original paper

haematologica 2001; 86:1186-1193
http://www.haematologica.it/2001_011/1186.htm

Immune tolerance in hemophilia with factor VIII inhibitors: predictors of success

GUGLIELMO MARIANI,* BARBARA KRONER,°
FOR THE IMMUNE TOLERANCE STUDY GROUP (ITSG)#
*Hematology and BMT Unit, Palermo University Hospital, Palermo, Italy; °Research Triangle Institute, Rockville MD, USA

#Members of the ITSG: RT Miller (Los Angeles, USA), NP Ewing (City of Hope, USA), R Butler (Philadelphia, USA), M Morfini (Florence, Italy), B Bell (Atlanta, USA), E Fressinaud (Angers, France), A Gringeri (Milan, Italy), CT Kisker (Iowa City,USA), C Kessler (Washington,USA), JC Gill (Milwaukee,USA), I Scharrer (Frankfurt, Germany), P Arcieri (Rome, Italy), R Zimmermann (Heidelberg, Germany), GC White (Chapel Hill, USA), G Mariani (Palermo, Italy), GH Growe (Vancouver, Canada), D Di Michele (New York, USA), T Nagao (Yokohama, Japan), HH Brackmann (Bonn, Germany), EP Mauser Bunschoten (Utrecht, The Netherlands), A Balocchi (Saint Alban Leysse, France), AR Thompson (Seattle, USA), MJ Inwood (London, Canada), DB Brettler (Worcester, USA), M Magallon Martinez (Madrid, Spain), E Scheibel (Copenhagen, Denmark), DS Beardsley (New Haven, USA), L Menza (Buffalo, USA), M Manco-Johnson (Denver, USA), J Tusell (Barcelona, Spain), M Chiu Poon (Calgary, Canada), I Walker (Hamilton, Canada),F Rodeghiero (Vicenza, Italy),M Bond (Montreal, Canada),V Rasi (Oulu, Finland), Y Sultan (Paris and Poitiers, France), SH Rubin (Moncton, USA), F Blei (New York, USA), C Guerois (Tours, France), N Ciavarella (Bari, Italy), M Berrettini(Perugia, Italy), K Peerlinck (Leuven, Belgium), J Lusher (Detroit, USA), BM Wicklund (Kansas City, USA), H Lenk (Leipzig, Germany), J Goudemand (Lille, France), H Ekert (Parkville, Australia), MA Korper (San Francisco, USA), R d'Oiron (Paris, France).

Correspondence: Prof. G. Mariani, Hematology and BMT Unit, University of Palermo, Medical School, 129, Via del Vespro, 90127, Palermo, Italy. Phone: international +39.091.6554401.
Fax: international + 39.091.6554402/4423.
E-mail: guglielmo.mariani@tin.it

Background and Objectives. Long term administration of high doses of factor VIII (FVIII) was shown to eliminate alloantibodies to FVIII (FVIII inhibitors). This procedure is widely referred to as *immune tolerance* (IT).

Design and Methods. In 1989 an international registry of IT protocols was created which recruited 314 patients with severe hemophilia A (HA) and an inhibitor who were given IT treatment.

Results. Fifty hemophilia care centers worldwide contributed data to the registry; 94.8% of the patients were high responders. The median inhibitor titer prior to IT (*pre-titer*) was 7 BU (range 0-720). The FVIII doses ranged from <50 International Units (I.U.)/kg/b.w./day to >200. At the end of IT, 140 patients had undetectable inhibitor titers, including 128 who also had normal FVIII kinetics. The remaining 174 patients included 66 with treatment failure, 23 who achieved partial responses, 48 patients in whom treatment was ongoing and 37 with data inadequate to evaluate outcome. Using logistic regression, the best predictive model for success included maximum inhibitor titer, pre-titer, dose and age at treatment. FVIII dose and pre-titer were also associated with treatment duration, as was the time between inhibitor detection and treatment. The risk of relapse was approximately 15% after 15 years of follow-up.

Interpretation and Conclusions. This study underscores the importance of starting IT as early as possible, at the lowest inhibitor titer and with high FVIII doses in order to maximize the chance of treatment success and minimize treatment costs.

Key words: FVIII, FVIII inhibitors, immune tolerance, FVIII alloantibodies.

The mainstay of hemophilia treatment is based on replacement therapy with FVIII concentrates prepared from plasma (pdFVIII) or through recombinant DNA technology (rFVIII). The majority of hemophilic patients are immunologically unresponsive to the administration of FVIII, that is they do not produce antibodies capable of inhibiting the FVIII procoagulant activity. There is a significant proportion of patients, however, in whom an immune response to FVIII mounts, leading to the production of alloantibodies (inhibitors) directed against specific epitopes on the FVIII molecule[1,2] and subsequently to the neutralization of the factor activity.

In a recent meta-analysis of 451 hemophiliacs followed prospectively in 8 different countries,[3] the average cumulative incidence of FVIII inhibitors, after exposure to pdFVIII replacement therapy, was 20% (range 0 to 45%) and in a prospective study the cumulative prevalence has been estimated to be as high as 33%.[4] After much debate, there is now agreement that the observed incidence of inhibitors after treatment with rFVIII concentrates is not different from that after treatment with plasma-derived products.

Today, the appearance of an inhibitor is considered by far the main complication of replacement therapy in hemophilia A (HA). What is more, it seems unlikely that the introduction of gene therapy in the management of HA will change this situation, so we will have to face this problem for many years to come. Beyond the various therapeutic strategies, the major goal remains suppression of the inhibitor and restoration of a state of unresponsiveness to FVIII. At present, this is achieved by administering large doses of factor until the inhibitor disappears. The mechanism which leads to inhibitor disappearance has not yet been identified; nonetheless, the restoration of unresponsiveness to FVIII is currently referred to as *immune tolerance* (IT).[5,6]

The original IT protocol, known as the *Bonn protocol*,[5-8] required administration of ≥ 200 I.U. of FVIII per Kg of body weight (IU/kg/b.w.) on a daily basis for an extended period of several months, if not years. Alternative protocols are based on low doses of FVIII[9-12] and the administration of non-specific therapies, such as steroids,[11] cyclophosphamide, high-doses of intravenous immunoglobulins (Ig) or extra-corporeal immuno-adsorption,[13,14] in order to reduce the IgG production/concentration or to interfere with their mechanism of action.

Since uncertainties still exist concerning the practice of IT and the possibility of predicting the outcome and as a considerable number of patients may have to bear the burden of an expensive and demanding treatment that could turn out to be unsuccessful, we have analyzed 314 cases treated with IT protocols collected and followed-up in the International Registry since 1989.

Design and Methods

Characteristics of the Registry

The International IT Registry was initiated in 1989.[15] Hemophilia Care Centers (HCC) worldwide were solicited to participate, and those agreeing were asked to complete a data collection form on each patient who had undergone immune tolerance at their center. The data form addressed the following issues: a) demographic characteristics, b) levels of inhibitor at discovery, pre- and post-treatment, and the highest anamnesis, c) dosage of FVIII to achieve tolerance and other medications used, d) type of concentrate used (virus inactivated [VI], non-VI, intermediate purity, high purity, recombinant), and e) clinical and laboratory data on HIV infection. Annual follow-up data were requested on patients who had not yet completed treatment at enrollment and those who were deemed successfully treated in order to determine the status of relapse.

Definitions and features of variables

Inhibitor titers were expressed in *Bethesda Units* (B.U.).[16] Maximum inhibitor titer was defined as the highest titer ever recorded. *Pre-titer* was considered the titer assayed before (within two months) the beginning of IT induction. A patient was defined as a *High Responder* (HR) when the inhibitor ever exceeded 10 B.U. and as a *Low Responder* (LR) if the inhibitor titer was consistently lower.[17] The dose (I.U./kg b.w./day) for IT induction was divided into four categories, <50, 50-99, 100-199, and > 200. Final treatment outcome was defined as failure, *high to low*, success, and relapse. Treatment failure indicated that there was no relevant change in the immune response, *high to low* indicated that a low inhibitor titer persisted despite treatment continuation, success indicated that the inhibitor could no longer be detected and FVIII kinetic parameters (recovery and half-life) had reverted to normal; relapse meant that there was a reappearance of the inhibitor after at least three negative inhibitor tests. A patient was said to have a *negative* BU titer when < 0.6 U were assayed, a normal recovery when > 80% of the FVIII was found shortly after the infusion and a normal FVIII half-life when this exceeded 6 h.

Additionally, the treatment outcome for some subjects was categorized as *ongoing* if treatment was not complete or *not evaluable* if required parameters were missing.

Statistical methods

Non-parametric chi-squared tests of significance were used to compare the treatment outcome for categorical variables and for continuous variables converted to categorical for simplicity of presentation. The best model predicting success in the multivariate analysis was determined from a stepwise procedure using a significance cut-off of 0.05. Standard actuarial methods were used to demonstrate the rates of success for predictive markers.

Results

Descriptive statistics

Up to 1999, 314 severe hemophiliacs with an inhibitor from 50 HCC worldwide had been enrolled in the IT registry. Twenty-four of the centers were in Europe and 19 in the United States. The other centers were in Canada (n=5), Japan (n=1) and Australia (n=1). Although 48% of the centers were located in Europe, they contributed 64% (n=201) of the patients. Forty-four of the 50 centers recruited fewer than 10 patients each. Of the 314 enrolled patients, the median age at the time the inhibitor was diagnosed was 4 years (mean 10, range <1 to 64) (Figure 1). The median number of months between the diagnosis and treatment was 17 (mean 53, range <1 to 379). The earliest IT treatment began in 1974 and the latest in 1998 (median year 1988). Seventy-two percent of the hemophilic subjects were HIV negative at the start of their treatment, and 8% of these eventually became positive. An intermediate purity product which was non-virus inactivated (VI) was used in 50 patients (15.9%); in 188 patients (60%), the concentrate used was an intermediate purity or high purity (chromatographic or monoclonal) plasma-derived VI product. In 37 patients (11.8%), both VI and non-VI concentrates were used. Recombinant concentrates were used in 39 (12.4%) of the procedures. The daily dose for IT in the Registry population varied widely and included 79 (25.2%) patients who received < 50 IU/kg/bw/day, 72 (22.8%) patients who received 50-99 IU, 62 (19.7%) patients who received 100-199 IU and 101 (32.1%) patients who were given > 200 IU. Seventy-eight (24.8%) patients enrolled in the Registry received activated prothrombin complex concentrates (APCC) during their treatment, 16 (5.1%) received steroids and 7 (2.2%) received both types of therapies. The vast majority of patients (213 or 67.9%) were treated with plain FVIII protocols. The median of the maximum inhibitor titers for patients in the Registry was 54 B.U. (mean 530, range 1-25,000). Low responders comprised just 6.2% of the Registry population. The median pre-titer was 7 B.U. (mean 41, range 0-720). Two hundred and seventy-five of the 314 Registry patients had an evaluable IT outcome (Table 1). Of the 140 patients who achieved an inhibitor titer of <1 B.U. as a result of treatment, 128 also reported normal FVIII kinetics, as measured by factor recovery and half-life. The remaining 12 patients with consistently absent inhibitor during follow up had one or more missing pieces of data, and therefore were excluded from the comparative analyses between successes and failures.

Treatment duration was evaluated by plotting the cumulative percent of patients who achieved tolerance by the number of months needed to achieve

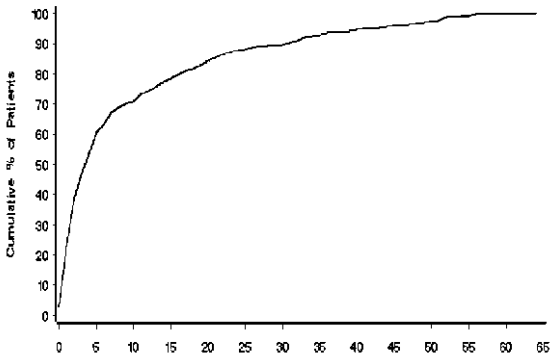

Figure 1. Age at inhibitor diagnosis.

Table 1. Overall outcome of IT protocols.

Outcome	*n.*	*%*
Success	140	50.9
Partial success (from a high titer to < 10 B.U.)	21	7.7
Failure	66	24.0
Ongoing	48	17.5
Not evaluable	39	

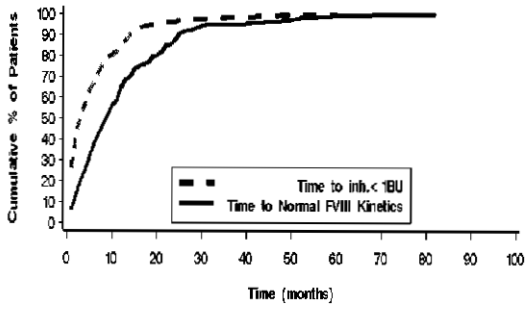

Figure 2. Treatment duration analysis. Time to achieve inhibitor titer < 1 B.U. (- - - -) and normal FVIII kinetics (——).

either the disappearance of the inhibitor or all the requirements defining success (absence of inhibitor + normal FVIII recovery and half-life) (Figure 2).

Statistical analyses

As shown in Table 2, treatment dose was strongly associated with outcome. In those patients receiving < 50 I.U., treatment was considered a success in 48% whereas in those patients given ≥ 200 I.U., treatment was considered a success in 86% (p <0.001). There were also highly significant associations (p = 0.001) found between treatment outcome and the following variables: maximum inhibiter titer, age at treatment and the pre-titer (Table 2). The time between inhibitor diagnosis and start of IT was also significantly associated with outcome (data not shown, p = 0.005); in those subjects treated within 5 years of inhibitor detection, there was a success rate of > 70%.

The variables associated with success from the univariate logistic regression analysis are shown in Table 3. The variables representing the model which best predicted success from the multivariate logistic regression analysis are shown in Table 4. Variables in these two tables continued to be the same as those that were individually found to be categorical variables, except for the time between inhibitor diagnosis and treatment in the non-parametric analysis previously described.

Of the 128 patients with a successful outcome, six relapsed between 1 and 15 years after treatment, as shown by the Kaplan-Meier inhibitor-free survival curve (Figure 3).

In order to ascertain whether treatment duration was affected by the variables associated with success, two groups of patients were established:

Table 2. Treatment age, maximum inhibitor titer, pre-titer and treatment dose as outcome predictors.

Variables	*Outcome*	
	Success	*Failure*
Age (yrs)*		
< 5	38 (70%)	16 (30%)
5-10	33 (73%)	12 (27%)
11-20	36 (78%)	10 (22%)
> 20	19 (40%)	28 (60%)
Max. Inh. Titer (B.U.)§		
< 10	39 (89%)	5 (11%)
10-20	22 (85%)	4 (15%)
21-99	32 (58%)	23 (42%)
100-500	20 (57%)	15 (43%)
> 500	15 (44%)	19 (56%)
Pre-titer (B.U.)^		
< 2	36 (78%)	10 (22%)
2-10	48 (79%)	13 (22%)
11-20	13 (65%)	7 (35%)
> 20	19 (41%)	27 (59%)
Dose (I.U./Kg/bw/day)°		
< 50	19 (48%)	21 (52%)
50-99	35 (66%)	18 (34%)
100-199	17 (49%)	18 (51%)
> 200	57 (86%)	9 (14%)

*Differences between successes and failures: *χ^2: 18.2; p =.001; §$\chi^2$2: 24.0; p =.001; ^$\chi^2$2: 20.2; p =.001; °χ^2: 23.0; p =.001.*

Table 3. Logistic regression: univariate analysis.

Variable	*direction*	χ^2	*"p"*
Maximum historical titer	negative	6.5	.01
Pre-titer	negative	4.6	.03
Time between diagnosis and treatment	negative	21.5	.0001
Treatment age	negative	7.9	.005
Dose	positive	15.8	.001

Dx: diagnosis; Tx: treatment.

those with a *favorable* prognosis (FVIII daily dose > 100 IU/Kg, pre-titer < 10 BU and time between inhibitor detection and treatment < 5 years) and the others with *poor* prognostic features. Figure 4 shows the comparison between the two groups with reference to the time needed to attain success (< 1BU and normal FVIII kinetics).

Discussion

The only approach which has the potential to eradicate allo-antibodies in hemophilic patients is the induction of a new immune tolerance status, through the administration of large doses of FVIII. This is in contrast to the situation in non-hemo-

Table 4. Logistic regression: multivariate analysis.

Variable	*Parameter*	*"p"*
Maximum historical titer	- 0.001	.04
Pre- titer	- 0.001	.04
Treatment age	- 0.047	.008
Dose	+ 0.51	.03

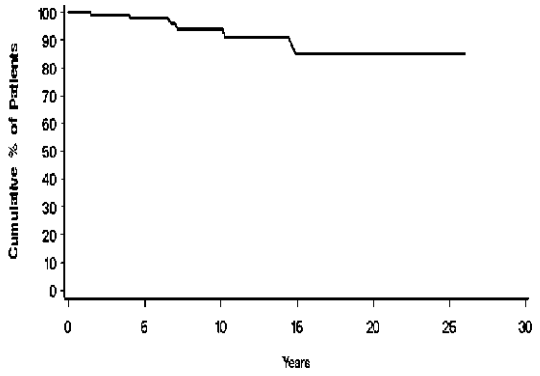

Figure 3. Kaplan-Meier inhibitor-free survival curve after IT.

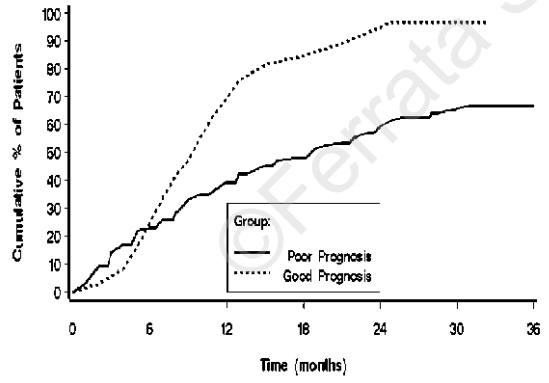

Figure 4. Time to success in patients with favorable (- - - -) and poor (——) prognostic indicators.

philic patients with auto-antibodies in whom the administration of steroids and/or other immunosuppressive therapies may reduce the inhibitor titer, or even lead to its disappearance.

Restoration of tolerance to FVIII in hemophilia represents an important aspect of immunotherapy, since it is one of the first examples of suppression of an alloimmune complication in humans. Tolerance in hemophilia can be considered a *specific* process induced by the administration of FVIII. This became evident, however, only after the use of the ultrapure and the recombinant concentrates for IT induction.[18,19] In fact, in the previous IT treatments performed with the low- or intermediate-purity concentrates, patients were receiving other immunogens which were present at various concentrations (i.e., fibrinogen, Ig, fibronectin, vWF) and therefore it could not be ruled out that these were also acting as toleragens. The underlying mechanism of this type of *peripheral* tolerance is thought to be a T-cell clonal deletion following a persistent antigenic stimulation, a process known as *activation-induced apoptosis*.[20]

IT regimens can be a successful treatment for inhibitors, but there are a number of factors which influence the outcome and the cost. In order to define a framework for optimal IT treatment, we created a Registry of over 300 inhibitor patients treated with protocols based, in most cases, on the administration of FVIII alone. Undoubtedly, the ideal study would have been a prospective randomized study as there has been much debate over the efficacy of specific types of concentrates, the recommended dosages, the duration of treatment and the likelihood of recruiting and gaining co-operation from the required numbers of patients and treaters. In the light of these problems, the creation of a Registry collecting cases from HCC worldwide was seen to be an interim, suitable alternative. The analysis of the Registry data will almost certainly help to shape future prospective studies.

In 50% of the Registry cases, the first immune response to FVIII developed before the age of 4 years old and in 70% of the cases it developed before the age of 9 (Figure 1). There was a higher proportion of HR (94%) than commonly reported among all inhibitor patients. This over-representation reflects a selection of the treaters concerning inhibitor patients who are eligible for IT treatment.

We defined a successful outcome in the most conservative manner, requiring both disappearance of the inhibitor and normalization of the FVIII recovery and half-life. Normal FVIII kinetics, in fact, allows the treater to rule out the presence of very low or transient inhibitor titers. Tolerance status was further documented by the inhibitor-free follow-up.

Our analysis, both at univariate and multivariate levels, demonstrated that a very strong predictor of

success was a low inhibitor pre-titer. This factor was probably intuitively recognized by most treaters, as the majority of cases began treatment at the lowest possible inhibitor level. The importance of starting with a low pre-titer implies that patients enrolled in a tolerance program should receive therapeutic materials not containing FVIII (i.e., recombinant, activated FVII, [rFVIIa], Novoseven™) before the start of IT. For the same reason, the use of rFVIIa is also advisable for the treatment of any bleeding episodes which occur during IT.[21-23]

Another important baseline factor was the age when treatment began. Procedures started during infancy or childhood were associated with greater success (Figure 4). In order to ascertain whether treatment was effective in both patients with long-standing inhibitors and newly diagnosed ones, we evaluated the *time between inhibitor detection and treatment*. This time frame was also shown to be a predictor of success, indicating that IT should be started as soon as possible after the inhibitor is detected. The fact that this variable was not a significant predictor in the multivariate analysis is probably due to its strong association with age at treatment and inhibitor diagnosis.

Dosage was found to be an important and significant predictor of success, a finding which was evident during the interim registry analyses.[15] Patients who were given higher dosages of factor were significantly more likely to become tolerant. The dose is also important in the management of bleeding episodes during IT. High doses of FVIII overcome the inhibitor more easily and allow for the maintenance of free FVIII levels for a progressively longer time. This leads to a rapid reduction and eventual cessation of bleeding episodes.

Two other, smaller registries were created more recently; the North American (NAITR)[24] and the German registries.[25] A comparative analysis of the international registry and the other two registries yielded the result that predictors of success were the same, but FVIII dose,[26] as in the NAITR patients treated with high dosages were very few and, *vice versa*, in the German one the vast majority of patients received > 200 IU/Kg of FVIII. However, a full comparison of the three registries cannot be made as the definition of the variables are not always the same.

Another important feature of the international registry analysis was the duration of treatment. The proportion of successfully treated patients increased steadily during the first months of treatment and leveled off at 90% after about 2 years (Figure 2). It is noteworthy that the disappearance of the inhibitor occurred earlier, within 15 months (Figure 2). This means that treatment must be continued for a considerably longer time after the inhibitor is not detectable and that if the inhibitor persists after two years of treatment, the probability of a success becomes virtually nil. Tolerance was shown to be a stable condition with relapses rarely occurring (4.3% of the registry cases).

In order to evaluate the combined conditions associated with success, we categorized all of the successes and failures alike into two groups with reference to the three most important outcome predictors. The first group was characterized by the favorable predictors of success (pre-titer < 10 BU, time from inhibitor detection to treatment < 5 years, treatment dose > 100 IU/kg) and the second group was defined by the opposite features associated with a poorer prognosis. The two cohorts were evaluated with reference to the probabilities of success and treatment duration as end-points. This analysis demonstrated that treatment duration was significantly shorter in patients with favorable predictors. In fact, most of the patients (65%) with good prognostic indicators achieved tolerance within one year, compared to the poor prognostic group in which a 65% success rate was not achieved for at least 31 months (Figure 4).

The length of time needed to achieve a successful outcome has significant economic implications.[27]

It is noteworthy that the criteria we have chosen to define the prognostic groups can be met in most of the patients. In fact, it is as feasible to start the procedure within a few years after the appearance of the inhibitor as it is to begin the treatment with low or undetectable levels of an inhibitor. As to the dose, one could argue that even lower doses could be used with successful results under the right conditions, but the increased costs for the high dose regimens would probably be balanced by a shorter treatment duration.

The high cost of IT cannot be overstated. In fact, the *on demand* treatment of a patient with an inhibitor can be extremely expensive as well, since bleeding episodes are treated with very costly products such as FEIBA®, rFVIIa and porcine FVIII. In addition, two recent studies[28,29] demonstrated that the highest costs for the management of inhibitor patients are due to hospitalization for the frequent occurrence of severe and life-threatening bleeding episodes. Furthermore, the quality of life of a patient with inhibitors can be very poor and should be a factor considered in any decision to place a patient on an IT program.[30] It is important to note that the

cost-benefit evaluation of an IT procedure appears to be documented well enough by the facts that tolerized patients can be treated again with FVIII and the relapse rate is very low.

In conclusion, the data accumulated in the Registry indicates that IT is effective and may be affordable provided that patients are treated as early as possible after the inhibitor detection, at the lowest possible inhibitor titer and using high-dose FVIII protocols.[31,32]

Contributions and Acknowledgments

GM co-ordinated the activities of the Registry on Immunetolerance. GM and BK have analyzed and interpreted the data, drafted the article, revised the drafts and jointly approved the final version. GM is the first name author as co-ordinator fo the Study Group. The authors thank Mrs. Sylvia Cohn for her assistance in carrying out the statistical analyses.

Disclosures

Conflict of interest: none.

Redundant publications: no substantial overlapping with previous papers.

Manuscript processing

This manuscript was peer-reviewed by two external referees and by Professor Vicente Vicente, who acted as an Associate Editor. The final decision to accept this paper for publication was taken joinbtly by Prof. Vicente and the Editors. Manuscript received July 13, 2001; accepted September 20, 2001.

Potential implications for clinical practice

Within the limits of this type of retrospective study, the analysis of the data and the follow up of the patients indicate that the most significant predictors of success are: i) a low titer inhibitor prior to treatment; ii) high dosages of factor VIII; iii. treatment age. These variables have an important impact on the treatment costs as well.

References

1. Scandella D, DeGraaf Mahoney S, Mattingly M, Roeder D, Timmons L, Fulcher CA. Epitope mapping of human factor VIII inhibitor antibodies by deletion analysis of factor VIII fragments expressed in Escherichia coli. Proc Natl Acad Sci USA 1988; 85: 6152-6.
2. Prescott R, Nakai H, Saenko EL, et al. The inhibitory antibody response is more complex in hemophilia A patients than in most nonhemophiliacs with factor VIII autoantibodies. Recombinate and Kogenate Study Groups. Blood 1997; 89:3663-71.
3. Briet F, Rosendaal FR, Kreuz W, et al. High titer inhibitors in severe haemophilia A. A meta-analysis based on eight long-term follow-up studies concerning inhibitors associated with crude or intermediate purity factor VIII products. Thromb Haemost 1994; 72:162-4.
4. Ehrenforth S, Kreuz W, Scharrer I, et al. Incidence of development of factor VIII and factor IX inhibitors in haemophiliacs. Lancet 1992; 339:594-8.
5. Brackmann HH, Gormsen J. Massive factor-VIII infusion in haemophiliacs with factor-VIII inhibitor, high responder. Lancet 1977; 2:933.
6. Brackmann HH. Induced immunotolerance in factor VIII inhibitor patients. Progr Clin Biol Res 1984; 150:181-95.
7. Brackmann HH, Oldenburg J, Schwaab R. Immune tolerance for the treatment of factor inhibitors: twenty years' "Bonn protocol". Vox Sang 1996; 70 (Suppl 1):30-5.
8. Oldenburg J, Shwaab R, Brackmann HH. Induction of immune tolerance in haemophilia A inhibitor patients by the "Bonn Protocol": predictive parameter for therapy duration and outcome. Vox Sang 1999; 77(Suppl 1):49-54.
9. Ewing NP, Sanders NL, Dietrich SL, Kasper CK. Induction of immune tolerance to factor VIII in hemophiliacs with inhibitors. JAMA 1988; 259:65-8.
10. Van Leeuwen EF, Mauser-Bunschoten E, Van Dijken PJ, Kok AJ, Sjamsoedin-Visser EJ, Sixma JJ. Disappearance of FVIII:C antibodies in patients with hemophilia A upon frequent administration of factor VIII in intermediate or low dose. Br J Haematol 1986; 64:291-7.
11. Aznar JA, Jorquera JJ, Peiro A, Garcia I. The importance of corticoids added to continuous treatment with factor VIII concentrates in the suppression of inhibitors in haemophilia A. Thromb Haemost 1984; 51:217-21.
12. Mariani G, Solinas S, Pasqualetti D, et al. Induction of immunetolerance for high-titre inhibitor eradication: a long-term follow-up. Thromb Haemost 1989; 62:835-9.
13. Nilsson IM, Berntorp E, Zettervall O. Induction of immune tolerance in patients with hemophilia and antibodies to factor VIII by combined treatment with intravenous IgG, cyclophosphamide, and factor VIII. N Engl J Med 1988; 318:947-50.
14. Knobl P, Derfler K. Extracorporeal immunoadsorption for the treatment of haemophilic patients with inhibitors to factor VIII or IX. Vox Sang 1999; 77: (Suppl 1)57-64.
15. Mariani G, Ghirardini A, Bellocco R. Immune tolerance in hemophilia-principal results from the International Registry. Thromb Haemost 1994; 72:155-8.
16. Anonymous. A more uniform measurement of factor VIII inhibitors. Thromb Diath Haemorrh 1975; 34:869-72.
17. Allain JP, Frommel D. Antibodies to factor VIII. V. Patterns of immune response to factor VIII in hemophilia A. Blood 1976; 47:973-82.
18. Rotschild C, Laurian Y, Satre EP, et al. French previously untreated patients with severe hemophilia A after exposure to recombinant factor VIII: inci-

dence of inhibitor and evaluation of immune tolerance. Thromb Haemost 1998; 80:779-83.
19. Rocino A, de Biasi R. Successful immune tolerance treatment with monoclonal or recombinant factor VIII concentrates in high responding inhibitor patients. Vox Sang 1999; 77(Suppl 1):65-9.
20. Shi YF, Szalay MG, Paskar L, et al. Activation-induced cell death in T cell hybridomas is due to apoptosis. Morphologic aspects and DNA fragmentation. J Immunol 1990; 144:3326-33.
21. Brackmann HH, Effenberger W, Hess L, Schwaab R, Oldenburg J. Immune tolerance induction: a role for recombinant activated factor VII (rFVIIa)? Eur J Haematol 1998; 61:18-23.
22. Petrini P, Klementz G. Treatment of acute bleeds with recombinant activated factor VII during immune tolerance therapy. Blood Coagul Fibrinolysis 1998; 9(Suppl 1):S143-6.
23. Hedner U. Treatment of patients with factor VIII and IX inhibitors with special focus on the use of recombinant factor VIIa. Thromb Haemost 1999; 82:531-9.
24. DiMichele DM, Kroner BL. Analysis of the North American Immune Tolerance Registry (NAITR) 1993-1997: current practice implications. ISTH Factor VIII/IX Subcommittee Members. Vox Sang 1999; 77(Suppl 1):31-2.
25. Lenk H. The German National Immune Tolerance Registry, 1997 update. Study Group of German Haemophilia Centres. Vox Sang 1999; 77(Suppl 1): 28-30.
26. Kroner BL. Comparison of the international immune tolerance registry and the North American immune tolerance registry. Vox Sang 1999; 77(Suppl 1):33-7.
27. Mariani G, Kroner B. Immune tolerance in hemophilia and inhibitors: a cost analysis. Transfusion 2000; 40:495-6.
28. Goudemand J. Pharmaco-economic aspects of inhibitor treatment. Eur J Haematol 1998; (Suppl) 63:24-7.
29. Chang H, Sher GD, Blanchette VS, Teitel JM. The impact of inhibitors on the cost of clotting factor replacement therapy in haemophilia A in Canada. Haemophilia 1999; 5:247-52.
30. Anonymous. Economic aspects of haemophilia care in the United States. Haemophilia 1999; 5:282-5.
31. Mariani G, Brackmann HH. Immune tolerance in hemophilia and treatment of hemophiliacs with an inhibitor. Haematologica 2001; 86:225-6.
32. Meeting report. Immune tolerance in hemophilia and the treatment of hemophiliacs with an inhibitor. Palermo, Italy, October 1999. Haematologica 2000; 85(10 Suppl):1-116.