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ORIGINAL ARTICLE

Factors influencing the late phase of recovery after bone mineral density loss in allogeneic stem cell transplantation survivors

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Accelerated bone mineral density loss (BMDL) occurs early after allogeneic stem cell transplantation (SCT) and is related to factors such as steroids and chronic GvHD. In order to understand the natural history of BMDL of SCT in the longer term, we evaluated a longitudinal cohort of 148 survivors with a median follow-up of 12 years (range 3–22 years). All women received hormone replacement therapy, and routine calcium/vitamin D supplementation was recommended but ~50% of patients still had suboptimal vitamin D levels and bisphosphonates were rarely utilized. BMD significantly improved from 5 to 20+ years but the femoral neck and forearm remained vulnerable sites. Younger age, higher pretransplant body mass index (BMI) and increment in BMI post transplant were significantly associated with increased BMD and protected against osteopenia/osteoporosis. These findings support consideration of BMD loss in SCT survivors in two phases, an early phase of BMD loss (3–5 years) followed by a later phase of BMD recovery, with different protective and aggravating factors. Treatment- and transplant-related factors (such as steroids, immunosuppressives, chronic GvHD, vitamin D) are known to impact the early phase of BMD loss but age and BMI are more influential in the late phase of BMD recovery.

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INTRODUCTION

Dramatic bone mineral density loss (BMDL) has been observed in patients undergoing autologous as well as allogeneic stem cell transplantation (SCT) and is attributable to varied risk factors ranging from prior therapy, cumulative corticosteroid exposure, preparative regimens, immunosuppressive drugs, hypogonadism, vitamin D deficiency, secondary hyperparathyroidism due to low serum calcium, malabsorption, renal dysfunction, aging, female gender, low body mass index (BMI) and reduced mobility.[1–7] Loss in BMD is not a benign entity. Pundole *et al.*[8] have recently shown that the BMD loss in autologous and allogeneic SCT ultimately translates into fractures affecting 8% of the transplant population at a rate that is approximately eightfold increased compared with the general population.

The rate of BMDL decline is extremely rapid in the first 4 to 6 months of transplant, slows down between 6 and 12 months and starts to recover only later.[9] Recovery begins in the lumbar spine followed by the femoral neck where BMD nadirs at 24 months.[4,10] In an earlier study from our group, Savani *et al.*[11] found that older age and prolonged immunosuppressive therapy for >3 years, a surrogate for a significant burden of chronic GvHD (cGvHD), significantly worsened BMDL in the first decade after transplant. Others have also shown that BMDL in the first decade after SCT is associated with cGvHD.[7,12] Several studies have also revealed the high prevalence of vitamin D deficiency in transplant recipients,[13,14] and this is of great interest in the field as it represents a powerful and modifiable risk factor.

Our study sought to identify trends in BMD and the factors influencing persistent BMD in later SCT survivors, when the impact of cGvHD, immunosuppression and early SCT-related variables are presumably less relevant than those related to the normal aging process. We analyzed a large longitudinal cohort of long-term survivors of allogeneic SCT in their second decade to understand long-term trends in BMD and the interplay between transplant- versus aging-related risk factors for osteoporosis or osteopenia.

MATERIALS AND METHODS

Patients

Recipients of allogeneic SCT at the National Institutes of Health between 1993 and 2011, who survived for >3 years from their date of transplant, were identified as long-term survivors and enrolled in the Institutional Review Board-approved long-term evaluation and follow-up natural history protocol (NHLBI 05-H-0130; ClinicalTrials.gov Identifier NCT00106925). All long-term survivors who returned for follow-up in our program were enrolled in this study and all subjects who enrolled had BMD at study entry and periodically thereafter. 148 survivors underwent dual-energy X-ray absorptiometry (DEXA) scanning at their scheduled follow-up clinic visits. BMD measurements were targeted for 3, 5, 7, 10, 15 and 20 years post transplant. Of 148 patients reported, 52 patients only had one available BMD scan at baseline, whereas the majority of patients ($n = 96$) had 2 or 3 repeated scans on longitudinal follow-up. Essentially, this resulted in a cross-sectional study at baseline followed by longitudinal evaluation.

BMD measurement

BMD was measured by serial DEXA scans at six anatomic sites (anteroposterior spine, femoral neck, trochanter, intertrochanter, total hip and forearm) and recorded as *T*-scores and *Z*-scores in adult patients and

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Z-scores in children (age <18 years). The same DEXA scanner was used for serial measurements.

The BMD *T*-score is defined as the number of SD from the normal values of young, healthy, gender-matched controls. *Z*-score is defined as the number of SD from the normal values of age- and gender-matched controls by the World Health Organization criteria.[15,16] Adult patients were classified by their BMD *T*-scores as: normal (*T*-score $\geqslant -1$) or BMDL: osteopenia (*T*-score <-1 and >-2.5) and osteoporosis (*T*-score $\leqslant -2.5$). Children was classified as osteopenia (*Z*-score <-1 and >-2) and osteoporosis (*Z*-score $\leqslant -2$). Radiological evaluation for fractures was performed in subjects as clinically indicated.

Supportive care

All females below the natural age of menopause (variably between the ages 45 and 50 years) received hormone replacement therapy (HRT). Lo-Ovral (0.3 mg norgestrel/0.03 mg ethinyl estradiol) or therapeutic equivalent was the preferred HRT. Male hypogonadism, when discovered, was referred to the primary care provider without firm recommendation for testosterone supplementation and none of our male subjects received long-term supplementation for more than 12 months. All survivors were encouraged to take calcium and vitamin D supplementation post SCT. Ionized calcium levels were not checked and supplemental doses of vitamin D were recommended to be increased when there was evidence of hypovitaminosis. Weight-bearing exercise (outside of general fitness) and compliance with exercise were not explicitly measured. Bisphosphonates were not administered except to three patients who continued taking bisphosphonate prescribed from before SCT. In general, the implementation of supportive care of BMD was delegated to outside primary medical practitioners.

Statistical analysis

Summary statistics such as proportions, medians, means and SEM were used to describe patient characteristics and the longitudinal BMD changes post transplant. A linear mixed effects modeling for repeated measures was used to analyze the BMD scan measures that allows for different numbers of measurements for individual patients and provides estimates of BMD changes over time and effects of baseline and longitudinal factors. The generalized estimating equation models were used to assess the change in percentages of BMDL and osteoporosis over time. Both linear mixed models and generalized estimating equation models adjusted for the intrasubject correlation among repeated measures on BMD scans. To identify the factors associated with the BMD change, baseline variables at transplant such as age, gender, BMI, C-reactive protein (CRP), primary diagnosis (ALL vs others), intensity of conditioning regimen and stem cell source, covariates at study enrollment (occurrence of cGvHD, severity of cGvHD and prolonged immunosuppressive therapy) and covariates at each BMD measurements (time since SCT, BMI change from baseline, vitamin D level and CRP level) were included as independent variables in the multivariate regression models. Both age and BMD measurement time were analyzed as continuous variables in the multivariate models. All tests were two sided, and a *P*-value of <0.05 was considered statistically significant. Statistical analyses were performed with the R statistical software, version 3.2.0 (R Foundation for Statistical Computing, Vienna, Austria).

RESULTS

Patient characteristics

Patient and transplant characteristics are summarized in Table 1. The median follow-up was 12 years post SCT (range: 3.1–21.5 years) for our cohort of 148 patients surviving >3 years after HLA-identical sibling T lymphocyte-depleted SCT.

Of the 148 long-term survivors, 109 patients developed some manifestation of cGvHD disease (43% of patients had extensive cGvHD and 57% patient had limited cGvHD). However, given the T cell-depleted nature of the majority of the transplants, cGvHD was generally mild and only 67 patients required systemic immunosuppressive therapy at 3 years post SCT. There was no uniform approach to treatment for cGvHD and information on steroid dosing and exposure was not available.

Table 1. Patient and transplant characteristics

Variable (n = *148*)	N *(%)*
Gender	
Male	85 (57%)
Female	63 (43%)
Ethnicity	
Non-Hispanic	77 (52%)
Hispanic	70 (47%)
Not stated	1 (1%)
Age at transplant, median (range), years	36 (7–69)
BMI, median (range), kg/m^2	25 (15–45)
Overweight (BMI >25 kg/m^2)	64 (43%)
CRP mg/dL, median (range)	0.40 (0.23–13)
Transplant indications	
CML/CMML	62 (42%)
AML/MDS	56 (38%)
ALL	16 (11%)
NHL/CLL	8 (5%)
Others (SAA, MM, mastocytosis)	6 (4%)
Graft source	
PBSC	133 (90%)
Bone marrow	15 (10%)
Intensity of conditioning	
Fully ablative	131 (88%)
Reduced intensity	7 (5%)
Nonmyeloablative	10 (7%)
TBI cGY	
1200–1360	131 (88%)
400	7 (5%)
0	10 (7%)
GvHD prophylaxis	
Cyclosporine alone	148 (100%)

Abbreviations: BMI = body mass index; CMML = chronic myelomonocytic leukemia; CRP = C-reactive protein; MDS = myelodysplastic syndrome; MM = multiple myeloma; NHL = non-Hodgkin's lymphoma; SAA = severe aplastic anemia.

Because of the irregular visit time for different patients, subsets of 88 patients were informative at 3–5 years, 112 patients at 5.1–10.0 years, 55 patients at 10.1–15.0 years and 12 patients at over 15 years. A total of 267 BMD scans were performed between 3 and 22 years post SCT. A total of 52 patients only had one available scan and 96 patients had two to three scans recorded. Seven (3%) scans for 5 children (median age 16 years (range 13–17) at the scans, 3 children had one scan each and 2 children had two scans each) were recorded and the *Z*-scores were used in place of *T*-scores for these scans in the statistical analysis.

BMD at the 5-year landmark

The 5-year landmark was selected as the study baseline when the majority of subjects were informative. In this landmark analysis, 88 patients with their initial BMD scans 3 to 5 years post SCT were included. At the 5-year landmark, the earliest time point for study enrollment, BMDL was observed in 76% of survivors: 58% had osteopenia and 18% were osteoporotic. Forearm (10%) and spine (8%) were the most common sites of osteoporosis followed by trochanter (4.7%) and femoral neck (3.4%). Femoral neck (50%), spine (41%) and total hip (42%) were the sites with higher percentages of BMDL (Table 2).

Table 2. Bone mineral density loss by anatomic site at 5 years post SCT ($n = 88$)

Sites	*BMDL (%)*	*Osteopenia (%)*	*Osteoporosis (%)*
AP spine	41.4	33.3	8.0
Femoral neck	50.0	46.6	3.4
Trochanter	36.0	31.4	4.7
Intertrochanteric	37.2	37.2	0.0
Total hip	42.0	40.9	1.1
Forearm	37.7	27.3	10.4
Overall (lowest *T*-score)	76.1	58.0	18.2

Abbreviations: AP = anteroposterior; BMDL = bone mineral density loss; SCT = stem cell transplantation.

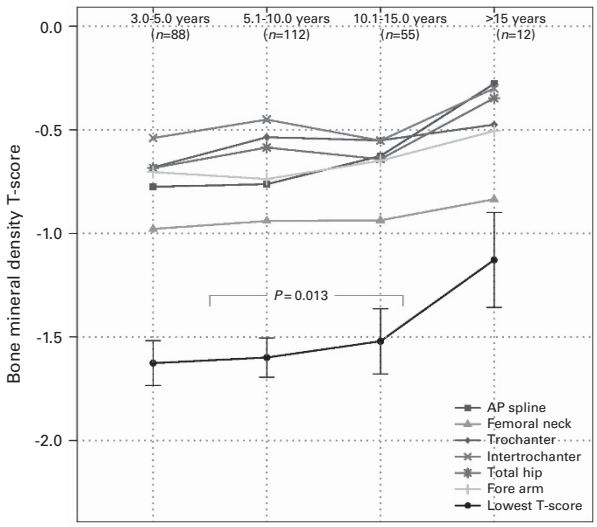

Figure 1. Mean BMD *T*-scores by anatomical site over time post SCT. Error bars represent the s.e.m. A full color version of this figure is available at the *Bone Marrow Transplantation* journal online.

Long-term trend in BMD

Serial DEXA scans showed a high proportion of bone loss already present at 3–5 years that subsequently stabilizes at 5–9 years and is gradually improved from 10 to 15 years (Figure 1). The mean ± SEM for the lowest *T*-scores (measured for an individual across all anatomic sites) were −1.63 ± 0.11, −1.60 ± 0.10, −1.52 ± 0.16 and −1.13 ± 0.13 at 3–5 years, 5.1–10.0 years, 10.1–15.0 years and over 15 years, respectively. The longitudinal analysis of BMD trend with a categorical time variable showed the lowest *T*-scores significantly increased after 10+ years post SCT compared with the scores between 3 and 10 years ($P = 0.013$). Femoral neck had the lowest BMD at ⩾10 years compared with other sites (all $P < 0.001$, paired *t*-test) apart from the forearm ($P = 0.07$). Although there was apparent improvement beyond 15 years, the numbers of subjects were too small to reach statistical significance.

Multivariable analyses of factors affecting BMD

Linear mixed models were used to examine the continuous time trend of BMD *T*-scores at various anatomical sites and the lowest *T*-scores across sites and to assess the factors associated with *T*-scores. In the multivariate model, the actual study visit time (when BMD scans were measured) was included in the model as a continuous variable, rather than a categorical variable or fixed time points, because the visit times were irregular and varied across different patients. The results of these multivariate regressions are shown in Table 3. A small but statistically significant increasing trend in *T*-scores was found at most sites during the long-term follow-up ($P < 0.05$ for the linear time effect on the lowest *T*-score, total hip, intertrochanteric and forearm sites; $P = 0.08$ and $P = 0.06$ for spine and femoral neck sites.) Younger age and greater BMI pretransplant and BMI gain post transplant were associated with increased *T*-scores at most sites and overall lowest *T*-score. Prolonged immunosuppressive therapy was significantly associated with decreased *T*-scores at femoral neck, total hip and intertrochanteric sites. There were also gender differences in *T*-scores for certain sites. BMD *T*-scores was not influenced by primary diagnosis, history of cGvHD, severity of cGvHD (extensive or limited), the intensity of conditioning regimen and stem cell source at the BMD scans.

Because the increases in the *T*-scores were relatively small, the percentages of BMDL and osteoporosis remained stable after 3 years post SCT at individual sites. The results of multivariate generalized estimating equation models for overall BMDL and osteoporosis are shown in Table 4. The percentages of overall BMDL and osteoporosis did not change with time since transplant. Similarly, younger age and greater BMI at transplant and BMI gain from baseline were associated with a decreased risk for overall BMDL and osteoporosis. Other factors were not found to be associated with BMDL and osteoporosis.

Vitamin D levels

We evaluated vitamin D levels in our study population, but routine measurement was introduced into the protocol only in recent years. Out of 148 patients, 48 patients had no vitamin D levels drawn, 78 had 1 vitamin D measure and 22 had 2–3 measures. Approximately 50% patients had a suboptimal vitamin D level (vitamin D level < 30 ng/mL) (Figure 2). The percentage of vitamin D deficiency did not change over the follow-up period. A separate multivariate analysis in a subset of 100 recent patients (123 vitamin D measures) showed that the vitamin D level was not associated with BMD change.

C-reactive protein

We evaluated CRP levels as a marker of general inflammatory response in our study population at transplant and at subsequent visits. CRP is increased after transplant and then becomes stabilized, with no significant changes during 3–22 years. A separate multivariate analysis in a subset of 110 patients (168 CRP measures) showed that CRP at transplant or CRP change after transplant did not have an effect on BMD min *T*-scores or anteroposterior spine and femoral neck scores.

Osteoporotic fractures

Five subjects (one femur and hip, one spinal compression, one humerus, one inferior pubic ramus and one ulnar) had fractures documented by radiography. Four out of these five subjects had concurrent BMDL documented by DEXA scans (Supplementary Table 1).

DISCUSSION

In our cohort study of very long-term survivors, we found that low BMD, the precursor to osteoporotic fractures, remains a significant clinical problem affecting the majority of survivors, with comforting evidence of improvement over time. BMD stabilized between 3 and 5 years and gradually improved between 5 and 15 years without bisphosphonates. BMD at the lumbar spine starts improving gradually at 5 years and this trend continues over to the second decade. However, the femoral neck is an anatomical site that improves minimally at 5 years and reaches a plateau in the second decade, representing an important vulnerability. We also show that persistent BMD loss in very long-term transplant

Table 3. Results of the multivariate regression analysis of BMD scores

Variables	*Dependent variable in each multivariate model*						
	AP spine	*Femoral neck*	*Total hip*	*Trochanter*	*Intertrochanteric*	*Forearm*	*Lowest T-score*
	regression coefficients						
Time since SCT	0.022†	0.016†	0.030**	0.008	0.029*	0.039**	0.026**
Age at SCT, per 10 years	0.017	−0.252**	−0.130*	−0.120†	−0.094	−0.120	−0.170*
BMI at SCT	0.055*	0.093**	0.091**	0.082**	0.089**	0.062**	0.077**
BMI change from baseline	0.033	0.070**	0.059**	0.048*	0.072**	0.049*	0.049**
Gender (male vs female)	0.373†	0.269†	0.348*	0.318†	0.353*	−0.846**	−0.121
Prolonged IST	−0.073	−0.320*	−0.315*	−0.255	−0.352*	−0.146	−0.167

Abbreviations: AP = anteroposterior; BMD = bone mineral density; IST = immunosuppressive therapy; SCT = stem cell transplantation. †$P < 0.1$ (a nonsignificant trend); *$P < 0.05$; **$P <$ 0.01. In each multivariate linear mixed model, time since SCT, baseline age, gender and body mass index (BMI) at SCT, prolonged IST and BMI change from baseline were included.

Table 4. Results of the multivariate regression analysis of overall BMDL and osteoporosis

Variables	*BMDL*		*Osteoporosis*	
	Odds ratio (95% CI)	P-*value*	*Odds ratio (95% CI)*	P-*value*
Time since SCT	0.95 (0.89 – 1.02)	0.15	1.02 (0.95 – 1.10)	0.62
Age at SCT, per 10 years	1.49 (1.09 – 2.05)	0.012	1.70 (1.24 – 2.34)	0.001
BMI at SCT	0.87 (0.81 – 0.94)	0.0001	0.86 (0.79 – 0.94)	0.001
BMI change from baseline	0.89 (0.81 – 0.98)	0.022	0.87 (0.77 – 0.97)	0.014

Abbreviations: BMDL = bone mineral density loss; BMI = body mass index; CI = confidence interval; SCT = stem cell transplantation. Overall BMDL and osteoporosis were defined by lowest *T*-score ⩽1 and ⩽2.5, respectively. In each multivariate generalized estimating equation (GEE) model, time since SCT, baseline age, BMI at SCT and BMI change from baseline were included.

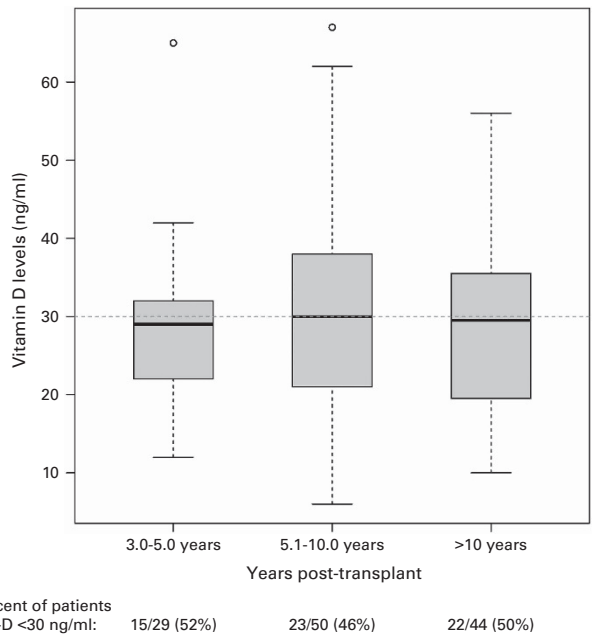

Figure 2. Vitamin D level over time post SCT. A full color version of this figure is available at the *Bone Marrow Transplantation* journal online.

survivors is more strongly influenced by standard nontransplant factors, such as advancing age and BMI (reflecting general health or nutritional status) than by transplant-related variables indicating cGvHD or immunosuppression. Chronic GvHD, identified in this same cohort but at an earlier time frame of median 6.5 years, was no longer a significant exacerbating factor.[11] Female gender was not an issue because of excellent compliance with HRT. The kinetics of recovery were slower than previously noted in other studies[12,17] but this could be attributed to suboptimal calcium/vitamin D supplementation and/or avoidance of bisphosphonates. The elevated risk of fractures in SCT survivors is now a proven entity[8] and mandates a need for closer transplant center involvement in BMD management in order to meet ASBMT (American Society for Blood and Marrow Transplantation) guidelines[18] with an emphasis on appropriate doses of calcium/vitamin D plus introduction of bisphosphonates according to FRAX (Fracture Risk Assessment Tool) score.[19]

Our data support the consideration of post SCT BMD loss in two phases. An 'early BMD loss phase' that commences with antineoplastic therapy before transplant typically lasting up to the first 3 to 5 years, followed by a 'later BMD recovery phase' in which recovery occurs from accumulated insults once GvHD and subclinical inflammation subside.[20–22] The 'early BMDL phase' is dramatic; Yao *et al.*[23] have shown that BMDL between baseline and day 100 DEXA scans is equivalent to 7–10 years of aging of bones for autologous and 13–17 years for allogeneic SCT recipients. Factors influencing the 'early BMD loss phase' include the intensity of antineoplastic therapy, conditioning, GvHD, immunosuppression and cumulative steroid exposure.[20–22] Management principles in the 'early BMD loss phase' include reducing exposure to corticosteroids, probably the strongest modifiable risk factor, and fracture risk returns to baseline 6–18 months after discontinuing corticosteroid exposure.[24] Calcium and vitamin D supplementation alone in the 'early BMDL phase' are unable to prevent BMDL[9,25,26] but probably play a critical role in the 'later BMD recovery phase'. HRT is of benefit in reducing BMDL hypogonadal patients[27] but should probably be

routinely supplemented in all eligible patients. Anti-resorptive bisphosphonate therapy is rapidly gaining acceptance in this difficult cohort of subjects, but its effects are variable; they have been shown to improve BMDL at the lumbar spine but not at the femoral neck.[28] Pamidronic acid reduces BMDL at the femoral neck and hip especially in patients receiving high doses of glucocorticoids for GvHD treatment.[26,29] Treatment with risedronate for 12 months increased BMD significantly at the lumbar spine and prevented further bone loss at the femoral neck in the long-term survivors after SCT.[30] Zoledronic acid reduces BMDL in most patients after SCT.[25,31–33] A randomized multicenter phase II trial showed intermittent Zoledronic acid preserved long- term bone health in adult SCT recipients at risk for osteoporosis.[34] We expect that the conclusive demonstration by Pundole *et al.*[8] of increased fractures in transplant patients should promote more aggressive efforts to preserve BMD and greater involvement by the transplant team.

There are unique strengths in this study related to the very long-term follow-up of survivors into 15+ years post transplantation, and cohort homogeneity from a uniform stem cell source, HLA-identical allogeneic sibling transplants, radiation-based conditioning, avoidance of severe cGvHD due to T-depleted transplants and perfect compliance with HRT in premenopausal women. Nevertheless, there are significant limitations to generalization: HLA-mismatched transplantation, nonmyeloablative conditioning, nonmalignant indications for transplant, severe cGvHD and pediatric subjects are underrepresented in our cohort and deserve separate study. We found that vitamin D supplementation was clearly inadequate, with half the population achieving low levels, and bisphosphonate therapy was uncommon. The precise influence of reduced vitamin D supplementation or bisphosphonates on BMD recovery could not be adequately studied in this cohort. Another significant limitation is that this study did not have multiple repeated measures on all subjects during the long-term follow-up and the findings based on these statistical models are hypothesis generating.

In conclusion, BMD stabilized between 3 and 5 years and gradually improved between 5 and 20 years in our long-term survivors without bisphosphonates. The femoral neck is an anatomical site of prolonged vulnerability to a decline in BMD. In the 'late phase of BMD recovery', age and BMI were more influential than transplant- or therapy-related factors. Closer transplant center involvement is necessary in BMD management.

CONFLICT OF INTEREST

The authors declare no conflict of interest.

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AUTHOR CONTRIBUTIONS

PA designed the study, collected data, analyzed and interpreted the data and wrote the manuscript; NAJ and PAP collected data and critically revised the manuscript; XT analyzed and interpreted the data and critically revised the manuscript; COW analyzed the data; BNS critically revised the manuscript; EK and SI took care of patients and critically revised the manuscript; JB and MB designed the study, analyzed and interpreted the data, wrote the manuscript and took care of patients.

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