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Eradication of Factor VIII Inhibitors in Patients with Mild and Moderate Hemophilia A

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Abstract

In hemophilia A, up to 25% of new anti-factor VIII (FVIII) inhibitory antibodies (inhibitors) occur in patients with mild or moderate disease [1]. Once the inhibitor develops, options for management include observation, immune modulation, and immune tolerance induction (ITI). Currently, there is little data to guide a clinician's management decisions. In a case series, 8/26 subjects with mild or moderate hemophilia complicated by an inhibitor underwent ITI; 2 successful, 2 unsuccessful and 4 partially successful [2]. In a systematic review of the literature, 12/16 patients with mild or moderate hemophilia responded to rituximab for treatment to eradicate the inhibitor [3]. To increase our understanding of treatment options for inhibitor eradication in patients with mild or moderate hemophilia A complicated by an inhibitor, a secondary analysis of clinical and treatment characteristics in a cohort of 36 patients with mild or moderate hemophilia A and inhibitor was undertaken. In multivariate analyses, rituximab alone (n=6) and other immune modulating treatments alone (n=2) were significantly associated with an increased likelihood of inhibitor clearance [hazard ratio (HR) of 4.4 (95% CI 1.06–20.03) and 10.21 (95% CI 1.17–78.28), respectively] whereas ITI alone (n=9) was not [HR 1.35 (95% CI 0.44–4.07)].

Keywords

Hemophilia A; Immunotherapy

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Authorship

C.K. designed the research, analyzed the data, and wrote the paper, G.A., J.H., R.KJ, R.P., C.W. and G.Y. performed the research and critically revised the manuscript, J.M.S. designed the research, analyzed the data, and critically revised the manuscript.

Conflict of interest

All other authors have no conflict of interest.

Data was originally collected as part of a case-control study [4]. For this analysis, complete data were available on 32 subjects [missing the peak inhibitor titer (n=1), effect of inhibitor on FVIII coagulant activity (n=2), and initial inhibitor titer (n=1)] (supplementary Table 1). The onset of inhibitor was after the year 2000 in 66% of subjects, but as early as 1970s in one subject [4]. The median age at inhibitor onset was 31.0 years [interquartile range (IQR), 41 years]. The majority of subjects were non-Hispanic white (83.3 %). Subjects had a median baseline FVIII coagulant activity was 4.0% (IQR 9%) and a median peak inhibitor titer of 17.0 Bethesda unit (BU)/ml (IQR 85.7 BU/ml).

Characteristics of subjects with cleared versus persistent inhibitors are shown in Table 1. The median duration of the inhibitor among subjects whose inhibitor cleared was 15.9 months (IQR 35.1 months) while the median duration of follow-up of subjects with a persistent inhibitor was 93.8 months (IQR 168.4 months). None of the differences between the two groups in the distribution of studied characteristics reached statistical significance.

Among the 8 subjects whose inhibitor spontaneously cleared, the median peak inhibitor titer was 4.4 BU/ml (IQR 12.4 BU/ml) and only 37.5% of these subjects had a high-titer inhibitor (>5 BU/ml). In contrast, among the 17 subjects whose inhibitor cleared following treatment, the median peak inhibitor titer was 16.0 BU/ml (IQR 40.8 BU/ml) (p=0.07) and 88.2 % (p=0.002) had a high-titer inhibitor. The median time to inhibitor clearance was 12.9 months (IQR 24.1 months) and 43.8 months (IQR 61.7 months) (p=0.15) in those who did or did not receive treatment respectively.

The impact of receiving any treatment to eradicate the inhibitor on the probability of having a persistent inhibitor over time is shown in Fig. 1a. Although subjects who received any treatment appeared to clear the inhibitor sooner, the results were not statistically significantly different (p=0.26). The affects of specific treatments, ITI, rituximab, and various other treatments (prednisone, cyclophosphamide, cyclosporine, and IVIg) alone or in combination, on the probability of having a persistent inhibitor are also shown in Fig. 1b–d. Although none of the differences in outcomes were statistically significant, the group that received ITI appeared to be more likely to have a persistent inhibitor when compared with all other subjects.

The effect of any ITI, rituximab or other treatments on the duration of inhibitor after adjustment for age, baseline FVIII coagulant activity, race and peak inhibitor titer was evaluated in 35 subjects. When comparing any use of ITI, rituximab or other treatments with those that did not receive ITI, rituximab, or other treatments respectively, none were statistically significant and only ITI trended toward having a lower likelihood of inhibitor clearance at any given point in time (HR 0.57, 95% CI 0.20–1.44).

The effect of rituximab, ITI, other treatments alone or combinations of treatments after adjustment for age, race, baseline FVIII coagulant activity, peak inhibitor titer and all other treatment parameters was evaluated in 35 subjects (Table 2). Rituximab alone and other treatments alone demonstrated a positive effect consistent with an increased likelihood of inhibitor clearance at any given point in time. There was no apparent effect of ITI alone on inhibitor clearance. Combinations of treatment used in 6 subjects (ITI + rituximab in 2, rituximab + other in 3, and ITI + other in 1) was associated with a HR of 0.30 (p=0.23, 95% CI 0.03–1.51). Age, baseline FVIII coagulant activity and peak inhibitor titer did not affect the likelihood of clearance of inhibitor (HR 0.99 for each). Being of white race trended toward greater likelihood of clearance of inhibitor at any given point in time (HR 1.6, 95% CI 0.45–8.82) however confidence intervals were wide reflective of the small number of non-white subjects enrolled in the study.

Among those that received ITI alone, the median time to the start of ITI was 1.3 months (IQR 2.7 months), the median age at start of ITI was 8.2 years (IQR 10 years) and the median peak inhibitor titer was 13 BU/ml (IQR 33.7 BU/ml). Among the 7 subjects in whom ITI was started within 3 months after onset of the inhibitor, the peak titer is likely a close approximation of the pre-ITI titer (median peak titer 7 BU/ml, IQR 47.2 BU/ml; <10 BU/ml in 4 subjects and >10 BU/ml in 3 subjects). In the remaining two subjects who started ITI after 6 months, it is possible that their pre-ITI inhibitor titer was lower than the peak inhibitor titer of 17 and 39 BU/ml.

In this cohort, R593C and N1922S were the most common FVIII gene (*F8*) mutations found. Among subjects with R593C, 4/6 cleared their inhibitor after a median of 20.0 months; although one subject classified as having a persistent inhibitor was a relatively recent inhibitor (recorded follow-up of only 0.5 months) and may not have had adequate time to clear spontaneously. Five of six with N1922S cleared their inhibitor titer after a median of 9.2 months and the one classified with persistent inhibitor had only 2.8 months of follow-up. There were three subjects with R2150H *F8* mutation and none cleared their inhibitor after 93.9, 117.5 and 191.8 months of follow-up.

This study has several limitations. First, it is a retrospective secondary analysis of subjects enrolled from 16 sites for another study of risk factors for inhibitor development [4]. Efforts were made to enroll all eligible subjects, however, 17 other subjects that had been identified as eligible for inclusion were not enrolled either because the hemophilia treatment center (HTC) declined to participate or the subject at a participating HTC declined enrollment. These 17 subjects were similar to those enrolled with regard to baseline FVIII coagulant activity and race. Second, there was limited temporal information, making it difficult to fully assess the direct effect of treatments on inhibitor eradication. Third, the sample size is small leading to a lack of power to detect weak associations. Fourth, the observational nature of the study leads to confounding by indication. Subjects perceived to be at lower risk were observed and those perceived to be at higher-risk were treated thus biasing the results against seeing a benefit of treatment.

Despite these limitations, given the rarity of this condition, this cohort provides an opportunity to explore and generate hypotheses that should be tested in subsequent studies. It appears that those subjects who spontaneously cleared their inhibitor tended to have a lower inhibitor titer compared with those that received treatment and ITI appears less beneficial than rituximab or other immune modulating agents alone. The HR associated with other treatments alone is likely to be an over-estimate since it is based on two subjects; however, our overall results suggest that immune modification may be more effective than ITI in eradicating inhibitors in patients with non-severe hemophilia A. These results are consistent with the poor response rate to ITI reported by Hay et al and the favorable response rate to rituximab reported by Franchini et al [2, 3] and do not appear to be the result of a delay between inhibitor onset and ITI initiation or that those that received ITI represented a poor risk group. The poor response rate to combination treatment most likely represents confounding by indication, i.e., patients with worse disease or a more persistent inhibitor, were more likely to have received several treatments either simultaneously or sequentially. Other single case reports or small case series have reported a positive experience with combination treatments that have included immune suppressive agents such as corticosteroids and/or cyclophosphamide, however, case reports are prone to reporting bias [5–7].

In this cohort, there were only three *F8* mutations that occurred in three or more subjects. Therefore, it was difficult to examine associations between genotype and inhibitor development. The mutation R2150H appears to be associated with a greater likelihood of

having a persistent inhibitor. Santagastino et al [8], reported a case of two brothers with R2150H mutation and an inhibitor that persisted for decades even in the absence of FVIII infusions. Despite numerous publications describing subjects with the R593C mutation, the responses to treatment and inhibitor duration in these cases are not clearly reported.

In conclusion, approximately 22% of subjects with mild/moderate hemophilia A complicated by an inhibitor will spontaneously clear the inhibitor. Such spontaneous clearance appears to be more likely to occur among those with a low-titer inhibitor. However, it is possible that care providers are more likely to observe rather than treat patients with a low-titer inhibitor thereby allowing spontaneous clearance to occur. Conversely, care providers may be more likely to quickly treat patients with high-titer inhibitors thereby not only masking spontaneous clearance but also incorrectly attributing the clearance to the treatment initiated. Our findings suggest that when treatment is used, strategies that modulate the immune system have greater benefit than ITI and deserve closer study. However, a single approach is unlikely to be appropriate for all patients. It is possible that a combination *F8* mutation along with other clinical characteristics, such as peak inhibitor titer, could better stratify those into who should be treated and who should be observed but further studies are needed.

Materials and Methods

This study is a secondary analysis of the cohort of case subjects enrolled as part of a retrospective case-control study approved by Institutional Review Boards at 16 participating sites. Subjects were enrolled during an 18 month period beginning July 2007 and ending December 2008 [4].

Case Selection

Subjects were defined as individuals with mild or moderate hemophilia A (FVIII 1–40%) based on local FVIII testing and a history of either two inhibitor titers ≥ 1 BU/ml or one such inhibitor titer followed by the initiation of ITI (reference). Subjects were initially identified through the Centers for Disease Control and Prevention Universal Data Collection data set. HTCs with potential subjects were invited to participate. Sixteen HTCs agreed to participate. Participating HTCs also reviewed their clinical data base and all patients that met eligibility criteria were invited to participate.

Data Collection

After written informed consent was obtained, staff at participating HTC completed a standard data collection form for each subject. Data collected included the following: age at inhibitor onset, self-identified race (Black or African American, White, Asian or other); ethnicity (Hispanic or non-Hispanic); FVIII genotype if known; first inhibitor titer, peak inhibitor titer, effect of inhibitor on FVIII coagulant activity; the type of treatment [rituximab, ITI, or other treatments (cyclophosphamide, prednisone, or IVIg)] used to eradicate the inhibitor and the duration of the inhibitor (time from first inhibitor onset to a consistently negative inhibitor titer of < 0.6 BU/ml). Subjects with a consistently negative inhibitor titer on all follow-up visits were classified as having cleared their inhibitor whereas all others were classified as having a persistent inhibitor. The duration of negative inhibitor titer or number of negative inhibitor titers were not recorded. An inhibitor was considered high-titer if it was > 5 BU/ml [9].

Laboratory Materials and Methods

Blood was collected via venipuncture into two 4.5 ml vacuum-sealed tubes containing 3.2% sodium citrate. Red cells and buffy coat were collected for DNA extraction. Samples were

either stored at 2–8°C and shipped to the Molecular and Hemostasis Laboratory at the Centers for Disease Control and Prevention (CDC) within 24 hours or stored at −70 °C until shipment after 24 hours.

FVIII genotyping for all subjects was performed by the Molecular and Hemostasis Laboratory at the CDC except in the case of two subjects for whom historical FVIII genotype data were available. All exons, intron-exon junction regions, and the 3' untranslated regions of *F8* were sequenced in both directions. The VariantSEQr™ protocol was used for resequencing on a 3730 DNA Analyzer from Applied Biosystems. The PCR primers and M13 sequencing primers are described at http://www.ncbi.nlm.nih.gov/sites/entrez?db=probe with a few modifications to the PCR primers to enhance throughput and reproducibility. Data were analyzed with SeqScape®. Inversions of intron 22 and intron 1 in *F8* were examined by PCR [10]. Mutations were assigned names based on both Human Genome Variation Society (HGVS) protein amino acid changes using Mutalyzer sequence variation nomenclature checker at http://www.humgen.nl/mutalyzer from reference sequences NM_000132.3 and NP_000123.1 and traditional mature protein amino acid changes.

Data Analysis

Continuous variables are reported as median and range. Wilcoxon rank test was used to compare medians. Categorical variables are reported as frequencies and were compared using the χ^2 distribution. For sparse data, Fisher exact test was used to calculate p-values. Survival curves were created using the Kaplan-Meier method. Subjects who did not clear their inhibitor titer were right censored at the date of last positive inhibitor titer. Survival curves were compared using the Log-rank test. The influence of treatment to eradicate the inhibitor on the duration of inhibitor with adjustment for clinically relevant covariates (age, race, baseline FVIII coagulant activity, and peak inhibitor titer) was evaluated using Cox proportional hazards model using the Firth modification [11]. Age, baseline FVIII coagulant activity and peak inhibitor titer were included in the model since: 1) older patients (> median 30 years) were more likely to receive rituximab and younger patients were more likely to receive ITI (p=0.03); and 2) subjects who received rituximab alone trended towards having a higher median FVIII coagulant activity than those who received ITI alone (5.0 vs 2.0%, p=0.26) and a higher peak inhibitor titer (26.5 vs. 6.5 BU/ml, p=0.39). Race was included because of the well documented effect of race on risk of inhibitor development. All analyses were performed using SAS version 9.2 (Cary, NC, USA).

Supplementary Material

Refer to Web version on PubMed Central for supplementary material.

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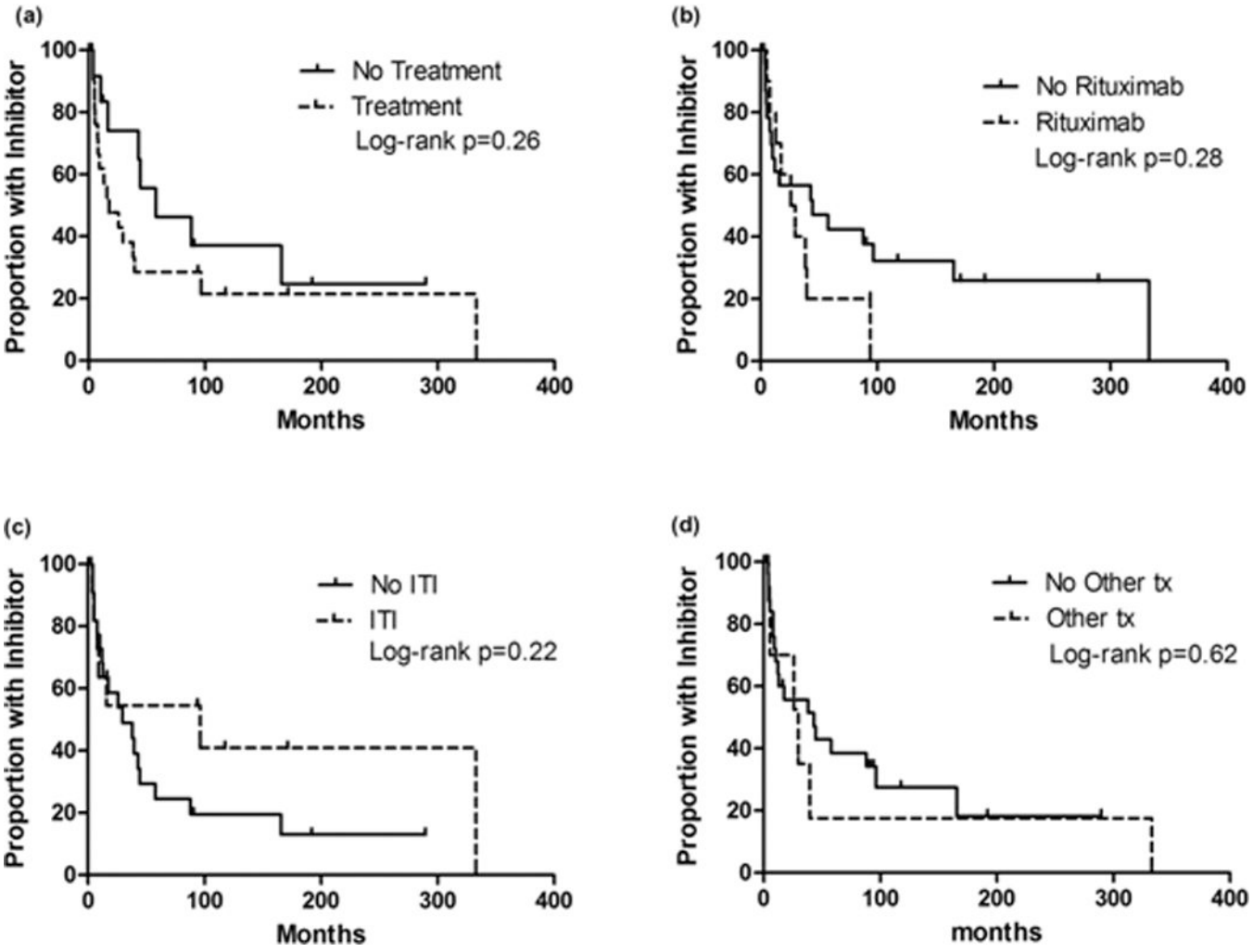

Figure 1.
Survival of inhibitor according to treatment received

Table 1

Subject characteristics according to inhibitor status

Characteristic	Cleared N=25	Persistent N=11	P value
Age at inhibitor onset (years)	30.0 (IQR 47)	32.0 (IQR 31)	0.86
White race	23 (92.0%)	9 (81.8%)	0.57
Non-Hispanic ethnicity	23 (92.0%)	11 (100%)	1.00
Baseline FVIII (%)	4.0 (IQR 9.0)	4.0 (IQR 9.2)	0.79 **
Initial inhibitor titer (BU/ml)	4.4 (IQR 9.9) *	4.8 (IQR 17.7)	0.97
Peak inhibitor titer (BU/ml)	14.9 (IQR 39.4)	49.0 (IQR 73.6) ^	0.12
High-titer inhibitor (> 5 BU/ml)	18 (72.0%)	9 (81.8%)	0.68 **
Received eradication treatment	17 (68.0%)	6 (54.6%)	0.47 **
FVIII reduced after inhibitor onset	22 (88.0%)	9 (81.2%) ***	0.54 **
Duration of inhibitor (months)	15.9 (IQR 35.1)	93.8 (IQR 168.4)	0.22

IQR=Interquartile range; FVIII=Factor VIII, BU=Bethesda Unit

* n=24

^ n=10

** Fisher exact test

*** FVIII after inhibitor onset unknown in two subjects

Table 2

Effect of each treatment on the likelihood of inhibitor clearance

Parameter	N	Parameter Estimate	Standard Error	Chi-square	P value	Hazard Ratio	95% CI
Immune Tolerance alone	9	0.30	0.59	0.26	0.61	1.35	0.44–4.07
Rituximab alone	6	1.49	0.76	3.81	0.05	4.44	1.06–20.03
Other treatments alone	2	2.32	1.12	4.27	0.04	10.21	1.17–78.28
Combination treatment	6	−1.19	0.99	1.42	0.23	0.30	0.03–1.51

*Adjusted for race, age, baseline factor VIII, maximum inhibitor titer and all other treatment parameters.