Lower Absolute Lymphocyte Count Before Conditioning Predicts High Relapse Risk in Patients After Haploidentical Peripheral Blood Stem Cell Transplantation With Low Dose Anti-Thymocyte Globulin/Post-Transplant Cyclophosphamide for GvHD Prophylaxis

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Abstract
Standard anti-thymocyte globulin (ATG) weight-based dosing often resulted in highly variable ATG exposure, which had profound effects on relapse and survival, especially in recipients with relatively low absolute lymphocyte count (ALC) before conditioning. Data regarding rabbit ATG pharmacokinetics and pharmacodynamics in the setting of HLA-haploidentical peripheral blood stem cell transplantation (haplo-PBSCT) is lacking. We conducted a retrospective study on 90 consecutive patients who underwent haplo-PBSCT with low dose rabbit ATG (5 mg/kg) plus low dose post-transplant cyclophosphamide (50 mg/kg) based regimen for graft-versus-host disease (GvHD) prophylaxis. We compared serum concentration of ATG and post-transplant results between patients with ALC<500/μl and ALC≥500/μl before conditioning. Patients with ALC<500/μl had higher ATG concentrations, delayed immune reconstitution, lower incidence of grade II-IV acute GvHD (0 vs. 19.42%, P = 0.043), higher risk of Epstein-Barr virus infection within 100 days post-transplant (47.78% vs. 22.22%, P = 0.020) and 1-year relapse rate (33.33% vs. 11.59%, P = 0.041), and lower 1-year overall survival (OS) (52.38% vs. 79.71%, P = 0.004), 1-year relapse free survival (RFS) (47.62% vs. 75.36% for RFS, P = 0.014), and 1-year GvHD free relapse-free survival (GRFS) (42.89% vs. 65.22%, P = 0.043). ALC<500/μl before conditioning was a significant poor risk factor for relapse, OS, RFS, and GRFS.

Keywords
absolute lymphocyte count, relapse, survival, haploidentical, peripheral blood stem cell transplantation, anti-thymocyte globulin

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Introduction

Allogeneic hematopoietic stem cell transplantation (allo-HSCT) is an effective method for the treatment of malignant hematologic diseases. HLA-haploidentical peripheral blood stem cell transplantation (haplo-PBSCCT) is more and more widely used. Disease relapse is the main cause of death after transplantation. There are many factors for disease relapse after transplantation, such as disease status before transplantation, conditioning regimen, and donor sources, and so on. Recent studies showed that body weight-based dosing of ATG often resulted in highly variable ATG exposures, which had profound effects on relapse and survival especially in recipients with low peripheral blood absolute lymphocyte counts (ALCs) before conditioning. A higher serum-level of rabbit ATG at early time post-transplant seemed to be a strong predictor for relapse and poor survival in patients with HLA-matched allo-HSCT. Anti-human thymocyte immunoglobulin (ATG) dosage based on body weight has been used widely for many years. Recent studies showed that body weight-based dosing of ATG often resulted in highly variable ATG exposures, which had profound effects on relapse and survival especially in recipients with low peripheral blood absolute lymphocyte counts (ALCs) before conditioning. A higher serum-level of rabbit ATG at early time post-transplant seemed to be a strong predictor for relapse and poor survival in patients with HLA-matched allo-HSCT. Data regarding rabbit ATG pharmacokinetics and pharmacodynamics in the setting of haplo-PBSCCT is sparse because available literature had substantive variability in total and active ATG concentrations. We hypothesized that lower ALCs before conditioning might be closely related to higher ATG concentration and higher risk of relapse in patients undergoing haplo-PBSCCT with low dose rabbit ATG (5 mg/kg) and low dose post-transplant cyclophosphamide (50 mg/kg) (low dose ATG/PTCy) based regimen for graft-versus-host disease (GvHD) prophylaxis.

Methods

Patients, Donors, and Stem Cell Sources

We conducted a retrospective study on recipients of haplo-PBSCCT from September 2017 to December 2019 in our center. All patients were diagnosed with hematologic malignancies. Family members selected as donors were typed on the HLA-A, -B, -C, -DRB1, and -DQB1 locus at high-resolution level with recipient-donor HLA mismatches ≥ 1 as haplo-type. All patients received peripheral blood stem cells (PBSCs) mobilized with granulocyte colony-stimulating factor. The target number of CD34+ cells in mobilized PBSC graft was a minimum of $8 \times 10^6$/kg. Some patients also received third-party 4/6-6/6 loci matched umbilical cord blood (UCB). The study was approved by the ethical committees of Shanghai General Hospital and conducted in accordance with the Declaration of Helsinki. All data originated from clinical trials was approved by patients with mandatory written informed consent.

Conditioning Regimens and GvHD Prophylaxis

Reduced-intensity conditioning was given to patients with acute myelocytic leukemia (AML) and myelodysplastic syndrome (MDS) aged 55 and above (≥55 years), while myeloablative conditioning was given to those aged under 55 years old (<55 years) [11-13]. Myeloablative conditioning regimens were given to patients with non-Hodgkin’s lymphoma (NHL) and acute lymphoblastic leukemia (ALL) [13]. All patients received low dose ATG/PTCy based regimen for GvHD prophylaxis [11-13], which included rabbit anti-thymocyte globulin (Thymoglobin®, Genzyme Polyclonals S.A.S) 2.5 mg/kg on day-2 and day-1, cyclophosphamide 50 mg/kg on day+3 (low dose ATG/PTCy) followed by cyclosporine A 2 mg/kg/d intravenously from day+4 and mycophenolate mofetil orally 720 mg three times per day from day+4 to day+34.

ALC and ATG Concentrations

Peripheral blood ALC was detected one day before conditioning. Blood serum for ATG concentrations test was collected and stored at -80°C from day -1 to day +27 after transplantation (d-1, d0, d+2, d+4, d+6, d+9, d+12, d+15, d+18, d+21, d+24, and d+27). ATG concentrations were tested with enzyme-linked immunosorbent assay (ELISA) by using a spectrophotometer (ATG ELISA Kit, You Xuan Biology, China).

CMV-DNA and EBV-DNA Detection

Quantitative real-time PCR assays for cytomegalovirus (CMV) DNA and Epstein-Barr virus (EBV) DNA in peripheral blood were performed weekly (Sino-American Biotech, Beijing, China). The cut-off value of CMV-DNA and EBV-DNA was set as 1000 copies/ml according to the manufacturer.

Definitions

The engraftment of neutrophil and platelet and graft failure were defined according to the literature. The chimerism analysis was performed with polymerase chain reaction (PCR) amplification of short tandem repeats on CD3+ T lymphocytes of bone marrow, or fluorescent in situ hybridization for patients with sex-mismatched donors every month within the first year post-transplant. Acute GvHD (aGvHD) and chronic GvHD (cGvHD) were diagnosed and graded according to the modified Glucksberg criteria and the 2014 National Institutes of Health consensus criteria, respectively. Relapse was defined by European Society for Blood and Marrow Transplantation criteria.

Statistical Analysis

SPSS 25.0 and GraphPad Prism 5 were used for data analysis and drawing. Baseline characteristics were summarized using descriptive statistics. Fisher exact and chi-square tests
were used to compare categorical variables and the Wilcoxon rank-sum test was used to compare continuous variables. The cumulative incidences of GvHD, relapse and non-relapse mortality (NRM) were calculated and compared using Fine-Gray test. Overall survival (OS), relapse free survival (RFS) and GvHD-free relapse-free survival (GRFS) were estimated by the method of Kaplan–Meier and compared by the log-rank test. The prognostic factors for GvHD, relapse, and NRM were analyzed by the proportional subdistribution hazards regression model in competing risks and for OS, RFS, and GRFS were examined in Cox proportional hazards models. Factors with $P$ value $<0.200$ in univariate analysis were included into multivariate analysis. ATG concentrations were compared by analysis of variance for repeated measurement data. A 2-sided $P$ value $<0.050$ indicated statistical significance.

**Results**

**Patient Characteristics**

A total of 90 consecutive recipients of haploidentical transplantation in our center enrolled the study, which included 52 patients with AML, 18 patients with ALL, 13 patients with MDS, and 7 patients with NHL. The median age was 40 (7–64) years old. Twenty-one patients had ALC $<500/\mu l$ before conditioning, while 69 patients had ALC $\geq 500/\mu l$. There were no significant differences in the clinical characteristics between ALC $<500/\mu l$ and ALC $\geq 500/\mu l$ groups. The details of patients’ characteristics were shown in Table 1.

**ATG Concentrations**

Ten consecutive recipients of haplo-PBSCT from October 2019 to December 2019 had ATG concentrations test in the study, which included 4 patients with ALC $<500/\mu l$ and 6 patients with ALC $\geq 500/\mu l$. ATG concentrations in peripheral blood serum were measured at 12 timepoints from the day-1 to day +27 after transplantation. The ATG concentrations were significantly higher in ALC $<500/\mu l$ group than that in ALC $\geq 500/\mu l$ group at the same timepoint ($d0$: 108.90 vs. 67.43, $P = 0.004$; $d+9$: 72.61 vs. 47.84, $P = 0.019$; $d+18$: 56.40 vs. 40.37, $P = 0.052$; $d+27$: 41.27 vs. 30.80, $P = 0.090$; ug/ml) (Fig. 1).

**Engraftment**

The median mononuclear cells and CD34+ cells in the PBSC grafts were 16.8 (3.7–33) $\times 10^9$/kg and 10.0 (2.8–29.5) $\times 10^6$/kg respectively. Fifty-six patients received third-party UCB with the median numbers of nucleated cells 2.2 (0.6–4.2) $\times 10^9$/kg and CD34+ cells 5.1 (1.1–12.2) $\times 10^6$/kg, respectively. Two patients in ALC $<500/\mu l$ group died of heart failure on day +5 and +9 after transplantation, respectively. Eighty-eight patients successfully engrafted and there was no primary graft failure. The median time of neutrophil and platelet engraftment were 13(10–21) and 15 (12–26) days, respectively. There were no differences in the time of neutrophil ($P = 1.000$) and platelet engraftment between the two groups ($P = 1.000$). All of the 88 patients achieved full donor chimerism on day +28 after transplantation.

**Immune Reconstitution**

The counts of CD3+, CD3+CD4+CD8−, CD3+CD4−CD8+, CD19+ and CD16+CD56+ (natural killer, NK) cells were higher in ALC $\geq 500/\mu l$ group than those in ALC $<500/\mu l$ group at 3.6, 9 and 12 months after transplantation, although there were no significant differences. The data of immune reconstitution post-transplant of patients between the two groups were displayed in Supplementary Fig. 1.

**GvHD**

The median onset time of aGvHD was 21 (11–95) day after transplantation. The cumulative incidences of grade II-IV and III-IV aGvHD were 12.22 and 5.56% within 100 days, respectively. The cumulative incidences of grade I-IV and II-IV aGvHD were significantly lower in ALC $<500/\mu l$ group than that in ALC $\geq 500/\mu l$ group (14.28% vs. 40.58%, $P = 0.009$; 0 vs. 19.42%, $P = 0.043$) (Fig. 2A, B). No patients developed III-IV aGvHD in ALC $<500/\mu l$ group, while 9.25% of patients in ALC $\geq 500/\mu l$ group developed III-IV aGvHD ($P = 0.171$) (Fig. 2C). Univariate analysis for grade II-IV aGvHD was showed in Supplementary Table 1 and multivariate analysis showed that ALC $\geq 500/\mu l$ before conditioning was an independent risk factor for grade II-IV aGvHD ($RR = 0.371$, $P = 0.048$) (Table 2).

The median onset time of cGvHD was 184 (123–952) days post-transplant. The 1-year cumulative incidences of all grades cGvHD and moderate to severe cGvHD were 31.11% and 16.67%, while the 3-year cumulative incidences were 33.33% and 17.78%, respectively. No significant difference between the two groups was noted in the cumulative incidence of moderate to severe cGvHD (10.53% vs. 20.00% for ALC $<500/\mu l$ vs. ALC $\geq 500/\mu l$, $P = 0.318$) (Fig. 2D). Univariate analysis for grade moderate to severe cGvHD was showed in Supplementary Table 1. Donor age ($\geq 40$ years) might be an independent risk factor for moderate to severe cGvHD by multivariate analysis ($P = 0.058$) (Table 2).

**CMV and EBV Infection**

The cumulative incidence of CMV reactivation within 100 days post-transplant was 30.00% and two patients (2.22%) developed CMV disease. There was no significant difference.
Table 1. Characteristics of Patients.

| Variables                                      | All patients (N = 90) | ALC<500/μl (N = 21) | ALC≥500/μl (N = 69) | P value |
|------------------------------------------------|-----------------------|---------------------|---------------------|---------|
| Recipient age, year, n (%)                     |                       |                     |                     | 0.879   |
| <55                                            | 74 (82.22)            | 18 (85.71)          | 56 (81.16)          |         |
| ≥55                                            | 16 (17.78)            | 3 (14.29)           | 13 (18.84)          |         |
| Recipient sex, n (%)                           |                       |                     |                     | 0.583   |
| Male                                           | 56 (62.22)            | 12 (57.14)          | 44 (63.77)          |         |
| Female                                         | 34 (37.78)            | 9 (42.86)           | 25 (36.23)          |         |
| Diagnosis, n (%)                               |                       |                     |                     | 0.643   |
| Myeloid                                        | 65 (72.22)            | 16 (76.19)          | 49 (71.01)          |         |
| Lymphoid                                       | 25 (27.78)            | 5 (23.81)           | 20 (28.98)          |         |
| Number of chemotherapy, median (range)         | 3 (0–13)              | 3 (0–10)            | 3 (0–13)            | 0.992   |
| R-DRI, n (%)                                   |                       |                     |                     | 0.557   |
| Low                                            | 7 (7.78)              | 2 (9.52)            | 5 (7.24)            |         |
| Intermediate                                   | 68 (75.55)            | 14 (66.67)          | 54 (78.27)          |         |
| High                                           | 15 (16.67)            | 5 (23.81)           | 10 (14.49)          |         |
| Disease status, n (%)                          |                       |                     |                     | 0.234   |
| CR                                             | 61 (67.78)            | 12 (57.14)          | 49 (71.01)          |         |
| NR                                             | 29 (32.22)            | 9 (42.86)           | 20 (28.98)          |         |
| HCT-CI, n (%)                                  |                       |                     |                     | 0.233   |
| < 3                                            | 89 (98.89)            | 20 (95.24)          | 69 (100.00)         |         |
| ≥3                                             | 1 (1.11)              | 1 (4.76)            | 0                   |         |
| Donor age, yr, n (%)                           |                       |                     |                     | 0.173   |
| < 40                                           | 62 (68.89)            | 17 (80.95)          | 45 (65.22)          |         |
| ≥40                                            | 28 (31.11)            | 4 (19.95)           | 24 (34.78)          |         |
| Donor-recipient sex match, n (%)               |                       |                     |                     | 0.766   |
| Female to male                                 | 17 (18.89)            | 3 (14.29)           | 14 (20.29)          |         |
| Others                                         | 73 (81.11)            | 18 (85.71)          | 55 (79.71)          |         |
| Donor sources, n (%)                           |                       |                     |                     | 0.117   |
| Parents                                        | 27 (30.00)            | 3 (14.29)           | 24 (34.78)          |         |
| Sibling                                        | 11 (12.22)            | 4 (19.04)           | 7 (10.15)           |         |
| Offspring                                      | 49 (54.45)            | 14 (66.67)          | 35 (50.72)          |         |
| Cousin                                         | 3 (3.33)              | 0                   | 3 (4.35)            |         |
| ABO blood type, n (%)                          |                       |                     |                     | 0.291   |
| Compatible                                     | 51 (56.67)            | 14 (66.67)          | 37 (53.62)          |         |
| Incompatible                                   | 39 (43.33)            | 7 (33.33)           | 32 (46.38)          |         |
| Conditioning regimen, n (%)                   |                       |                     |                     | 0.580   |
| MAC                                            | 51 (56.67)            | 13 (61.91)          | 38 (55.07)          |         |
| RIC                                            | 39 (43.33)            | 8 (11.59)           | 31 (44.93)          |         |
| PBSC graft, median (range)                     |                       |                     |                     | 0.065   |
| Mononuclear cells, x10⁹/kg                     | 16.8 (3.7–33.0)       | 15 (3.7–31.0)       | 17 (4.3–33.0)       |         |
| CD3⁺ cells, x10⁹/kg                            | 2.6 (2.2–6.0)         | 2.2 (0.8–4.3)       | 2.7 (0.7–6.0)       | 0.098   |
| CD3⁴ cells, x10⁶/kg                            | 10.0 (2.8–29.5)       | 12.9 (2.8–22.7)     | 10.1 (2.8–29.4)     | 0.057   |
| UCB, n (%)                                     |                       |                     |                     | 0.320   |
| Yes                                            | 56 (62.22)            | 15 (71.43)          | 41 (59.42)          |         |
| No                                             | 34 (37.78)            | 6 (28.57)           | 28 (40.58)          |         |
| UCB, median (range)                            |                       |                     |                     | 0.078   |
| NCs, x10⁸/kg                                   | 2.2 (0.6–4.6)         | 2.0 (0.8–3.3)       | 2.5 (0.6–4.2)       |         |
| CD3⁴ cells, x10⁶/kg                            | 5.1 (1.1–12.2)        | 4.1 (1.1–10.7)      | 5.6 (1.2–12.2)      | 0.083   |

ALC: absolute lymphocyte count; R-DRI: refined disease risk index; CR: complete remission; NR: non-remission; HCT-CI: hematopoietic cell transplantation comorbidity index; PBSC: peripheric blood stem cell; MAC: myeloablative conditioning; RIC: reduced-intensity conditioning; UCB: umbilical cord blood; NC: nucleated cell.
in the cumulative incidences of CMV reactivation between the 2 groups within 100 days after transplantation (33.33% vs. 25.56% for ALC < 500/μl vs. ALC ≥ 500/μl, P = 0.926).

However, the mean CMV viral load by PCR was significantly higher in ALC < 500/μl group than that in ALC ≥ 500/μl group (2 852 025 vs. 90 701 copies/ml, P = 0.001) (Fig. 3A). The cumulative incidence of EBV reactivation within 100 days was 28.89% and only one two patients (1.11%) developed post-transplant lymphoproliferative disorders. The cumulative incidence of EBV reactivation within 100 days and mean EBV viral load after transplantation were significantly higher in ALC < 500/μl group than that in ALC ≥ 500/μl group, respectively (47.78% vs. 22.22%, P = 0.020 for EBV reactivation; 275 005 vs. 52 824 copies/mL for EBV-DNA load, P = 0.001) (Fig. 3B).

**Relapse and NRM**

A total of 17 (18.89%) patients relapsed, the median time to relapse were 229 (75–875) days. The 1-year cumulative incidence of relapse was significantly higher in ALC < 500/μl group than that in ALC ≥ 500/μl group (33.33% vs. 11.59%, P = 0.041) (Fig. 4A). Univariate analysis for relapse was shown in Supplementary Table 2. ALC < 500/μl was the only risk factor for relapse in multivariable analysis (RR = 2.713, P = 0.047) (Table 2).

**Figure 1.** Serum concentrations of anti-thymocyte globulin (ATG) in ALC < 500/μl group and ALC ≥ 500/μl group from day -1 to day +27 posttransplant. (Data were shown as mean ATG concentration, Pd1 = 0.006; Pd2 = 0.004; Pd4 = 0.008; Pd6 = 0.016; Pd8 = 0.017; Pd10 = 0.020; Pd11 = 0.018; Pd12 = 0.038; Pd14 = 0.052; Pd16 = 0.066; Pd18 = 0.086; Pd20 = 0.090). ALC: absolute lymphocyte count.

**Figure 2.** The cumulative incidences function (CIF) of graft versus host disease (GvHD) in ALC < 500/μl group and ALC ≥ 500/μl group. (A) The CIF of grade I-IV acute GvHD (aGvHD). (B) The CIF of grade II-IV aGvHD. (C) The CIF of grade III-IV aGvHD. (D) The CIF of grade moderate-severe chronic GvHD (cGvHD). aGvHD: acute graft versus host disease; ALC: absolute lymphocyte count; cGvHD: chronic graft versus host disease; HSCT: hematopoietic stem cell transplantation.
Fifteen patients died of non-relapse causes (16.67%), 9 of them died of infection, 2 of heart failure, 2 of graft rejection, 1 of cerebral hemorrhage and 1 of grade IV aGvHD. The 1-year NRM in ALC <500/μl group was similar with that in ALC ≥500/μl group (19.05% vs. 13.00%, P = 0.722) (Fig. 4B). Univariate analysis for NRM was shown in Supplementary Table 2. Recipient aged ≥55 years old was the only independent risk factor for NRM in multivariable analysis (RR = 3.935, P = 0.009) (Table 2).

**OS, RFS and GRFS**

After a median follow-up of 540 (12~1213) days, 12 patients (57.14%) in ALC <500/μl group and 17 patients (24.64%) in ALC ≥500/μl group died. The 1-year OS and RFS were significantly lower in ALC <500/μl group than that in ALC ≥500/μl group (52.38% vs. 79.71% for OS, P = 0.004; 47.62% vs. 75.36% for RFS, P = 0.014) (Fig. 5A, B). The 1-year GRFS in ALC <500/μl group was also significantly lower than that in ALC ≥500/μl group.
lower than that in ALC $\geq 500/\mu l$ group (42.89% vs. 65.22%, $P = 0.043$) (Fig. 5C). Univariate analysis for OS, RFS, and GRFS was shown in Supplementary Table 3. Multivariable analysis showed that ALC $< 500/\mu l$ was the only significant risk factor for OS ($RR = 4.090$, $P = 0.022$), RFS ($RR = 4.180$, $P = 0.018$), and GRFS ($RR = 1.966$, $P = 0.047$) (Table 2).
Discussion

The present study demonstrated that ALC before conditioning was an important prognostic factor for patients undergoing haplo-PBSCT with low dose ATG/PTCy based regimen for GvHD prophylaxis. The patients with ALC <500/μl before conditioning had higher blood concentration of ATG, lower incidence of GvHD, higher incidence of relapse, and lower 1-year OS and RFS, than patients with ALC ≥500/μl. Furthermore, ATG destroys not only reactive donor T lymphocytes that mediate GvHD but also B lymphocytes, NK cells, and even dendritic cells, which led to severe non-specific immunosuppression and delayed immune reconstitution. In the present study, the immune reconstitution was delayed in patients with ALC <500/μl as compared with patients with ALC ≥500/μl.

Remberger et al. reported a similar result that patients with higher ATG concentrations had higher risk of relapse than those with lower ATG concentrations (82% vs. 17%, P < 0.010). A multicenter retrospective study found excessive exposure to ATG resulted in higher risk of relapse with substantially lower ALC. For recipients with lower ALC before conditioning, the graft versus leukemia effect might be attenuated, which would increase the risk of relapse.

In terms of survival, our study showed ALC <500/μl was related to lower 1-year OS, RFS and GRFS. ALC <500/μl was the only significant risk factor for OS, RFS, and GRFS, but not for NRM. A study on matched related donor allo-HSCT with rabbit ATG (4.5 mg/kg) showed that the 1-year NRM was significantly higher in ALC <500/μl group as compared with ALC ≥500/μl group (28.6% vs. 8.6%; P = 0.031). The study also showed that the median survival in ALC <500/μl group was significantly inferior to that in ALC ≥500/μl group (291, 217.7 to 364.3 days vs. OS not reached; P = 0.001). Podgorny et al. reported that higher ATG concentration was a risk factor for infectious complications and increased mortality, which might increase the NRM, mostly via an infectious complication. However, in the present study, there was no significant difference in NRM between the two groups, the reason might be the different GvHD prophylaxis. Our previous study showed the low dose ATG/PTCy produced lower incidence of infection compared with classic GvHD prophylaxis with full dose of ATG.

Nevertheless, this study had some limitations. First, it was a retrospective analysis with limited number of patients from a single institution. Second, the study included patients with different hematological malignancies. Third, only a small part of patients had ATG concentration testing.

In conclusion, our study demonstrated that for patients undergoing haplo-PBSCT with low dose ATG/PTCy based regimen for GvHD prophylaxis, ALC <500/μl before conditioning was related to higher ATG concentration, lower risk of GvHD, but higher risk of relapse and poor survival. Weight based ATG dosing would lead to over exposure in patients with low ALC. ATG dosage adjustment on ALC before conditioning might be a more reasonable and precise strategy, which needs further prospective studies.

Ethical Approval

This study was approved by Clinical Research Ethics Committee of Shanghai General Hospital, China.
Statement of Human and Animal Rights
All procedures in this study were conducted in accordance with the Shanghai Municipal Clinical Research Ethics Committee approved protocols.

Statement of Informed Consent
Written informed consent was obtained from the patients for their anonymized information to be published in this article.

Declaration of Conflicting Interests
The author(s) declared no potential conflicts of interest with respect to the research, authorship, and/or publication of this article.

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Supplemental Material
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