Reduced dose of lenograstim is as efficacious as standard dose of filgrastim for peripheral blood stem cell mobilization and transplantation: A randomized study in patients undergoing autologous peripheral stem cell transplantation

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In vitro studies have demonstrated a 27% increased efficacy of lenograstim over filgrastim. However, equal doses of 10 μg/kg/day of filgrastim and lenograstim have been recommended for mobilization of CD34+ cells without associated chemotherapy. In this study, we investigated whether a 25% reduced dose of lenograstim at 7.5 μg/kg/day is equavalent to 10 μg/kg/day filgrastim for autologous peripheral blood stem cell (PBSC) mobilization and transplantation. A total of 40 consecutive patients were randomized to either filgrastim (n = 20) or lenograstim (n = 20). The two cohorts were similar in regard to disease, sex, body weight, body surface area, conditioning regimens, previous chemotherapy cycles and radiotherapy. Each growth factor was administered for 4 consecutive days. The first PBSC apheresis was done on the 5th day. In the posttransplant period, the same G-CSF was given at 5 μg/kg/day until leukocyte engraftment. Successful mobilization was achieved in 95% of patients. Successful mobilization with the first apheresis, was achieved in 10/20 (50%) patients in the filgrastim group versus 9/20 (46%) patients in the lenograstim group. No significant difference was seen in the median number of CD34+cells mobilized, as well as the median number of apheresis, median volume of apheresis, percentage of CD34+ cells, and CD34+ cell number. Leukocyte and platelet engraftments, the number of days requiring G-CSF and parenteral antibiotics, the number of transfusions were similar in both groups in the posttransplant period. Lenograstim 7.5 μg/kg/day is as efficious as filgrastim 10 μg/kg/day for autologous PBSC mobilization and transplantation. Am. J. Hematol. 83:644–648, 2008. © 2008 Wiley-Liss, Inc.

Introduction

In our country two forms of recombinant human granulocyte-colony stimulating factor (G-CSF) are available for clinical use: filgrastim (Neupogen®, F Hoffmann-La Roche, Basel, Switzerland) and lenograstim (Granocyte®, Chugai-Aventis Pharmaceuticals). The two products have differences in their chemical structures and physicochemical properties. They are both produced by recombinant DNA technology: Lenograstim is produced in culture from Chinese hamster ovary cells and filgrastim is produced in culture from *Escherichia coli* [1].

The difference in the production processes accounts for the differences in the amino acid sequences and glycosilation between the two molecules [2]. Lenograstim is glycosylated making the G-CSF molecule more stable to variations in pH, temperature, and proteolysis [3–5]. Similarly, it has been demonstrated that lenograstim has a greater capacity to stimulate the colony growth in vitro of both purified CD34+ and unmanipulated peripheral blood stem cells (PBSCs) [6].

In vitro studies indicate that lenograstim is more potent than filgrastim on a weight for weight basis [6–8]. It has been shown that glycosilation improves the in vitro priming effect exerted by G-CSF on superoxide production by human neutrophils which accounts for the higher activity of glycosylated G-CSF. One microgram of filgrastim is equivalent to 100,000 units of activity, whereas 1 μg of lenograstim is equivalent to 127,750 units of activity, which represents a 27% difference; that means that, in vitro, lenograstim is as more potent than filgrastim [9]. However, both products are recommended at the same dosage for PBSC mobilization as 10 μg/kg if used without chemotherapy [10]. No data comparing these two products at those reduced doses are present in the literature yet.

The objective of this trial was to evaluate if lenograstim, which is 27% more potent than filgrastim, has the same efficacy of filgrastim when is used at a 25% lower dose than filgrastim; in other words, we compared lenograstim at 7.5 μg/kg/day and filgrastim at 10 μg/kg/day in terms of potency in the mobilization of PBSCs in patients undergoing high-dose chemotherapy (HDC) and autologous PBSC transplantation.

Results

Patients

The patient characteristics are detailed in Table I. Forty consecutive patients (12 females and 28 males) were enrolled and randomly assigned to the filgrastim arm (n = 20)

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TABLE I. Patient Characteristics

	Filgrastim ($n = 20$)	Lenograstim ($n = 20$)	P
Age (years) (median, range)	33 (18–62)	24.5 (15–70)	0.52
Disease			
Hematologic malignancy (%)	15 (75)	12 (60)	0.73
Solid tumor (%)	5 (25)	8 (40)	0.49
Sex			1
Male	14 (70)	14 (70)	
Female	6 (30)	6 (30)	
Height (cm)	169 (142–180)	171.5 (161–181)	0.22
Weight (kg)	73.5 (59–105)	68.5 (47–120)	0.23
BSA (m2)	1.88	1.9	0.95
Conditioning			
TBI (%)	6 (30)	2 (10)	0.13
Non-TBI (%)	14 (70)	18 (90)	0.62
Number of patients with previous radiotherapy (%)	3 (15)	6 (30)	0.45
Median number of previous chemotherapy cycles (range)	9 (4–25)	10 (2–17)	0.92

TBI, total body irradiation; BSA, body surface area.

TABLE II. Apheresis Results

	Filgrastim (median, range)	Lenograstim (median, range)	P
Number of apheresis (n)	1 (1–3)	2 (1–3)	0.56
One apheresis (%)	10 (50)	9 (45)	0.35
Two apheresis (%)	9 (45)	10 (50)	0.35
Three apheresis (%)	1 (5)	1 (5)	1
Duration of apheresis (min)			
1st apheresis	270 (150–360)	270 (90–360)	0.57
2nd apheresis	240 (120–360)	285 (120–360)	0.73
Total volume (ml)			
1st apheresis	250 (150–300)	250 (100–300)	0.61
2nd apheresis	200 (100–300)	185 (100–300)	0.85
Number of TNC ($\times 10^{10}$)			
1st apheresis	7.40 (3.8–19.08)	6.29 (2.94–16.32)	0.38
2nd apheresis	5.04 (2–11.3)	4.25 (2.24–11.34)	0.62
Number of CD34 cells ($\times 10^{8}$)			
1st apheresis	2.02 (0.10–14.04)	1.04 (0.32–8.37)	0.07
2nd apheresis	0.70 (0.22–3.39)	0.54 (0.11–3.48)	0.90
White blood cell count (10^9/L)			
1st apheresis	31.6 (7.8–106)	29.1 (12.6–62.3)	0.36
2nd apheresis	26.4 (13.5–50.5)	22.5 (15–44.8)	0.34
Percentage of CD34 cells (%)			
1st apheresis	0.12 (0.02–0.35)	0.10 (0.04–0.33)	0.35
2nd apheresis	0.12 (0.03–0.55)	0.10 (0.05–0.90)	0.86
CD34+ cells/kg (10^6)	3.15 (1.54–5.06)	2.01 (1.02–17.10)	0.07

TNC, total nucleated cells.

or the lenograstim arm ($n = 20$). The median age of patients was 33 (18–62) in the filgrastim arm and 24.5 (15–70) in the lenograstim arm ($P = 0.52$). Fifteen patients had hematologic malignancies and 5 patients had solid tumors in the filgrastim arm versus 12 and 8, respectively, in the lenograstim arm. All patients had received chemotherapy previously. The median number of chemotherapy cycles was 9 in the filgrastim arm and 10 in the lenograstim arm ($P = 0.92$). The number of patients with previous radiotherapy was three in the filgrastim arm and six in the lenograstim arm ($P = 0.45$).

Apheresis

Apheresis results are listed in detail in Table II. The median number of apheresis was 1 (range, 1–3) in the filgrastim arm and 2 (range, 1–3) in the lenograstim arm ($P = 0.56$). Mean 11 L of blood volumes were processed during these collections. Successful mobilization was achieved in 95% of patients in both cohorts. Successful mobilization was achieved with the first apheresis, in 10/20 (50%) of patients in the filgrastim arm versus in 9/20 (46%) patients in the lenograstim arm ($P = 0.35$) and with the second apheresis in 9/20 (45%) patients in the filgrastim arm versus 10/20 (50%) patients in the lenograstim arm ($P = 0.35$). The third apheresis was required only in one patient in both arms ($P = 1$). Those two patients who underwent the third apheresis were poor mobilizers and did not achieve the minimum target dose of 2×10^6 cells/kg.

The median duration of apheresis was 270 min after the first apheresis in both cohorts ($P = 0.57$) and was 240 min versus 285 min for the second apheresis in filgrastim and lenograstim arm, respectively ($P = 0.73$).

The total volume of apheresis product was 250 mL in both groups after the first apheresis ($P = 0.61$) and

TABLE III. Results of Engraftment and Posttransplant Supportive Requirements

	Filgrastim (median, range)	Lenograstim (median, range)	*P*
Leucocyte engraftment (day)	10 (9–17)	11 (9–15)	0.23
Platelet engraftment (day)	11 (9–25)	12 (6–15)	0.40
Growth factor use (day)	9 (4–16)	10 (0–15)	0.93
Parenteral antibiotic use (day)	9 (6–18)	11 (7–17)	0.14
Transfusion of erytrocyte suspension (unit)	2.5 (0–8)	2 (0–6)	0.83
Transfusion of platelet suspension (unit)	2 (0–6)	2 (0–4)	0.74

200 mL in the filgrastim arm versus 185 mL in the lenograstim after the second apheresis ($P = 0.85$).

The number of CD34+ cells in the first and the second apheresis were not statistically different ($P = 0.07$ and $P = 0.90$). The median number of CD34+ cells after mobilization was 3.15×10^6/kg (range, 1.54–5.06 $\times$ 10^6/kg) in the filgrastim arm versus 2.01×10^6/kg (range, 1.02–17.10 $\times$ 10^6/kg) in the lenograstim arm ($P = 0.07$) (Table II).

The WBC count on the apheresis day (31.6 in the filgrastim arm versus 29.1 in the lenograstim arm, $P = 0.36$ after the first apheresis and 26.4 in the filgrastim arm versus 22.5 in the lenograstim arm, $P = 0.34$ after the second apheresis), total nucleated cells (TNC) (7.40 in the filgrastim arm versus 6.29 in the lenograstim arm, $P = 0.38$ after the first apheresis and 5.04 in the filgrastim arm versus 4.25 in the lenograstim arm, $P = 0.62$ after the second apheresis), and the percentage of CD34+ cells (0.12 in the filgrastim arm versus 0.10 in the lenograstim arm, $P = 0.35$ after the first apheresis and 0.12 in the filgrastim arm versus 0.10 in the lenograstim arm, $P = 0.86$ after the second apheresis) showed no statistical differences between the cohorts. Although the difference was not statistically significant, the total number of CD34+ cells in the first apheresis and the number of CD34+ cells per kilogram were in favor of the filgrastim arm (2.02 versus 1.04, $P = 0.07$ and 3.15 versus 2.01, $P = 0.07$).

Results of engraftment and posttransplant supportive requirements

G-CSF was used for 9 days in the filgrastim arm and for 10 days in the lenograstim arm ($P = 0.93$). The median time to WBC recovery ($> 1 \times 10^9$/L) was 10 days (range, 9–17) in the filgrastim arm, and 11 days (range, 9–15) in the lenograstim arm ($P = 0.23$). The median time to platelet recovery ($\geq 20 \times 10^9$/L) was 11 days (range, 9–25) in the filgrastim arm and 12 days (range, 6–15) in the lenograstim arm ($P = 0.40$). The median number of days requiring parenteral antibiotic therapy was 9 days in filgrastim arm and 11 days in lenograstim arm ($P = 0.14$).

The median number of erythrocyte suspension transfusion was 2.5 in the filgrastim arm and 2 in the grastin lenograstim arm ($P = 0.83$). The median number of platelet transfusions was 2 in both cohorts ($P = 0.74$) (Table III).

Discussion

The main endpoint of this study was to compare filgrastim 10 μg/kg/day with lenograstim 7.5 μg/kg/day in patients undergoing HDC and autologous PBSC transplantation, in terms of PBSC mobilization, number of apheresis procedures required for a sufficient yield of CD34+ cell, and engraftment results. Our aim was to assess the efficacy of lenograstim at a dose 25% lower than the recommended dose for PBSC mobilization. To our knowledge, this is the first randomized trial comparing the mobilizing potency of these two G-CSF products at doses different from the recommended doses for mobilization in patients undergoing HDC and PBSC transplantation.

Our results in this study showed that lenograstim which is shown to be 27% more potent than filgrastim in vitro has an equivalent efficacy to filgrastim when it is used at a 25% lower dose for PBSC mobilization for autologous PBSC in transplantation setting. Although some randomized trials are conducted in healthy volunteers comparing these two G-CSF products, no enough data on the mobilization capacity of these products in patient populations are yet available.

de Arriba et al. conducted an in vivo prospective randomized study in 30 patients diagnosed with stage II–IV breast cancer, and compared the efficacy of bioequivalent doses-in terms of biological activity of lenograstim and filgrastim for mobilization of PBSCs (0.82 MU/kg/day lenograstim or 0.84 MU/kg/day filgrastim for 4 days or until completion of leukapheresis) [11]. The cohorts were well-balanced mostly in terms of previously given chemotherapy. When they compared the effect of the administration of the same number of IU of each product, they saw that 31% more filgrastim was needed to achieve the same result (8.4 μg/kg/day versus 6.4 μg/kg/day).

In another study, Schiødt et al. compared the efficacy of filgrastim and lenograstim in 44 patients with lymphoid malignancies. The study design was based on daily enumeration of CD34+ cells in peripheral blood, in leukapheresis product and in bone marrow progenitors after priming with either filgrastim or lenograstim [12]. However, this study was not well-balanced regarding the number of patients administered growth factor (10 patients were treated with lenograstim and 33 patients were treated with filgrastim on a dose of 10 μg/kg/day in each arm). Evaluation of blood CD34+ levels by flow-cytometry did not reveal significant differences during the mobilization and no difference was found between the two groups. Although in vitro studies have suggested that glycosylation confers higher potency to lenograstim, they did not found any differences in the number of CD34+ cells harvested by leukapheresis after administration of equal doses of growth factors.

The preliminary results from another similar study conducted in patients with hematological malignancies and breast cancer showed no superiority of glycosylated G-CSF over non-glycosylated G-CSF [13]. In our study, we presented all the numerical data for blood CD34+ cell concentration, percentage of CD34+ cells, CD34+ cells per kilogram, volumes collected, number of TNC collected at each apheresis, number of aphereses, and duration of aphereses. Compared to previous in vivo studies, these numerical data misleading in some of them, are very informative and should be accounted in the design of future randomized trials.

The basis of our study, which makes it different from previous published ones, is the use of a dose of lenograstim of 7.5 μg/kg/day which is 25% lower than the recommended 10 μg/kg/day dose for PBSC mobilization [2,9,14,15]. In other words, lenograstim which is shown as 27% more potent than filgrastim was used on a 25%

reduced dose compared to filgrastim. That means that we used less vials of lenograstim per patient during a mobilization period, which allowed us an economic benefit in terms of lenograstim vials necessary for a successful mobilization. Among 40 patients which were included in the study, the successful mobilization rate was 95% in both cohorts with a median apheresis number of one (range, 1–3). Moreover, the two cohorts were well-balanced in terms of disease characteristics, meaning that solid and hematologic malignancies including lymphomas and multiple myeloma were in similar proportion in both groups. The median number of previously given chemotherapy cycles or radiotherapy administration were also similar between the groups ($P = 0.92$ and $P = 0.45$). The similar properties of the groups in our study probably lead similar results of mobilization and apheresis. We showed that even on the reduced dose of lenograstim, both products yielded similar number of CD34+ cells/kg (3.5×10^6/kg versus 2.01×10^6/kg, $P = 0.07$) or total number of CD34+ cells (2.72×10^8 versus 1.58×10^8, $P = 0.48$).

In a prospective randomized study, Kopf et al. compared filgrastim versus lenograstim versus molgramostim plus chemotherapy to evaluate their ability to mobilize CD34+ cells in peripheral blood [16]. The investigators used the three myeloid growth factors at 5 μg/kg/day following administration of disease-specific chemotherapy. They used different mobilizing chemotherapy regimens according to tumor type, and they administered myeloid growth factor 24 hr after the last day of the mobilizing chemotherapy. The mobilization failure reported in their study was 20%, which is quite high and they speculated that this high rate may be related to the significant percentage of germ cell tumor (GCT) (26%) and NHL (13%) patients who could have a mobilization failure rate of about 20–30% [17,18]. We included in our trial 40% NHL and 10% GCT patients. Only 2 out of 40 patients failed to achieve 2×10^6 CD34+ cells/kg, these patients being heavily pretreated NHL patients, one in each arm.

Essentially, it has also been confirmed by previous studies that prior treatment influences negatively the ability to mobilize PBSCs [17]. In our study, all patients were treated previously with cytotoxic agents (9–10 cycles) and some of them had also received radiotherapy. Kopf et al. had 43% chemonaïve patients, a factor which does not affect negatively the success of the mobilization. Their reported higher median CD34+ cell yield may be related to the fact that 43% of their patients were chemonaïve. In fact, in regard to mobilizing PBSCs, these myeloid growth factors on the standard dosage were found as efficacious as each other and no statistically significant difference between CD34+ cell yield was found between the products [14,16].

Höglund et al. showed in a dose finding trial in healthy volunteers that lenograstim 10 μg/kg/day for 6 days mobilizes PBSCs more efficiently than 3.5 and 7.5 μg/kg/day and the average CD34+ cell number was higher with lenograstim administration. Lenograstim produced 103.6 cells/μg versus 82.2 cells/μg with filgrastim, meaning that, an additional 27% CD34+ cells were produced with lenogarstim [19].

In normal donors, three randomized trials indicated the superior specific activity of lenograstim when compared with the same dosage of filgrastim. Höglund et al. administered G-CSF at 10 μg/kg/day for 5 days and found a significant difference in favor of lenograstim for mobilization of both CD34+ cells and CFU-GM [14]. In a similar crossover study by Watts et al., filgrastim and lenograstim were administered at a dose of 5 μg/kg/day subcutaneously for 6 days [20]. The blood peak level of WBC and CFU-GM were significantly higher with lenograstim compared to filgrastim. Ings et al.'s study showed similar results to those previous ones [21].

On the contrast of these studies, Martino et al. compared the efficiency of filgrastim at 300 μg and lenograstim at 263 μg (87% of filgrastim dosage) in healthy donors [22]. In this study, the majority of patients (>60%) were harvested on Day 4 and the remaining patients were harvested on Day 5, 6, or 7 rather than commencing on a fixed day. Their study revealed no significant difference between the two groups in terms of either the absolute number of CD34+ cells collected or the number of CD34+ cells calculated per kilogram of donor weight.

We started apheresis on the 4th day in 50% of patients and the second apheresis was done in 45% of patients on the 5th day. One another apheresis was required in one patient in each group on the 6th day. Our study revealed no statistically significant difference in terms of the number of TNC ($P = 0.38$ and $P = 0.62$), total number of CD34+ cells ($P = 0.07$ and $P = 0.90$), WBC count ($P = 0.36$ and $P = 0.34$), percentage of CD34+ cells ($P = 0.35$ and $P = 0.86$), or the number of CD34+ cells per kilogram ($P = 0.07$).

Martino et al. emphasized that their results are not in line with literature data. However, the similarity between our study and theirs is probably due to the fact that we both used lenograstim at a lower dose than filgrastim. We think that this finding is important in regard to demonstartaing that lenograstim may be efficacious even at a lower dose than the standard recommended dosage which may provide an economic benefit when taking into consideration the total number of vials used per patient for each mobilization.

However, our study showed another important finding that is the trend to a higher number of aphereses per patient in the lenograstim arm (median 2 aphereses) versus the filgrastim arm (median 1 apheresis). Although this difference did not reach statistical significance ($P = 0.56$), the relatively low number of patients included is likely the reason for the lack of statistically significant differences. If this trend is confirmed, the cost of the additional apheresis procedures could outweigh the economic benefit of a lower dose of lenograstim and be more strenuous for the patient.

In conclusion, our study revealed that filgrastim 10 μg/kg/day and lenograstim 7.5 μg/kg/day resulted in successful mobilization of CD34+ cells in patients undergoing HDC and PBSC transplantation. In comparison to filgrastim, priming with lenograstim at 25% lower dose does not negatively affect the number of CD34+ stem cells harvested, or engraftment results. By this way, using lenograstim at 7.5 μg/kg/day instead of 10 μg/kg/day may achieve an economic benefit in regard to G-CSF requirement or number of vials needed for a successful mobilization and PSCT.

Patients and Methods

Patients

This was a single center trial. Forty consecutive patients were enrolled in the trial at the Bone Marrow Transplantation Unit of GATA (Gulhane Faculty of Medicine) between January 2005 and February 2006. Patients older than 15 years and younger than 70 years were eligible for the study. Patients with bone marrow involvement and diagnosis of leukemia were excluded. All patients had an Eastern Cooperative Oncology Group (ECOG) performance status ≤2, with adequate hepatic, cardiac, and renal function. The diagnoses were osteosarcoma ($n = 2$), germ cell tumor ($n = 4$), Ewing's sarcoma ($n = 3$), non-Hodgkin's lymphoma ($n = 16$), Hodgkin's disease ($n = 7$), multiple myeloma ($n = 4$), rhabdomyosarcoma ($n = 2$), medulloblastoma ($n = 1$), and angiosarcoma ($n = 1$). Miscellaneous chemotherapy regimens were administered to the patients for their diseases. Patients were prospectively randomized in two cohorts according to the G-CSF product administered: filgrastim at 10 μg/kg/day or lenograstim at 7.5 μg/kg/day.

The two cohorts were well balanced in terms of age, gender, body weight, body surface area, disease, prior administration of chemotherapy/radiotherapy, number of previous chemotherapy cycles, and total body irradiation as a conditioning regimen. Complete blood counts were monitored daily each morning prior every G-CSF administration. The study was approved by the local Ethic Committee. Written informed consent was obtained from all patients. Patient characteristics are shown in Table I.

PBSC mobilization

The patients received subcutaneous injection of either non-glycosylated rhG-CSF (filgrastim) at a total dose of 10 μg/kg/day (cohort I, *n*: 20) or glycosylated rhG-CSF (lenograstim), at a dose of 7.5 μg/kg/day (cohort II, *n*: 20). G-CSF administration was started 3 weeks after the last chemotherapy during a hospital stay and was given on a single dose at the same time in the morning for 4 consecutive days. Apheresis commenced on the 5th day. If the target CD34+ cell yield was not achieved, an additional injection of G-CSF was given on the 5th and, when necessary, on the 6th day, and a second and, if necessary, a third apheresis were done after administration of the last dose of G-CSF.

Apheresis

The methodology of PBSC harvest was identical in all patients. Venous access was obtained by a jugular catheter placed in the internal jugular vein, such that high flow rates were achieved. PBMCs were harvested with a continuous flow cell separator, COBE Spectra (COBE BCT, Aphaeresis System, Lakewood, CO, USA). For each leukapheresis, whole blood was processed at a flow rate of 50–70 mL/min. The duration of apheresis depended on the blood flow and the number of apheresis procedures depended on the number of CD34+ cells collected.

All leukapheresis products were processed; frozen and stored on the day of collection.

Enumeration of CD34+ cells and flow cytometric analysis

One milliliter of fresh sample was removed from each apheresis product and diluted 1:10 in autologous plasma and white blood cell (WBC) counts were determined. The apheresis samples were lysed (FACS lysis solution, Becton Dickinson, San Jose, CA) and incubated with anti-CD34 phycoerythrin (PE) and anti-CD45 HLe-1 fluorescein isothiocyanate (FITC); 1×10^6 cells were stained simultaneously with the phycoerythrin (PE)-conjugated CD34) (HPCA-2)-antibody and anti-CD45 FITC (Becton Dickinson). A FACSCalibur analyzer (Becton Dickinson) was used, and data acquisition was performed with FACSCalibur Cellquest software (Becton Dickinson). A total of 60,000 cells were acquired and enumeration of the CD34+ cells was performed using the gating strategies according to ISHAGE guidelines. CD34+ cell number was determined by multiplying WBC count in the apheresis product by the percentage of CD34+ cells. Results were presented as number of CD34+ cells per milliliter of leukapheresis product.

Engraftment

Leukocyte engraftment was considered the day when the WBC count was $\geq 1 \times 10^9$/L), and platelet engraftment was considered when platelet count was $\geq 20 \times 10^9$/L).

Statistical analysis

The clinical and laboratory data of the patients were analyzed according to standard statistical methods using the statistical package SPSS for Windows (SPSS 10.01, SPSS, Chicago, IL). Data are presented as median (range) or percentage. Differences in the clinical characteristics of patients were assessed by the Fisher's exact test, and differences between the cohorts in regard to duration of aphereses, number of aphereses, as well as CD34+ cell count were determined by the Kruskal-Wallis test and Mann-Whitney *U* test. A two-tailed *P* value less than 0.05 was considered statistically significant for all statistical calculations.

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