII recoveries by up to 30% and/or a reduction of the inhibitor titer to less than 5 BU.

The MBMP treatment
A total of 35 patients with AH were treated with the MBMP. This treatment included:

i.) Large-volume immunoadsorption (IA) (2.5-3x total plasma volume on days 1-5)
ii.) ivIg substitution (0.3 g/kg body weight (BW)/d, on days 5-7)
iii.) Immunosuppressive therapy with cyclophosphamide (1-2 mg/kg BW/d) and prednisolone (1 mg/kg BW/d) from day 1 until remission (dose reduction)
iv.) Administration of FVIII (100 U/kg body weight, and in exceptional cases (BMI>40), up to 200 U/kg body weight) every 6 h. Optional dose reduction on the basis of clinical signs and the level of recovery achieved (50-80% FVIII residual activity after 4-6 h) throughout the treatment cycle.

The treatment cycles (from day 1 to day 7) were repeated several times, depending on the clinical response and coagulation factor activity.

Immunoadsorption was accomplished by apheresis of sheep-derived polyvalent anti-human immunoglobulin bound to sepharose CL 4B (Amersham Pharmacia, Biotech AB, Uppsala, Sweden), using a dual-column system (Ig Miltenyi Biotec GmbH, Plasmaselect Division, Bergisch Gladbach, Germany). Blood was drawn from an antecubital vein on one arm at a rate of up to 70 ml/min, and returned after processing via an antecubital vein on the other arm. Alternatively, in the case of inadequate antecubital vein access, a biluminal central venous catheter was placed. Plasma was continuously separated at a flow rate of up to 80 ml/min using either of two apheresis systems (Cobe Spectra, Cobe Labs Inc., Lakewood, CO, USA or
Autopheresis-C® Therapeutic Plasma Systems, Baxter Healthcare Corp., Round Lake, Illinois USA), with acid-citrate-dextrose (ACD-A, Baxter Healthcare Corp., Round Lake, IL, USA) as an anticoagulant diluted 1:30 or 1:40, respectively, in the two systems. The separated plasma was passed through the columns. The adsorptive capacity of the columns was 1.25 g for all IgG subclasses.

The target of processing was 2.5 times the plasma volume, and apheresis was continued for 5 d in each treatment cycle. After apheresis, either plasma-derived or recombinant human FVIII was administered, typically 100 IU/kg every 6 h, or in one exceptional case (BMI>40), up to 200 IU/kg every 6 h. The FVIII dosage could be optionally reduced in cases of satisfactory clinical response and 4-6 h recoveries of 50-80% throughout the treatment cycle. The magnitude of such dose reductions was equal to 20% of the coagulation factor dose administered on the preceding day.

Treatment-related side effects due to concomitant chemotherapy were scaled as follows: 0 = no side effects, 1 = mild side effects (nausea, hair loss, loss of appetite), 2 = severe side effects (fever, infection, alopecia, neutropenia, thrombopenia), and 3 = sepsis, in which case MBMP was immediately halted.

**Conventional treatment**

Three patients received the following treatments between January of 1993 and February 1996: Cyclophosphamide (n=3), corticosteroids (n=3), immunoglobulin (n=3), azathioprine (n=3); vincristine (n=1); Factor VIII inhibitor bypassing treatment (n=2) (FEIBA, Baxter Immuno AG, Vienna Austria) and FVIII substitution (n=3). MBMP treatment started in March 1996 and the follow-up continued until March 2004.

**Data analysis**

All statistical analyses were performed using the Statistical Package for Social Sciences SPSS, version 11.0 (SPSS, Inc., Chicago, Illinois, USA). Non-parametric statistics, the Spearman rank correlation (r_s) and the Mann-Whitney U test were used.

The primary study endpoints were the time of apheresis at which (a) activity of the inhibitor was first undetectable, (b) the factor substitution was discontinued upon relevant normal Factor VIII activity levels for 24-48 h after a single 30 IU/kg FVIII dose, and (c) termination of the MBMP treatment without the requirement for further apheresis. Kaplan-Meier analysis was performed to evaluate the time at which these endpoints were reached. The median time to reach these endpoints was calculated on the basis of the associated 95% confidence intervals (95% CI).
Results

Patient Characteristics
Upon admission, all patients exhibited life-threatening bleeding that required blood transfusions and intensive care monitoring. The FVIII level at initial diagnosis and at the beginning of the MBMP was < 1% of normal (70%-140%). The types of bleeding observed included muscle bleeding (n=25), which was associated with compartment syndrome (n=4), retroperitoneal bleeding (n=6), retropharyngeal bleeding, which required artificial respiration (n=3), and hematuria (n=2).

An overview of all enrolled patients is given in Fig. 1. A total of 35 patients (19 female, 16 male) with AH due to high-titer inhibitor levels (>5 BU) were diagnosed in our hospital. Three patients initially received conventional therapy and subsequently crossed over to the MBMP treatment. The mean age of the patients was 61 yrs ± 16,7 yrs (mean ± SD; median: 65 yrs, range: 28-81 yrs).

The mean inhibitor titer in the MBMP-treated patients was 369 BU ± 816,3 BU (median: 146 BU, range: 6-3600 BU). Patients with paraneoplastic syndrome (n=3) had significantly lower inhibitor levels (mean: 18,7 BU ± 14,8 BU, median: 18.7 BU, range: 6-35 BU, p=0.021), as compared to patients without cancer that received MBMP (mean: 402 BU ± 847 BU, median: 156 BU, range: 20-3600 BU).

Of the 35 patients that received MBMP, 33 completed the treatment. In two patients, the MBMP was interrupted in the 3rd and 12th treatment cycles, respectively, because of co-morbidities (epileptic seizure, obesity).

Underlying diseases were detected in 11 patients (28,9%). In four women (10,5%), the inhibitor was diagnosed peripartally (i.e.within 3 months of childbirth). Four patients (10,5%) suffered from another autoimmune disease (mixed connective tissue disease n=1, psoriasis n=1, polymyalgia rheumatica n=1, Sjögren Syndrome n=1) and in three patients (7,8%) the inhibitor occurred as paraneoplastic syndrome (lung cancer n=1, paraproteinemia n=2, lymphoma n=1).

Immunoadsorption
A total of 695 immunoadsorption procedures (apheresis) were carried out with an average of 19.6 apheresis sessions (range: 3-62) per patient. The extracorporeal treatment was well tolerated. Mild side effects, such as hypotension, hypaesthesia due to citrate anticoagulation (citric reactions) and allergic reactions occurred in less than 1% of all apheresis sessions that did not require an interruption of treatment. A median plasma volume of 5034 ml (range: 3500-9500 ml) was used, resulting in an average inhibitor reduction of up to 75%. The median reduction during one apheresis session of immunoglobulin was 407 mg/dl for IgG (range: 156-1000 mg/dl), 50 mg/dl for IgA (range: 0-157 mg/dl), and 19 mg/dl for IgM (range: 0-328 mg/dl).

In patients without paraneoplastic syndrome, the number of apheresis sessions correlated with the inhibitor level (r_S=-1, p=0.0046).

Factor Substitution
The total amount of factor substitution during MBMP (all patients) and conventional treatment (Pat.33, 34, 35) is summarized in Tab.1.

The average amount of FVIII that had to be substituted during the MBMP to achieve complete remission in patients was 0.26 x 10^6 IU ± 0.35 x 10^6 IU (mean ± SD, median: 0,14 x 10^6 IU ,
range: 0.024-1.87 x 10^6 IU). Patients with partial remission received an average of 0.39 x 10^6 IU ± 0.26 x 10^6 IU (median: 0.41 IU x 10^6, range: 0.1-0.7 x 10^6 IU) FVIII concentrate. The FVIII substitution therapy correlated with the inhibitor titer and with the patient’s plasma volume (r_s=1, p=0.002; r_s=1, p=0.03). Five of the MBMP patients required supplementary therapy with rFVIIa. A mean of 14 x 10^3 kilo-international units (kIU) rFVIIa ±10,1 x 10^3 (median: 12,50 x 10^3 kIU, range: 2.2-30.0 x 10^3 kIU) was administered to five patients (Pat.10,30,31,32,35) for a median duration of 3 d (range 2-5 d). Two patients undergoing conventional therapy (Pat. 34, 35) received 0.7 x 10^6 IU and 4.2 x 10^6 IU of FEIBA.

The time course of the development of the FVIII activity and the administered dosages of FVIII for one representative patient are shown in Fig. 2. This 68-year-old patient (Pat.14) had a pre-treatment Factor VIII inhibitor titer of 327 BU, and the inhibitor was eliminated before the third treatment cycle.

**Clinical outcome, treatment efficiency and side effects**

Fig. 3 indicates the time points at which undetectable inhibitor levels were achieved (Fig. 3a), coagulation factor concentrates could be discontinued (Fig. 3b), and a stable inhibitor elimination was achieved (Fig. 3c). The median number of apheresis days required to reach these endpoints was 3 d (95% CI 2-4 d), 12 d (95% CI 11-17 d) and 14 d (95%CI 12-17), respectively.

In four patients (Pat 29, 31, 32, 35), the MBMP induced partial remission with a median decrease of the inhibitor levels to 2.3 BU (range: 1-4.5 BU), resulting in a median FVIII recovery of 30% (range: 27-35%). In two patients, the MBMP was interrupted as consequence of other concomitant diseases. An 81-year old female (Pat.29) suffering from grand mal epilepsy and renal dysfunction developed neutropenia, and MBMP treatment had to be interrupted several times. This patient died 5 months later of an epileptic seizure and stroke. The second case was a 38-year-old male (Pat.30) who had an excessive plasma volume, as a result of obesity (BMI 49). This patient had an inhibitor titer of 3550 BU. After 62 immunoadsorption cycles, he still had a low inhibitor titer of up to 2.5 BU, but experienced remission 6 months later.

In three patients (Pat. 31, 32, 35), a malignant disorder with a poor prognosis was diagnosed during the course of the MBMP. The improvement of blood clotting due to the MBMP permitted patients to undergo diagnostic steps for tumour staging, including pleurodesis (Pat.35), lymph node biopsy (Pat.32), or mediastinoscopy (Pat.31) without bleeding events. The overall complete response rate was 88% (31/35). When patients with an underlying cancer were excluded, the CR was 97% (31/32).

In all 35 patients treated with MBMP, the acute bleeding episodes were rapidly controlled within the first 2-3 apheresis procedures and no subsequent episodes were encountered. This was also the case for the three patients who received conventional treatment between 1993 and 1995. During conventional treatment, they experienced a median of 17 events (range:12-27) (Tab.1). Of the 35 patients, 30 tolerated the MBMP treatment very well with only one patient experiencing moderate side effect such as nausea and slight hair loss. The remaining five patients exhibited severe side effects (infection n=3, neutropenia n=1, mucositis n=1), which were successfully managed by antibiotics without interrupting MBMP.

Patients who achieved a CR due to MBMP had a mean hospital stay of 23 d ± 16,75 d (median: 17 d, range: 8-68 d), MBMP-patients with partial remission were hospitalized for 49 d.
(range 23-80 d). The three patients who received a conventional treatment prior to undergoing MBMP treatment had spent a median of 102 d (range 25-155 d) in the hospital (Tab.1). During a long-term follow-up (median 44 months, range: 5-86 months), there was no evidence of any inhibitor relapse in 33 patients. The remaining two patients experienced a period in which FVIII declined to 10-50%, without any bleeding events 10 and 12 months after the initial MBMP treatment. Both patients had received a conventional therapy prior to the MBMP treatment (Pat.33,34). Relapses were managed by apheresis for 5-6 d, as well as immunosuppressive therapy. These interventions succeeded in restoring normal Factor VIII levels, and the clinical condition of the patients remained stable over a follow-up period of 4.5 years. None of the patients died as a direct consequence of bleeding events. However, two patients suffering from paraneoplastic disease (lung cancer Pat.35, lymphoma combined with paraproteinemia Pat.31) died of malignant disease 15 and 12 months after the beginning of the MBMP.
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**Tab.1:** Clinical and laboratory features of patients with acquired hemophilia treated by MBMP (ID 1-35), the total amount of factor substitution and previous conventional regimes (ID 33-35) are given. (): values under previous conventional treatment.

Pretreatment: **a:** corticosteroids, cyclophosphamide, immunoglobulin, azathioprine; **b:** FVIII substitution, **c:** vincristine, **e:** Factor VIII inhibitor bypassing treatment (FEIBA).

Patients suffering from cancer (31,32,35). Patients achieving partial remission under MBMP (29,31,32,35).
Discussion

This report describes the successful MBMP treatment of 35 AH patients. All patients exhibited high inhibitor titers and critical bleeding at presentation. AH is a rare condition, but high-titer patients with AH are seen even less frequently. Since our center is well-known and has a good reputation for the treatment of patients with hemophilia, physicians in the surrounding area frequently refer difficult cases to us. This has allowed us to document, for the first time, the treatment of such a large population of these high-titer patients.

We achieved a CR in these "difficult to treat" patients within 2 weeks (12-17d). Various conventional treatment regimens are reported to require 3 to 12 weeks to achieve CR in a population of mainly low-titer patients. The mean time to achieve cessation of bleeding is reported to be 3 weeks for various conventional treatment regimes, as compared to 3 days with our protocol (19, 21,22).

We attribute the high efficacy of the MBMP to the immunoadsorption that was included in our AH treatment protocol. The intensive large-volume immunoadsorption removes high levels of auto-antibodies and circulating immune complexes from patients' plasma. Overnight redistribution results in a mobilization of the inhibitor from the extravascular space and allows its removal in the subsequent apheresis sessions.

The major advantage of immunoadsorption versus other extracorporeal treatments, such as plasma exchange, is its high efficiency in removing pathogenic antibodies without a simultaneous loss of coagulation factors. As Fischer et al. reported recently (23), plasma exchange can lead to fatal bleeding. Even though these acute bleedings can be controlled with FEIBA, rFVIIa, and porcine FVIII, these approaches do not eliminate the inhibitor. These are symptomatic treatments that require the additional use of expensive clotting factors. Furthermore, we assume that recurring bleeding events increase the inhibitor titer, thereby delaying inhibitor elimination (8).

After apheresis, FVIII substitution is an essential element of the MBMP. FVIII infusions did not cause an increase in pathogenic antibodies in the MBMP-treated patients. There are several possible mechanisms that could explain this phenomenon. First, high concentrations of FVIII may selectively stimulate auto reactive cell clones and make them therefore more sensitive towards cytotoxic agents (1,11). Second, immunoadsorption allows high levels of FVIII recovery, which may then trigger the elimination of auto-reactive cell clones via Fas-mediated cytotoxicity (24). Third, the presence of low titer anti-FVIII and anti-idiotypic antibodies in healthy individuals has been well established (25,26). Finally, the concentration of natural inhibitors in normal plasma is low and regulated by natural anti-idiotypic antibodies. Although the role of natural inhibitors in maintaining hemostasis and tolerance is not clearly understood, it is possible that they may regulate plasma FVIII levels. In contrast to the highly specific inhibitor antibodies, these antibodies (known as natural antibodies) are polyclonal and polyreactive, and they therefore recognize several types of different antigenic structures. Based on these findings, we postulate that the FVIII substitution in the MBMP allows a sufficient biosynthesis of anti-idiotypic antibodies controlling the inhibitor via idiotypic suppression and therefore ensures a permanent inhibitor control (27).

Considering these potential "immune stimulating aspects" of the MBMP, the beneficial effects of concomitant immunosuppressive therapy may be questioned. Nevertheless, based on our experience, the immunosuppression is obligatory for successful elimination of the inhibitor. Auto-reactive cell clones stimulated by the presence of FVIII are likely to have a higher
turnover and become more sensitive towards immunosuppressive drugs. Modifications of the MBMP, such as excluding or reducing the FVIII substitution component, may initially save costs, but will ultimately lead to higher total costs, due to the necessity of bypassing agents in controlling bleeding.

The rapid response to MBMP permits the immunosuppressive drugs to be quickly reduced, thereby lowering the cumulative dose. This issue is of particular importance given the potential risk of leukaemia and the pro-carcinogenic activity of cyclophosphamide doses of up to 25 g, especially in younger patients (28, 29, 30, 31). As Delgado et al. (1) recently reported, side effects are frequently observed after an average duration of 3.5 months of chemotherapy, especially in elderly patients. A reduction in levels of immunosuppressive drugs will result in fewer adverse side effects.

Improvements in the MBMP in future should include a more specific knock-out of auto-reactive cell clones. Rituximab (Mabthera), a chimeric monoclonal antibody that targets the CD 20 antigen, is a potential candidate to substitute the current immunosuppression. It has already been reported to be used in AH in several case reports and one prospective clinical study (32, 33, 34). Rituximab induces apoptosis primarily in Pre B cell clones showing no immediate effect on the antibody-bearing plasma cells (35,36). The blockade of B cell proliferation allows a reestablishment of an intact B cell pool after an average of 3 to 6 months after treatment (35,36). Stasi et al. (32) describe in their prospective study of 10 patients an elimination of the inhibitor within 3 to 12 weeks from the start of Rituximab. Critical bleeding events were managed with plasmapheresis and/or rFVIIa before/during Rituximab treatment. Complete remission was seen in 8 patients with low titers. Three of these patients relapsed after restoration of the immune system. In 2 high-titer patients, Rituximab alone failed to eliminate the inhibitor, but was successful in a second rechallenge in combination with cyclophosphamide. Detailed data on the long-term follow up of these patients (>5 months) would be of considerable interest, especially considering that restoration of the B-cell pool requires a minimum of 3-6 months.

In 35 patients that received MBMP treatment, 31 exhibited stable elimination of inhibitor. In the remaining four patients, a partial remission was achieved; three of them were suffering from cancer. The spontaneous formation of inhibitors in patients with malignancy represents a clinical and diagnostic dilemma for the physician. According to Sallah et al. (5) 50% of cancer patients die as a result of bleeding, rather than from the cancer itself. In 2 of our patients, an IgG kappa paraproteinemia was diagnosed, indicating that the inhibitor arose from a monoclonal cell clone. Immunoadsorption can reduce inhibitor mass, but the treatment with cyclophosphamide as a part of MBMP was not sufficiently potent to suppress a monoclonal cell clone. Finally, the MBMP achieved a partial remission, allowing FVIII recovery of up to 30% in these patients, which permitted invasive diagnostics to determine exact tumor staging.

Sallah et al. (5) found significantly lower inhibitor titers in cancer patients compared to patients without cancer, a finding that was confirmed in our study. In our cancer patients, a discrepancy was seen between inhibitor titers and bleeding severity. Bleeding events are often fulminant in cancer patients, although inhibitor titers are low (14). In the study of Sallah et al. (5), CR was only achieved in up to 70% of cancer patients. Cancer treatment alone eradicated the inhibitor in 22% of the cases. Patients who continued to exhibit the inhibitor developed a more severe disease state. According to Sallah et al. (5), successful inhibitor elimination was limited to patients who were diagnosed with early and potentially treatable tumors (65%). Furthermore, a strong correlation between types of malignancy and inhibitor remission has been observed. For example, immunohematological disorders had higher response rates than did solid tumors.
In cancer patients, the inhibitor exists as a paraneoplastic syndrome subject to an immunological mechanism that differed from that seen in patients without cancer. In these cases, chemotherapy and/or surgery may have a profound influence on elimination of the inhibitor. The MBMP approach is applied symptomatically here without expecting or intending to attain CR. Therefore, the results were calculated separately, by both excluding and including these patients.

During the long-term follow-up of all patients, only two had an episode of reduced Factor VIII activity without bleeding. These relapses were successfully managed by the MBMP. Both patients had been pre-treated with diverse conventional regimens, suggesting that failed treatment regimens delay the elimination of auto-reactive cell clones and propose MBMP as first line choice.

Our detailed documentation of the clinical parameters (severity/number of bleeding events, the use of clotting factors, days of hospitalization) gives enough transparency to enable a cost-benefit analysis to compare MBMP with other treatment strategies. The average consumption of $0.26 \times 10^6$ IU FVIII accounted for the major share of the total MBMP treatment costs. The cost of an average dose of $14 \times 10^3$ kIU supplementary rFVIIa, as well as costs of approximately 1000 USD for each immunoadsorption should be included. Even in high-titer patients with AH, these costs occur only once, whereas with conventional treatments, recurring costs accumulate due to further bleeding events and a longer hospital stays.

Our treatment approach has already been implemented successfully by other institutions (37). Our findings show that MBMP offers a new and safe approach to retain hemostasis and to cure high-titer AH patients, especially in cases with critical bleeding. Considering the high efficacy of the MBMP, there are legitimate reservations about carrying out randomized trials. Due to the low incidence of AH and the resulting lengthy recruitment process, randomized clinical trials would delay the introduction of a successful treatment by many years.

In conclusion, AH is a rare, but highly interesting disorder. In contrast to most other autoimmune diseases, the pathogenic antibody involved in AH is identified and quantifiable. Furthermore, this pathogenic antibody can be removed from patients’ plasma, and the antigen is identified and available for infusion therapy. These features provide a unique opportunity to study the relationship between natural auto-reactivity, autoimmunity, and antigen-driven immune responses to a single protein in vivo. Therefore, the treatment of AH can perhaps facilitate the understanding of the pathogenesis and treatment of autoimmune diseases in general.
References

Figure Legends

Fig. 1. Number of Patients undergoing the MBMP and treated by conventional therapy. Schematic summary of the number of enrolled patients, the number of patients who received MBMP and/or conventional treatment and the clinical outcome (CR= complete remission, PR= partial remission).

Fig. 2. MBMP treatment eliminated the inhibitor and allowed coagulation factor administration to be discontinued. On the left y-axis ( ), inhibitor titer (BU/ml) is shown over the course of apheresis procedures in a representative patient (Pat.14). On the right y-axis ( ), the change in measured FVIII activity is shown over the course of apheresis procedures. The dose of administered FVIII IU x1000 ( ) and the time points of immunoglobulin substitution ( ) are marked. In this 68-year-old patient pre-treated with 327 BU of FVIII inhibitor, the inhibitor was completely eliminated before the third treatment cycle (after the 14th apheresis session).

Fig. 3. Treatment endpoints were rapidly reached in the MBMP group. Kaplan-Meier plots of (a) median time to reduce inhibitor to undetectable levels =3 d (95% CI, 2-4 d), (b) median time of factor substitution =12 d (95% CI, 7-17 d) and (c) median time of treatment =14 d (95% CI, 11-17 d). The abscissa shows time in apheresis days. Numbers above the Kaplan-Meier curves represent patients concluding the MBMP within the corresponding time period. Vertical excursions of the curves signify the occurrence of events.
Fig. 1

Patients enrolled: 35

Initially Treated by MBMP: 32
Initially Treated Conventionally: 3

Cross over to MBMP: 3

MBMP: 35

CR: 31
PR: 1
PR cancer: 3
Fig. 2
Fig. 3a
Fig. 3b
Fig. 3c
Treatment of acquired hemophilia by the Bonn-Malmo Protocol: documentation of an in-vivo immunomodulating concept

Heike Zeitler, Gudrun Ulrich-Merzenich, Lothar Hess, Eligius Konsek, Christoph Unkrig, Peter Walger, Hans Vetter and Hans-Hermann Brackmann

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