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Low-dose immune tolerance induction for severe hemophilia A inhibitor patients: Immunosuppressants are generally not necessary for inhibitor-titer below 200 BU/mL

Zhengping Li[1,2*] | Jie Sun[1,2*] | Zekun Li[1,2] | Zhenping Chen[2] | Guoqing Liu[1] | Wanru Yao[1] | Xiaoling Cheng[3] | Gang Li[2] | Yingzi Zhen[1] | Di Ai[1,2] | Yaohan Zhou[1,2] | Qianqian Mao[1,2] | Man-Chiu Poon[4] | Runhui Wu[1]

[1]Hemophilia Comprehensive Care Center, Hematology Department, Hematology Center, Beijing Key Laboratory of Pediatric Hematology-Oncology, Key Laboratory of Major Diseases in Children, National Key Discipline of Pediatrics (Capital Medical University), Ministry of Education, Beijing Children's Hospital, Capital Medical University, National Center for Children's Health, Beijing, China
[2]Hematologic Disease Laboratory, Hematology Department, Hematology Center, Beijing Key Laboratory of Pediatric Hematology-Oncology, Key Laboratory of Major Diseases in Children, National Key Discipline of Pediatrics (Capital Medical University), Ministry of Education, Beijing Pediatric Research Institute, Beijing Children's Hospital, Capital Medical University, National Center for Children's Health, Beijing, China
[3]Department of Pharmacy, Beijing Children's Hospital, Capital Medical University, National Center for Children's Health, Beijing, China
[4]Departments of Medicine, Pediatrics and Oncology, University of Calgary Cumming School of Medicine, Calgary, Canada

Correspondence
Runhui Wu, Hemophilia Comprehensive Care Center, Hematology Department, Hematology Center, Beijing Children's Hospital, Capital Medical University,

ABSTRACT

Importance: It remained unclear that the efficacy comparison between low-dose immune tolerance induction (LD-ITI) incorporating immunosuppressants (IS) when severe hemophilia A (SHA) patients had inhibitor-titer ≥200 Bethesda Units (BU)/mL (LD-ITI-IS^{200} regimen) and LD-ITI combining with IS when SHA patients had inhibitor-titer ≥40 BU/mL (LD-ITI-IS^{40} regimen).

Objective: To compare the efficacy of the LD-ITI-IS^{200} regimen with that of the LD-ITI-IS^{40} regimen for SHA patients with high-titer inhibitors.

Methods: A prospective cohort study on patients receiving LD-ITI-IS^{200} compared to those receiving LD-ITI-IS^{40} from January 2021 to December 2023. Both received LD-ITI [FVIII 50 IU/kg every other day]. IS (rituximab + prednisone) was added when peak inhibitor tier ≥200 BU/mL in the LD-ITI-IS^{200} regimen and ≥40 BU/mL in the LD-ITI-IS^{40} regimen. Success is defined as a negative inhibitor plus FVIII recovery ≥66% of the expected.

Results: We enrolled 30 patients on LD-ITI-IS^{200} and 64 patients on LD-ITI-IS^{40}, with similar baseline clinical characteristics. A lower IS-use rate was discovered in the LD-ITI-IS^{200} regimen compared to the LD-ITI-IS^{40} regimen (30.0% vs. 62.5%). The two regimens (LD-ITI-IS^{200} vs. LD-ITI-IS^{40}) had similar success rate (70.0% vs. 79.7%), median time to success (9.4 vs. 10.6 months), and annualized bleeding rate during ITI (3.7 vs. 2.8). The cost to success was lower for LD-ITI-IS^{200} than for LD-ITI-IS^{40} (2107 vs. 3256 US Dollar/kg). Among patients with peak inhibitor-titer 40–199 BU/mL, 10 non-IS-using (on LD-ITI-IS^{200} regimen) and 28 IS-using (on LD-ITI-IS^{40} regimen) had similar success rates (70.0% vs. 78.6%) and time to success (9.0 vs. 8.8 months).

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National Center for Children's Health, Beijing 100045, China.
Email: runhuiwu@hotmail.com

Man-Chiu Poon, Departments of Medicine, Pediatrics and Oncology, University of Calgary, Foothills Medical Centre, 1403-29th Street NW, Calgary, Alberta T2N 2T9, Canada.
Email: mcpoon@ucalgary.ca

*These authors contributed equally to this work.

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Interpretation: In LD-ITI, IS are not necessary for inhibitor titer <200 BU/mL.

KEYWORDS
Hemophilia A, High-titer inhibitor, Immune tolerance induction, Immunosuppressant, Low-dose

INTRODUCTION

Neutralizing alloantibodies (inhibitors) against coagulation factor VIII (FVIII), is a severe complication of FVIII replacement therapy.[1] Immune tolerance induction (ITI) by frequent exposure to FVIII concentrates is currently the only effective regimen to eradicate inhibitors.[2,3]

The international ITI study showed that high-dose (FVIII 200 IU·kg^{-1}·day^{-1}) and low-dose (LD)-ITI regimens (FVIII 50 IU/kg thrice weekly) had similar tolerization rates in patients with high-titer inhibitors.[4] However, all enrolled patients belonged to the "good-ITI-risk" group in that ITI was not initiated until the inhibitor-titer had dropped to <10 Bethesda Units (BU)/mL. Waiting for the inhibitor-titer to fall before ITI exposes the patient to a period of bleeding risk. Clinicians now tended to use high-dose ITI regimen[5] instead of waiting for the inhibitor titer to drop and for other "poor-ITI-risk" patients. Unfortunately, the internationally recommended high-dose ITI regimen is too costly to be widely applied in developing countries like China. In the international ITI randomized controlled trial, peak inhibitor-titer >36 BU/mL apparently contributed to a lower success rate and longer time taken to achieve success irrespective of low- or high-dose ITI.[6] Addition of immunosuppressants (IS) to ITI regimens has been shown to improve efficiency.[7] We, therefore, explored adding IS to a more affordable LD-ITI regimen (FVIII 50 IU/kg every other day) for patients with "poor-ITI-risk" which we defined as having peak inhibitor-titer ≥40 BU/mL. We showed in a previous study using LD-ITI, the addition of IS consisting of rituximab and prednisone (to become LD-ITI-IS) on "poor-ITI-risk" patients achieved efficacy similar to that using high-dose ITI (without IS)[4] but at nearly one-tenth treatment cost.[8] However, in this study[8] as many as 36 (64.3%) patients received LD-ITI-IS.

Recently the Future of Immunotolerance Treatment (FIT) group considered patients with peak inhibitor-titer <200 BU/mL as a "good prognosis" group and recommended treatment with LD-ITI or intermediate-dose ITI (FVIII 100 IU·kg^{-1}·day^{-1}).[9] Both the United Kingdom Hemophilia Centre Doctors' Organization consensus[10] and the international ITI study[4] also recommended that high-dose ITI be used only for peak inhibitor-titer ≥200 BU/mL.

This new FIT recommendation raised the question of whether IS was required for our LD-ITI regimen for patients with peak inhibitor-titer <200 BU/mL and what the outcomes would be if IS was used with our LD-ITI only for patients with peak inhibitor-titer ≥200 BU/mL. We, therefore, designed a prospective cohort study on SHA inhibitor patients to compare the LD-ITI outcomes between the group adding IS only when the peak inhibitor-titer was ≥200 BU/mL and the group incorporating IS when the peak inhibitor-titer was ≥40 BU/mL.

METHODS

Ethical approval

The study was approved by the Beijing Children's Hospital Ethics Review Board ([2022]-E-098-Y). Informed consent

was obtained from the parents or legal guardians of each recruited patient.

Participants

This prospective cohort study was conducted at Hemophilia Comprehensive Care Center of Beijing Children's Hospital on SHA patients with high-titer inhibitors recruited from January 2021 to December 2023 (ClinicalTrials.gov: NCT03598725). This was a non-randomized controlled trial because of the limited funds. All patients received LD-ITI but with IS added for peak inhibitor-titer (observed before or during ITI) ≥200 BU/mL (as LD-ITI-IS^{200} group) or peak inhibitor-titer ≥40 BU/mL (as LD-ITI-IS^{40} group) in accordance with the patients' preferences. To avoid selection bias, the study designers, patient recruiters, and data statisticians were different individuals. Participants were enrolled by the specialist investigators at the hemophilia clinic. Outcomes analysis was performed in December 2023.

Study design

Inclusion and exclusion criteria

The inclusion criteria were: (i) SHA (FVIII clotting activity [FVIII: C] <1% before inhibitor development)[11]; (ii) patients ≤14 years of age at ITI-initiation; (iii) patients with high-titer inhibitors (≥5 BU/mL). The exclusion criteria were: (i) patients with other congenital or acquired bleeding disorders; (ii) patients with comorbidity of autoimmune or chronic infectious disease.

Coagulation assay

FVIII inhibitor titers were determined using the Nijmegen modification of the Bethesda assay.[12] During ITI, the inhibitor was monitored every 1–2 weeks until there was a steady inhibitor-titer decline, then monthly until normal FVIII recovery, thereafter every 3 months. In-vivo FVIII recovery was assessed when two consecutive inhibitor titers were <0.6 BU/mL.

FVIII recovery was assessed by administering a single dose of plasma-derived FVIII/von Willebrand factor concentrate (pd-FVIII/VWF) at FVIII 50 IU/kg after a 48–72 h washout period.[13] For the individual, the pd-FVIII/VWF product used for FVIII recovery and ITI was the same brand.

ITI regimen

All study patients received LD-ITI with pd-FVIII/VWF at FVIII 50 IU/kg every other day. The choice of brands of pd-FVIII/VWF in accordance with patients' preferences or at the discretion of the managing clinician. All pd-FVIII/VWF products used were local Chinese manufactured products.

LD-ITI-IS^{200} (New regimen/protocol): IS consisting of rituximab and prednisone were added to LD-ITI (to become LD-ITI-IS) if (i) any peak inhibitor-titer (historical, pre-ITI or during ITI) was ≥200 BU/mL; or if (ii) the inhibitor titer decline was <20% of the initial titer in the first three months of ITI-initiation.

LD-ITI-IS^{40} (Original regimen/protocol)[8]: IS was added to LD-ITI (to become LD-ITI-IS) if (i) the peak inhibitor-titer (historical, pre-ITI or during ITI) was ≥40 BU/mL; or if (ii) the inhibitor titer decline was <20% of the initial titer in the first three months of ITI-initiation.

ITI was not suspended when patients received IS. IS used for each regimen included rituximab 375 $mg \cdot m^{-2} \cdot week^{-1}$ (maximum 600 mg) for 4 weeks, together with prednisone 2 $mg \cdot kg^{-1} \cdot day^{-1}$ (maximum 60 mg) for one month, then tapered over 2 months. For patients receiving rituximab, intravenous immunoglobulin (200 $mg \cdot kg^{-1} \cdot month^{-1}$ for 6 months) was administered for infection prophylaxis.[14,15] Notably, all patients were screened negative for Hepatitis B surface antigen and positive for Hepatitis B surface antibody before starting IS. Bleeding episodes during ITI were managed with domestically manufactured prothrombin complex concentrate (PCC). Activated PCC (APCC) which is not available in China, and activated recombinant FVII which is not affordable in China were not used.

Definition of ITI outcomes

(i) Success: achieving both inhibitor elimination (FVIII inhibitor titer of <0.6 BU/mL in at least two consecutive measurements) and normal FVIII recovery of ≥66% of the expected values within 24 months of treatment; (ii) Partial success: achieving inhibitor elimination but with persistently abnormal FVIII recovery within 24 months of treatment; (iii) Failure: failure to achieve the criteria for success and partial success; (iv) Non-success: including partial success and failure.

Once the patient had achieved ITI success, the pd-FVIII dose would be reduced gradually to 25 IU/kg three times a week, then changed to recombinant (r) FVIII at this dosage for long-term prophylaxis.[10]

Venous access

For children with poor vascular access, peripherally inserted central catheter (PICC) for intravenous access was allowed at the discretion of the investigators. Following PICC implantation, vascular ultrasound was conducted at month one, then once every 3 months. Generally, the PICC implantation was replaced after one year.

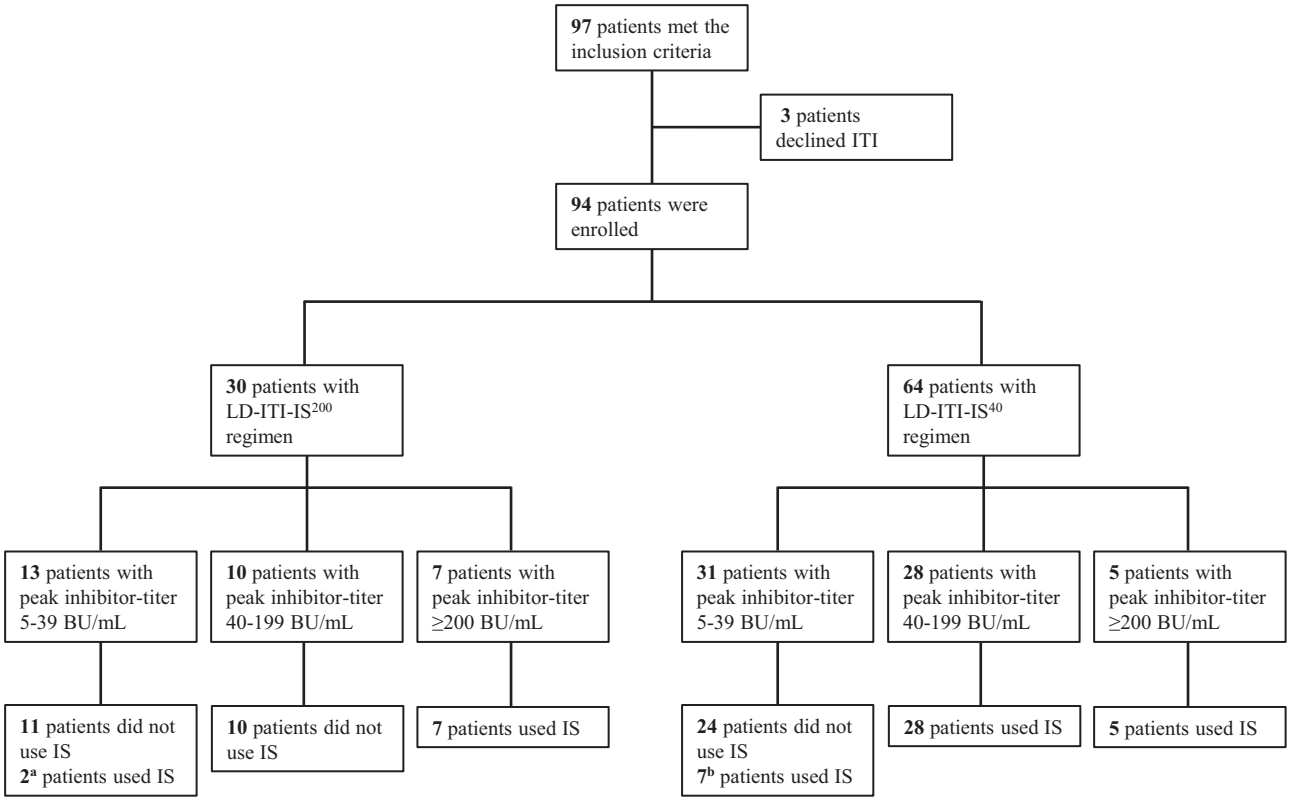

FIGURE 1 Flow diagram showing the inhibitor features and immune tolerance induction (ITI) regimen disposition of all 94 patients. [a] Two patients used IS with peak inhibitor-titers 5.9 and 27.5 BU/mL respectively but had insufficient inhibitor-titer decline during ITI. [b] Seven patients used IS for inhibitor-titer between 10.4 and 38.1 BU/mL but had insufficient inhibitor-titer decline during ITI. Abbreviations: ITI, immune tolerance induction; IS, immunosuppressants; BU, Bethesda Units; LD-ITI-IS^{200} regimen, low dose-ITI (Factor VIII [FVIII] 50 IU/kg every other day), adding rituximab-based IS for peak inhibitor-titer ≥200 BU/mL; LD-ITI-IS^{40} regimen, low dose-ITI adding rituximab-based IS for peak inhibitor-titer ≥40 BU/mL.

Treated-breakthrough bleeding

The treated-breakthrough bleeding episodes collection was done in a pretested case record form through face-to-face interviews and from patients' records. The annualized bleeding rate (ABR) was calculated for all patients as the number of all reported treated bleeding events divided by the number of months elapsed (12 months). The annualized joint bleeding rate (AJBR) was calculated for all patients as the number of reported treated joint bleeding events divided by the number of months elapsed (12 months).[16]

Statistical analysis

Categorical variables, expressed as frequency and percentage, were compared by chi-square or Fisher's exact test. Continuous variables, expressed as the median and interquartile range (IQR), were compared by the Student's *t*-test (for normal distribution) or the Mann-Whitney *U* test (for nonnormal distribution). Kaplan–Meier curve was compared using the log-rank test. The reported *P*-values were two-sided, and <0.05 was considered statistically significant. All statistical analysis was conducted using IBM SPSS version 26.0 for Windows (IBM Corp.).

RESULTS

Study population and baseline clinical characteristics

Totally 97 patients met the inclusion criteria, of whom three (3.1%) declined ITI for inability to follow frequent visits. The remaining 94 patients were enrolled (30 patients receiving the LD-ITI-IS^{200} regimen, and 64 patients using the LD-ITI-IS^{40} regimen) with a median follow-up period of 29.6 (IQR, 24.6–32.9) months since ITI-start without any dropouts (Figure 1).

Patients in the two regimens had similar baseline characteristics, including information before inhibitor development, inhibitor-related information, and ITI-related information (Table 1). However, IS-adding in the LD-ITI-IS^{200} group was introduced at a significantly lower rate (30.0%) than that in the LD-ITI-IS^{40} regimen group (62.5%) ($P = 0.003$).

TABLE 1 The baseline characteristics of patients in the low-dose immune-tolerance induction immunosuppressant (LD-ITI-IS)^{200} and LD-ITI-IS^{40} regimen

Variables	LD-ITI-IS^{200} regimen (n = 30)	LD-ITI-IS^{40} regimen (n = 64)	P
Information before inhibitor development			
Age of initial bleeding (months)	8.0 (5.3–18.0)	7.7 (3.5–11.1)	0.164
Age of initial exposure (months)	13.5 (8.3–36.0)	13.0 (8.0–25.0)	0.446
Prophylaxis	10 (33.3)	12 (18.8)	0.190
FVIII concentrates			
Plasma-derived FVIII	21 (70.0)	40 (62.5)	0.499
Recombinant FVIII	9 (30.0)	21(37.5)	
Inhibitor related information			
Age at inhibitor diagnosis (years)	3.2 (1.9–5.2)	2.5 (1.3–4.6)	0.183
EDs before inhibitor diagnosis (days)	15 (6–23)	14 (8–22)	0.216
Titer at inhibitor-diagnosis (BU/mL)	14.9 (4.5–55.4)	16 (4.3–31.5)	0.543
Historical peak inhibitor-titer (BU/mL)	19.7 (11.2–118.9)	33.7 (21.4–69.7)	0.265
Pre-ITI inhibitor titer (BU/mL)	12.8 (5.2–40.7)	21.4 (10.4–48.4)	0.152
Peak inhibitor-titer during ITI (BU/mL)	18.5 (10.0–170.9)	34.1 (10.0–78.6)	0.789
Interval-time[a] (months)	3.5 (0.5–15.4)	4.8 (0.7–23.5)	0.463
ITI related information			
Age of ITI-start (years)	4.2 (2.7–7.0)	3.7 (2.3–4.5)	0.309
Follow-up time (months)	29.3 (24.3–32.6)	30.4 (24.7–33.1)	0.871
Treatment regimen			
ITI-alone	21 (70.0)[b]	24 (37.5)[d]	0.003
ITI-IS	9 (30.0)[c]	40 (62.5)[e]	
ABR from inhibitor diagnosis to ITI-start	15.7 (4.1–29.3)	12.8 (8.0–22.8)	0.575
AJBR from inhibitor diagnosis to ITI-start	6.0 (3.4–9.7)	6.3 (3.8–9.9)	0.553

Data are presented as n (%) or median (interquartile range).
[a]Interval-time from inhibitor-diagnosis to ITI-start.
[b]Included 11 patients with peak inhibitor-titer 5–39 BU/mL, and 10 patients with peak inhibitor-titer 40–199 BU/mL;
[c]Included two patients with peak inhibitor-titer 5–39 BU/mL, and seven patients with peak inhibitor-titer ≥200 BU/mL. The two patients with inhibitor titer <200 BU/mL received IS because of inadequate inhibitor titer decline (<20%) in the first 3 months of ITI;
These 24 patients had peak inhibitor-titer 5–39 BU/mL;
[e]Included seven patients with peak inhibitor-titer 5–39 BU/mL, 28 patients with peak inhibitor-titer 40–199 BU/mL, and five patients with peak inhibitor-titer ≥200 BU/mL. Those with inhibitor titer <40 BU/mL received IS because of inadequate inhibitor titer decline (<20%) in the first 3 months of ITI.
Abbreviations: ABR, annualized bleeding rate; AJBR, annualized joint bleeding rate; BU, Bethesda Units; EDs, exposure days; ITI, immune tolerance induction; IS, immunosuppressant; LD-ITI-IS^{200} regimen, low dose-ITI (Factor VIII [FVIII] 50 IU/kg every other day), adding IS (rituximab + prednisone) for peak inhibitor-titer ≥200 BU/mL. LD-ITI-IS^{40} regimen, low dose-ITI (FVIII 50 IU/kg every other day), adding IS (rituximab + prednisone) for peak inhibitor-titer ≥40 BU/mL.

Comparison of overall ITI outcomes between the LD-ITI-IS^{200} and LD-ITI-IS^{40} regimens

ITI success/partial success rates

Patients on the LD-ITI-IS^{200} regimen and LD-ITI-IS^{40} regimen had similar success rates (70% vs. 79.7%, $P = 0.309$), and similar partial success rates (76.7% vs. 82.8%, $P = 0.576$) (Table 2).

Time taken to achieve success/partial success

Similar time to success (9.4 vs. 10.6 months, $P = 0.260$) and time to partial success (7.2 vs. 8.7 months, $P = 0.665$) were discovered between patients on LD-ITI-IS^{200} and LD-ITI-IS^{40} regimens (Table 2). The Kaplan–Meier curves (Figure 2) also demonstrated that patients in the two regimens achieved similar time to success ($P = 0.560$).

TABLE 2 Comparison of immune-tolerance induction (ITI) outcomes between patients in the low-dose immune-tolerance induction immunosuppressant (LD-ITI-IS)^{200} and LD-ITI-IS^{40} regimen

Characteristics in ITI outcomes	LD-ITI-IS^{200} regimen (n = 30)	LD-ITI-IS^{40} regimen (n = 64)	P
Success			
Number of patients	21 (70.0)	51 (79.7)	0.309
Time to success (months)	9.4 (5.5–12.3)	10.6 (6.0–13.1)	0.260
ABR until success during ITI	3.7 (1.4–7.1)	2.8 (1.8–6.5)	0.640
AJBR until success during ITI	2.3 (1.0–3.6)	2.2 (1.1 – 3.0)	0.961
Success + Partial success			
Number of patients	23 (76.7)	53 (82.8)	0.576
Time to partial success (months)	7.2 (4.0–11.7)	8.7 (4.6–11.5)	0.665
ABR until partial success during ITI	4.7 (1.5–7.4)	3.8 (1.9–6.5)	0.958
AJBR until partial success during ITI	2.7 (2.0 – 4.0)	2.6 (2.4 – 3.6)	0.074
Patients receiving IS	9 (30.0)	40 (62.5)	0.003
Total cost per kg to success			
RMB (¥)	14 960 (8105–23 647)	23 120 (13 634–29 609)	0.048
US Dollar ($)	2107 (1141–3330)	3256 (1920–4170)	

Data are presented as n (%) or median (interquartile range).

ITI success: defined as achieving both inhibitor elimination (FVIII inhibitor titer of <0.6 BU/mL in at least two consecutive measurements) and normal FVIII recovery of ≥66% of the expected values within 24 months of treatment. Partial success: patients achieving inhibitor elimination but with persistently abnormal FVIII recovery within 24 months of treatment.

Cost (per kg body-weight) for each regimen was calculated as follows: median number (n) of treatment doses up until success (including FVIII, rituximab, prothrombin complex concentrate (PCC) for treatment of breakthrough bleeds) × cost per unit or milligram × units or milligrams per kilogram per dose. The cost calculation of intravenous immunoglobulin was based on 6 months of usage (mg per kilogram body-weight) × cost per milligram. Not included in the calculation are: the cost of (i) PCC (for bleed prophylaxis) used only in very few patients with the inconsequential average cost for the groups and (ii) prednisone (for IS) which is very inexpensive in China with inconsequential cost. The foreign exchange rate used in the analysis was US$1 = RMB¥ 7.1012 (2023/12/21).

Abbreviations: ABR, annualized bleeding rate; AJBR, annualized joint bleeding rate; ITI, immune tolerance induction; LD-ITI-IS^{200} regimen, low dose-ITI (Factor VIII [FVIII] 50 IU/kg every other day), adding IS (rituximab + prednisone) for peak inhibitor-titer ≥200 BU/mL. LD-ITI-IS^{40} regimen, low dose-ITI (FVIII 50 IU/kg every other day), adding IS (rituximab + prednisone) for peak inhibitor-titer ≥40 BU/mL.

Treated breakthrough bleeding and adverse events

During ITI, both the ABR and the AJBR were similar between patients on the two regimens. The ABR for LD-ITI-IS^{200} and LD-ITI-IS^{40} from ITI-start to partial success was 4.7 vs. 3.8 (P = 0.958), and from ITI-start to success was 3.7 vs. 2.8 (P = 0.640) (Table 2 and Figure 3A). The AJBR for LD-ITI-IS^{200} and LD-ITI-IS^{40} from ITI-start to partial success was 2.7 vs. 2.6 (P = 0.074), and from ITI-start to success was 2.3 vs. 2.2 (P = 0.961) (Table 2 and Figure 3B).

Among patients taking LD-ITI-IS in the two regimens (over a median follow-up period of 25.3 months since IS-adding) rituximab infusion-related side effects like rash were similar, being 22.2% (2/9) for the LD-ITI-IS^{200} and 22.5% (9/40) for the LD-ITI-IS^{40} patients. All these side effects could be resolved and subsequently prevented by antihistamine drugs.

Only a 1-year-old patient on the LD-ITI-IS^{40} regimen developed severe infection manifested as continuous cough, fever, and diarrhea during the 8th week of receiving prednisolone, requiring treatment with cephalosporin antibiotics. Up to the latest follow-up visits, no patients developed other prednisolone-related side effects such as high blood pressure, diabetes, cushingoid features, peptic ulcer, or edema.

Relapse

Among the 64 patients on the LD-ITI-IS^{40} regimen, 51 achieved success over a median of 10.6 months. Of these 51 patients, 46 remained inhibitor-free over a median follow-up period of 20.1 months after success, but five (9.8%) relapsed after a median (IQR) of 4.0 (3.2–4.4) months following success. Among these five relapsed patients, four (80.0%) were using IS. These four patients (using IS) continued LD-ITI and received one partial round of rituximab-prednisone rescue IS (using only two weekly doses of rituximab at 375 $mg \cdot m^{-2} \cdot week^{-1}$). The single non-IS-using patient with a baseline inhibitor-titer of 10.3 BU/mL received the full 1st round of LD-ITI-IS, with IS

TABLE 3 Comparison of patients with peak inhibitor-titer 40–199 BU/mL in the low-dose immune-tolerance induction immunosuppressant (LD-ITI-IS)^{200} and LD-ITI-IS^{40} regimens

Variables	LD-ITI-IS^{200} regimen (n = 10)	LD-ITI-IS^{40} regimen (n = 28)	P
Clinical characteristics			
Age at inhibitor diagnosis (years)	2.8 (1.8–3.6)	2.6 (1.5–5.3)	0.804
Age at ITI-start (years)	3.9 (2.4–9.4)	4.3 (2.3–6.8)	0.529
Titer at inhibitor-diagnosis (BU/mL)	58.3 (11.1–118.9)	23.2 (8.6–48.7)	0.194
Historical peak inhibitor-titer (BU/mL)	90.4 (16.0–146.1)	66.3 (40.7–155.8)	0.807
Pre-ITI inhibitor titer (BU/mL)	38.0 (13.0–43.9)	48.1 (21.9–103.1)	0.205
Interval-time[a] (months)	7.6 (0.3–72.2)	8.0 (0.6–28.1)	0.613
ITI outcome			
Success	7 (70.0)	22 (78.6)	0.673
Success + Partial success	8 (80.0)	23 (82.1)	1.000
Time to success (months)	9.0 (5.0–11.8)	8.8 (5.0–15.1)	0.650
Time to partial success (months)	6.0 (1.5–8.3)	7.3 (3.0–11.8)	0.346
Cost			
Total cost per kg to success			
RMB (¥)	18 577 (10 352–24 588)	19 459 (11 649–32 758)	0.454
US Dollar ($)	2616 (1458–3463)	2740 (1640–4613)	
Total cost per kg to partial success			
RMB (¥)	6148 (2703–17 577)	19 459 (7443–26 354)	0.046
US Dollar ($)	866 (381–2475)	2740 (1048–3711)	

Data are presented as n (%) or median (interquartile range).
[a]Interval-time: time from inhibitor-diagnosis to ITI-start.
Abbreviations: BU, Bethesda Units; IS, immunosuppressant; ITI, immune tolerance induction; LD-ITI-IS^{200} regimen, low dose-ITI (Factor VIII [FVIII] 50 IU/kg every other day), adding IS (rituximab + prednisone) for peak inhibitor-titer ≥200 BU/mL. LD-ITI-IS^{40} regimen, low dose-ITI (FVIII 50 IU/kg every other day), adding IS (rituximab + prednisone) for peak inhibitor-titer ≥40 BU/mL.

consisting of prednisolone and four weekly rituximab. At the time of data analysis, the two who received rescue IS had achieved success, while the other three had persistent low-titer inhibitors but without breakthrough bleeding over a median of 17.6 months since relapse. None of the 21 successful patients on LD-ITI-IS^{200} relapsed over a median of 20.2 months since achieving success.

Comparison of cost (to success) between the LD-ITI-IS^{200} and LD-ITI-IS^{40} regimens

Cost (per kg body-weight) for each regimen was calculated as follows: median number (n) of treatment doses up until success (including FVIII, rituximab, PCC for treatment of breakthrough bleeds) × cost per unit or mg × units or mg per kg per dose. The cost calculation of intravenous immunoglobulin was based on 6 months of usage (mg per kg body-weight) × cost per mg. Costs not included in the calculation are the cost of (i) PCC (for bleed prophylaxis) used only in very few patients with inconsequential average cost for the groups and (ii) prednisone (for IS) which is very inexpensive in China with inconsequential cost.

The per kg treatment cost from ITI-start to success in the LD-ITI-IS^{200} (US$ 2107) was significantly lower than that in the LD-ITI-IS^{40} (US$ 3256) (P = 0.048) (Table 2).

Comparison of the two groups of patients with peak inhibitor titer 40–199 BU/mL: those not using IS in the LD-ITI-IS^{200} regimen vs. those using IS in the LD-ITI-IS^{40} regimen

Between the two regimens, there were 38 patients with peak inhibitor-titer 40–199 BU/mL, 10 not using IS in the LD-ITI-IS^{200} protocol, and 28 using IS in the LD-ITI-IS^{40} protocol. We analyzed these two groups to determine if IS was necessary in patients on LD-ITI and with peak inhibitor-titer between 40 and 199 BU/mL (Table 3).

The success rate was not significantly different between the 10 non-IS-using patients and the 28 IS-using patients (70% vs. 78.6%, P = 0.673).

The cost per kg to attain partial success was significantly lower in the non-IS-using patients (US$ 866) than in the IS-using patients (US$ 2740) (P = 0.046). However, the

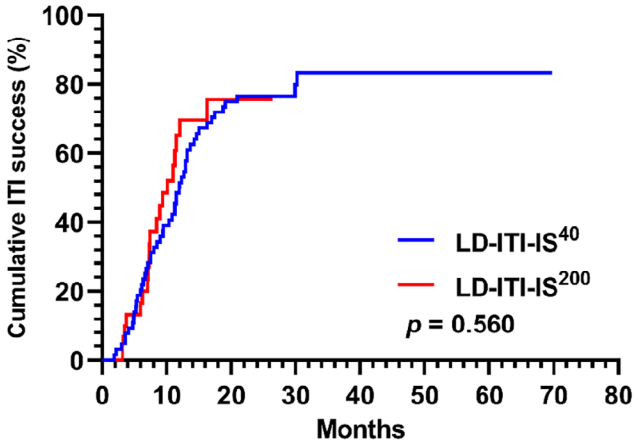

FIGURE 2 Time to immune tolerance induction (ITI) success by treatment regimen. Kaplan-Meier plot showing similar time to ITI success between the two treatment regimens ($P = 0.560$). Abbreviations: ITI, immune tolerance induction; IS, immunosuppressants; LD-ITI-IS^{200} regimen, low dose-ITI (Factor VIII [FVIII] 50 IU/kg every other day), adding rituximab-based IS for peak inhibitor-titer ≥200 BU/mL; LD-ITI-IS^{40} regimen, low dose-ITI adding rituximab-based IS for peak inhibitor-titer ≥40 BU/mL; ITI success: defined as achieving both inhibitor elimination (FVIII inhibitor titer <0.6 BU/mL in at least two consecutive measurements) and normal FVIII recovery of ≥66% of the expected values within 24 months of treatment.

cost per kg to achieve success between the two subgroups was not significantly different (US$ 2616 vs. US$ 2740, $P = 0.454$). Of the 6 non-IS using patients in the LD-ITI-IS^{200} protocol who achieved success, two had FVIII recovery evaluation delayed by nearly five months after achieving two consecutive negative inhibitor-titers because of the COVID-19 pandemic. The prolonged time to success in these two patients likely impacted the time to success for the whole group of six patients, increasing the apparent cost to achieve success.

ITI outcomes of patients with peak inhibitor-titer ≥200 BU/mL

Between the two protocols, there were 12 patients with peak inhibitor-titer ≥200 BU/mL (all using IS), seven in the LD-ITI-IS^{200} protocol, and five in the LD-ITI-IS^{40} protocol. Among them, eight patients (66.7%, four on each regimen) achieved success over a median of 10.1 months, and none relapsed at a median of 18.5 months follow-up since achieving success.

ITI outcomes of patients with peak inhibitor-titer <40 BU/mL

Between the two protocols, there were 44 patients (13 in LD-ITI-IS^{200} and 31 in LD-ITI-IS^{40}) with peak inhibitor-titer 5–39 BU/mL. Their overall success rate was 79.5% (35 of 44). Nine of the 44 patients (two in LD-ITI-IS^{200} and seven in the LD-ITI-IS^{40}) received IS because they failed to have adequate inhibitor titer decline in the first 3 months of ITI. All nine achieved success at a median of 11.3 months from ITI-start with no relapse after a median 22.6 months follow-up since success.

PICC access

A total of 61 (64.9%) patients had PICC implantation for a median of 625 (IQR, 471–783) days. None had implanted venous ports. None had acute complications within the first week after implantation. Only three catheter-related infections were documented during the study accounting for 0.09 infections per 1000 PICC days (defined as the number of new infections per total 'PICC days' of observation). Following antibiotic treatment, the infected PICC lines were removed. There were no severe life-threatening complications. The success rate was not significantly different between the patients with PICC and the patients without PICC (78.7% [48/61] vs. 72.7% [24/33], $P = 0.611$).

DISCUSSION

In this study, we compared the efficacy between the LD-ITI-IS^{200} regimen and the LD-ITI-IS^{40} regimen. The IS use rate was as expected lower in the LD-ITI-IS^{200} group (30.0%) than in the LD-ITI-IS^{40} group (62.5%). Importantly, the success rate and time to success of the LD-ITI-IS^{200} regimen with more restrictive use of IS was similar to those of the LD-ITI-IS^{40} regimen while at a lower cost.

There were a total of 38 patients in the two regimens with peak inhibitor-titer between 40 and 199 BU/mL, 10 non-IS using in the LD-ITI-IS^{200} regimen, and 28 IS-using in the LD-ITI-IS^{40} regimen. Both groups had similar baseline characteristics including peak inhibitor-titer before and during ITI, and similar success rate as well as time taken to achieve success. The findings therefore suggest that IS was not necessary for these patients with peak inhibitor-titer <200 BU/mL while taking LD-ITI. This is in line with the recommendation of the FIT group[9] that patients with peak inhibitor-titer <200 BU/mL, as a "good prognosis" group, should be treated with low- or intermediate-dose ITI. It should be noted that in our protocol, IS would still be used for patients in this group who have inadequate (<20%) inhibitor titer decline in the first 3 months of ITI. Similarly, some patients in the 5−39 BU/mL inhibitor group whose inhibitor titer did not decline adequately during the first 3 months of ITI also received IS.

Patients with a peak inhibitor-titer ≥200 BU/mL have been found to be associated with worse ITI outcomes.[2,17–19] The recommendations for these patients were to use higher ITI doses by itself[9] with or without IS[2] or emicizumab[10] to improve prognosis. LD-ITI was not recommended by the FIT group because there were no reports of success when LD-ITI regimens were used on these patients.[9] Here, we showed the efficacy of adding the rituximab/prednisone-based IS to our LD-ITI for our patients with peak

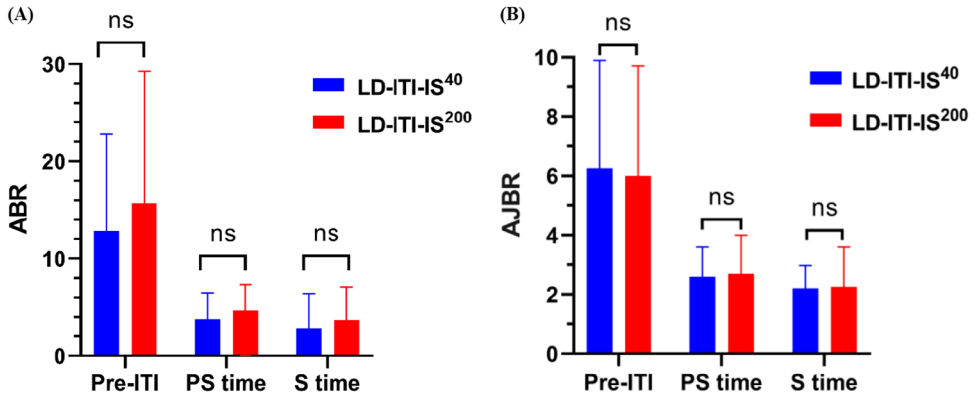

FIGURE 3 Annualized bleeding rate (ABR) and annualized joint bleeding rate (AJBR) by treatment regimen and phase of immune tolerance induction (ITI). (A) ABR (median with interquartile range) between the two treatment regimens was similar: during the period from inhibitor-diagnosis to ITI-start (Pre-ITI); during the period from ITI-start to achieving partial success (PS time); and during the period from ITI-start to achieving success (S time). (B) AJBR (median with interquartile range) between the two treatment regimens was similar: during Pre-ITI; during PS time; and during S time. Abbreviations: ITI, immune tolerance induction; IS, immunosuppressants; LD-ITI-IS^{200} regimen, low dose-ITI (Factor VIII [FVIII] 50 IU/kg every other day), adding rituximab-based IS for peak inhibitor-titer ≥200 Bethesda Units (BU)/mL; LD-ITI-IS^{40} regimen, low dose-ITI adding rituximab-based IS for peak inhibitor-titer ≥40 BU/mL; ITI success: defined as achieving both inhibitor elimination (FVIII inhibitor titer <0.6 BU/mL in at least two consecutive measurements) and normal FVIII recovery of ≥66% of the expected values within 24 months of treatment; Partial success: patients achieving inhibitor elimination but with persistently abnormal FVIII recovery within 24 months of treatment; ns, not significant.

inhibitor-titer ≥200 BU/mL. Rituximab as the B-cell suppressor has been described to positively affect outcomes for rescuing patients who failed ITI.[20] There were a total of 12 patients in our two regimens with peak inhibitor-titer ≥200 BU/mL and using IS. Among them, eight (66.7%) patients achieved success within a median of 10.1 months. Although none had FVIII half-life evaluation, none of these patients relapsed over a median follow-up period of 18.5 months after achieving success, suggesting the high likelihood of their having achieved full "tolerance". The success rate for patients with high-titer inhibitors (≥200 BU/mL) has been reported as 75.9% when using high-dose ITI (FVIII ≥200 IU·kg^{-1}·day^{-1}) without IS[19] and 18.9% when using intermediate-dose ITI (FVIII 50–199 IU·kg^{-1}·day^{-1}) without IS.[19] Our success rate of 66.7%, however, cannot be compared directly with these published success rates[19] given that our definition of success lacks the usually required half-life measures, although none of our patients had a relapse over 18.5 months of follow-up.

After rituximab, B-cells regenerate by about 6 months, which therefore represents a time of high relapse risk.[7,21] Indeed, four of the patients on IS relapsed over a median of 6.1 months post-rituximab. However, two of them were successfully rescued with additional IS courses while continuing the LD-ITI. The other two had persistent low-titer inhibitors without breakthrough bleeding over a median of 17.1 months since relapse.

Only one patient taking IS had infections while on prednisone and required antibiotics. Our low infection rate could be related to the routine use of intravenous immunoglobulin for infection prophylaxis. We do need a larger study followed for a longer period to assess the long-term side effects of IS.

Our study has some limitations. First, ours was a non-randomized single-center study. However, the study designers, patient recruiters, and data statisticians were different individuals to avoid selection bias. Also, the key baseline data between the two regimens were compared, and no statistical differences were found. Certainly, IS was not commonly used and not necessary in North America and Europe where high-dose ITI is affordable. LD-ITI (without IS) might work in poor-ITI-risk patients but would take much longer time. Emicizumab which could be used up front to prevent bleeding during the ITI course is expensive and generally not affordable in China. Second, the success definition did not include the FVIII half-life >6h, so true tolerance could not be defined. The difference in success definition makes comparison of outcomes in our study with those of others difficult. Nonetheless, all but five of the LD-ITI-IS^{40} success patients remained inhibitor-free over 20.1 months since success and no patient on LD-ITI-IS^{200} regimen relapsed over 20.2 months since success. These successful patients had a high likelihood of having achieved tolerance. Third, the number of patients on the new regimen was inevitably small given the rarity of the disease. Fourth, we did not perform Synacthen testing to assess steroid adverse effects at the end of the steroid course.

We have optimized the use of IS in our low-dose ITI protocol for SHA patients with high-titer inhibitors. We showed that more restrictive use of IS only for patients with peak inhibitor titer≥200 BU/mL (instead of at a lower titer of ≥40 BU/mL) maintained the same efficacy while decreasing the cost.

CONFLICT OF INTEREST

Man-Chiu Poon declares no conflict of interest for this work. However, he has otherwise received an honorarium for events sponsored by Takeda and for attending advisory Board meetings for KVR Pharm, Novo Nordisk, Octapharma, and Sobi. The other authors declare no conflict of interest.

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