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Whole Blood Titanium Concentration after Limb Salvage Surgery with Three-Dimensional-Printed Ti6Al4V Implants

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Background: Three-dimensional (3D)-printed customized implants can be fabricated and utilized for all bones with massive bone defects. The main safety issues with 3D-printed implants made of Ti6Al4V alloy are related to the release of metal debris and residual powder. In this study, we investigated the perioperative titanium concentrations in whole blood and peri-implant fluid samples of patients who underwent limb salvage surgery with a 3D-printed Ti6Al4V implant.

Methods: Nineteen patients who underwent limb salvage surgery with 3D-printed Ti6Al4V implants were divided into two groups: the serial samples group and the follow-up group. To observe metal distribution and clearance in the body, serial samples of blood and peri-implant fluid from the surgical drain were prospectively collected for five patients in the serial samples group. For the remaining 14 patients who were followed up for more than a year, blood samples were collected only once.

Results: In the serial samples group, the mean baseline titanium concentration was 0.78 μg/L (range, 0.1–2.2 μg/L): 3 patients showed peak concentration before the third postoperative month, while 2 patients still showed an increasing pattern at this point. Total titanium mass in the surgical drain showed a wash-out phenomenon in a week, with a significant uniform decrease ($p = 0.04$). In 14 patients in the follow-up group, the mean titanium concentration in the whole blood was 10.8 μg/L (range, 0.3–36.6 μg/L). For the 14 patients with a long-term follow-up, the aluminum and vanadium concentrations were all negligible.

Conclusions: Whole blood titanium concentrations were higher after surgery using 3D-printed implants than after that using conventional orthopedic implants, but markedly lower than in patients with implant failure. None of the patients developed serious clinical adverse effects during follow-up.

Keywords: *Limb salvage surgery, Titanium, Concentration, 3D printing, Endoprosthesis*

Three-dimensional (3D)-printed, custom-made metal implants are a surgical option in orthopedic oncology for skeletal reconstruction after wide excision involving the bone tissue. Compared to traditional surgical reconstructive methods, such as enthoprosthesis, structural bone allograft, or recycled autograft, 3D-printed metal implants have a short clinical history.[1-3]

The titanium alloy Ti6Al4V is the most commonly used material for the construction of 3D-printed implants in practice.[2-4] Ti6Al4V is a widely used metal for the fabrication of conventional orthopedic implants. It is a 3D-printable metal, which is considered biocompatible because of its high osteointegration, resistance to corrosion,

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and modulus of elasticity similar to cortical bone.[5] The 3D printers used to fabricate Ti6Al4V implants in orthopedics are commonly powder-based, with an electron beam melting (EBM) or selective laser melting (SLM) energy source.[4,6] The diameters of the metal powders of Ti6Al4V for EBM and SLM 3D-printing range from 45–100 μm and 45–75 μm, respectively. One of the major concerns regarding orthopedic implants fabricated using powder-based 3D-printers is metal debris, either residual or related to wear. In EBM, the entire process occurs in vacuum following a preheating phase; EBM thus carries the advantage of a low thermal gradient, but unmelted powders can sinter to the surfaces of products.[7,8]

Regarding the biological aspect, sintered and unmelted powder leads to roughness of the external surfaces, resulting in two sides. The roughness of EBM products ranges from 20 to 50 μm in experimental results.[7,9] However, in clinical orthopedic papers, 3D-printed implants were mainly fabricated by EBM and utilized after post-processing, involving sanding and polishing without any stress-relieving processes. The surface roughness of implants has seldom been reported. The rough external surface of orthopedic implants provides advantages for cell proliferation and bone integration.[10-12] However, sintered and unmelted powders could be possible factors in crack initiation.[6-8,13,14]

Sintered and unmelted metal powders may cause a large amount of metal debris in patients who have undergone orthopedic surgeries using 3D-printed implants. The systemic adverse effects of the titanium ions and local adverse reactions to metal debris have not yet been established.[5] The titanium concentration in the whole blood is used to assess the junction performance of orthopedic implants, which includes implant wear and corrosion. A high concentration of titanium alloys in the whole blood indicates implant wear in conventional orthopedic implants.[5,15-17] Although titanium is known to be a metal with low toxicological effects, if the postoperative metal systemic concentration is too high, adverse effects may occur. It is possible that the baseline concentrations of the metal components of the 3D-printed implant may be higher than those of conventional orthopedic implants. However, the metal concentration and distribution of 3D-printed Ti6Al4V implants have not yet been reported.

The aims of this study were to quantify the level of increase of metal concentrations in blood and in peri-implant fluid collected from the surgical drain and observe the timing of clearance after surgery using 3D-printed Ti6Al4V implants.

METHODS

The study was approved by the Institutional Review Board of National Cancer Center (No. NCC2017-0129). Written informed consent was obtained from all patients.

Sample Collection

We prospectively enrolled 19 patients who had undergone limb salvage surgery with a 3D-printed Ti6Al4V implant. Patient demographics, laboratory test, and surgical information, including surgical site, size, weight, and surface area of the implants, were collected (Table 1). Eight of

Table 1. Characteristics of Patients and Surgeries

Characteristic	Value
Age (yr)	45.7 (13.2–67.8)
Sex (male:female)	9 : 10
Diagnosis	
Chondrosarcoma	7
Osteosarcoma	2
Ewing sarcoma	1
Other bone tumor	4
Metastatic carcinoma	3
Non-oncological causes	2
Location	
Pelvis	8
Femur	4
Tibia	1
Scapula	2
Humerus	4
Hybridization of impalnts	
3D-implant only	11
With hip arthroplasty	4
With shoulder arthroplasty	2
With intramedullary nailing	2
Follow-up (mo)	
SS group	7.0 (5.8–9.6)
LT group	26.7 (12.0–45.8)

Values are presented as mean (range) or number.
3D: three-dimensional, SS group: serial sample group, LT group: long-term follow-up group.

these had undergone limb salvage surgery with the hybridization of 3D-printed implants and conventional orthopedic implants. Assembly between metals was performed using bone cement in all patients. Of these 19 patients, serial samples of blood and postoperative surgical drain fluid were collected for 5 patients (serial sample group; SS group) and single blood samples were collected for 14 patients (long-term group; LT group). For the SS group, blood samples were collected preoperatively, 1 day, 3 days, 7 days, 14 days, 1 month, and 3 months postoperatively. For peri-implant fluid, samples from surgical drains were collected 1 day, 3 days, 7 days, and 14 days postoperatively. If all surgical drains were removed before 14 days, the collection of peri-implant fluid samples was terminated early. For the drain samples, the measured metal concentrations were diluted as the daily drain amounts were different for each day; therefore, the metal mass was calculated from the concentration and drain amount. For the LT group, single blood samples were collected for 14 patients who underwent limb salvage surgeries with 3D-printed Ti6Al4V implants at least 12 months (range, 12.0–45.8 months) after the index surgery. There were no cases of implant failure in either of the groups. All implants were fabricated by an EBM type 3D printer (ARCAM A1; General Electrics, Boston, MA ,USA) and were composed of Ti6Al4V extra-low interstitial powder (Fig. 1).

Metal Concentration Measurement

For the elemental analysis, the whole blood and surgical drain samples were collected in a 3 mL BD Vacutainer tube with a royal-blue cap (Trace element K2 EDTA; Becton, Dickinson and Company, Franklin Lakes, NJ, USA). The inner wall of the sampling tubes was coated with

Table 2. Operating Conditions Employed in ICP-MS

ElementXR ICP-MS	Parameter
Plasma forward power (W)	1,250
Argon flow rate (L/min)	
Plasma	16
Auxiliary	0.8
Nebulizer	1
Sample introduction system	
Nebulizer	PFA micronebulizer
Spray chamber	PFA Scott type
Carrier flow rate (mL/min)	1.08
Mass resolution	Medium-4,000
Isotope	^{27}Al, ^{47}Ti, ^{51}V

ICP-MS: inductively coupled plasma mass spectrometer, PFA: paraformaldehyde.

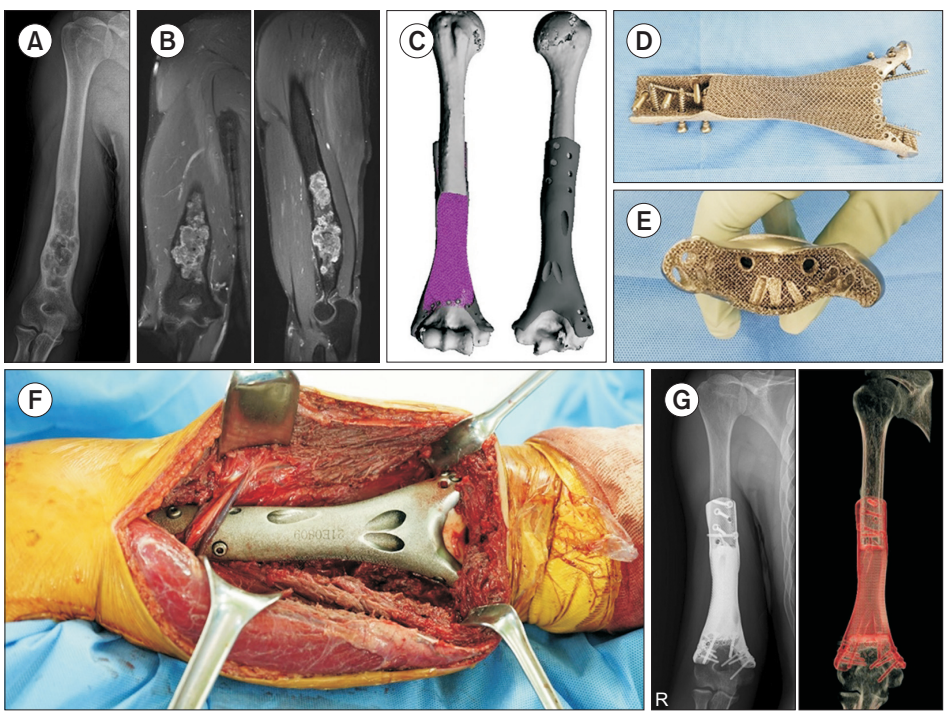

Fig. 1. Images of a patient who underwent limb salvage surgery using the three-dimensional (3D)-printed implant in the distal humerus. A preoperative plain radiograph (A) and T1-weighted gadolinium-enhanced magnetic resonance images (B) showing chondrosarcoma in the distal humerus, except in the elbow joint. (C) Graphical designs of the 3D-printed implant. Photographs of the anterior side (D) and the distal junction (E) of the implant. (F) Intraoperative photograph showing skeletal reconstruction. Postoperative plain radiograph (G, left) and computed tomography reconstruction image (G, right) showing humeral reconstruction.

spray-dried K3-ethylenediaminetetraacetic acid as an anticoagulant with calcium ions. First, 0.75 mL of the sample was moved to the 100 mL polytetrafluoroethylene vessel. Then, 2.25 mL nitric acid (HNO_3; Distill grade, TAMAPURE-AA-100, TAMA chemicals, Kawasaki, Japan) was added to the polytetrafluoroethylene vessel. The sample vessel was heated in a hot plate at 200 °C for 2 hours. The digested sample was then transferred to a 15 mL Falcon Conical tube (STEMCELL Technologies Inc., Vancouver, Canada) and diluted to a total volume of 15 mL with deionized water. All sample treatments were performed in a class 1,000 clean room.[17-19]

The sample solutions were measured by high-resolution inductively coupled plasma mass spectrometer (HR-ICP-MS; ElementXR, Thermo Fischer Scientific, Dreieich, Germany) for elemental analysis. To minimize isobaric and polyatomic interferences by Ca, S, N, O, etc., the HR-ICP-MS was tuned to obtain maximum and stable ion signals at medium resolution (R = 4,000). The instrument-operating conditions and data-acquisition parameters are presented in Table 2. The concentrations of the three constitutive elements (Ti, V, and Al) in the samples were quantified by external calibration using certified reference material solutions (AccuStandard, New Haven, CT, USA).

Table 3. Titanium Concentration in Serial Samples

Sample	Preoperative	1 day	3 day	7 day	14 day	1 mo	3 mo
Patient 1: pelvis type I + II + III							
Blood concentration (μg/L)	2.2	2.7	2.6	2.5	4.3	7.2	8.1
Drain							
Concentration (μg/L)	-	11.3	8.9	5.6	7.2	-	-
Mass (μg)	-	11.8	5.4	1.7	2.8	-	-
Patient 2: pelvis type I							
Blood concentration (μg/L)	1.2	0.6	< 0.1	0.5	2.3	2.6	2.0
Drain							
Concentration (μg/L)	-	31.4	46.3	10.8	-	-	-
Mass (μg)	-	9.1	0.7	0.6	-	-	-
Patient 3: scapula							
Blood concentration (μg/L)	< 0.1	0.8	< 0.1	5.9	5.6	3.7	2.9
Drain							
Concentration (μg/L)	-	38.6	65.2	88.0	-	-	-
Mass (μg)	-	4.6	2.0	0.9	-	-	-
Patient 4: proximal humerus							
Blood concentration (μg/L)	< 0.1	< 0.1	< 0.1	0.9	0.9	0.9	2.7
Drain							
Concentration (μg/L)	-	34.8	59.5	98.6	-	-	-
Mass (μg)	-	9.8	4.0	0.5	-	-	-
Patient 5: distal humerus							
Blood concentration (μg/L)	0.3	1.6	0.9	8.3	2.9	2.2	2.9
Drain							
Concentration (μg/L)	-	147.3	154.2	58.8	-	-	-
Mass (μg)	-	41.3	10.8	1.2	-	-	-

The coefficient of determination of the calibration curves of all elements was more than 0.999. A recovery test was performed with the spik method with blood matrix. The results of recovery ranged from 100% to 130%. The limit of quantitation of the concentration was 0.1 μg/L in blood and drain samples and 5 ng/L in the titanium dissolution test.

Statistical Analysis

Data are presented as means and ranges. The Wilcoxon signed-rank test was used to compare metal concentrations at the different sampling points for the SS group. For the LT group, Student *t*-test was used to compare metal concentrations to normal ranges. Mann-Whitney *U*-test and Spearman correlation analysis were performed to evaluate the relationship between metal concentration and possible affecting variables: age, surgical location, preoperative creatinine (Cr) level, implant volume, implant weight, and implant surface area. All statistical analyses were performed using IBM SPSS ver. 22.0 (IBM Corp., Armonk, NY, USA), and $p < 0.05$ was considered statistically significant.

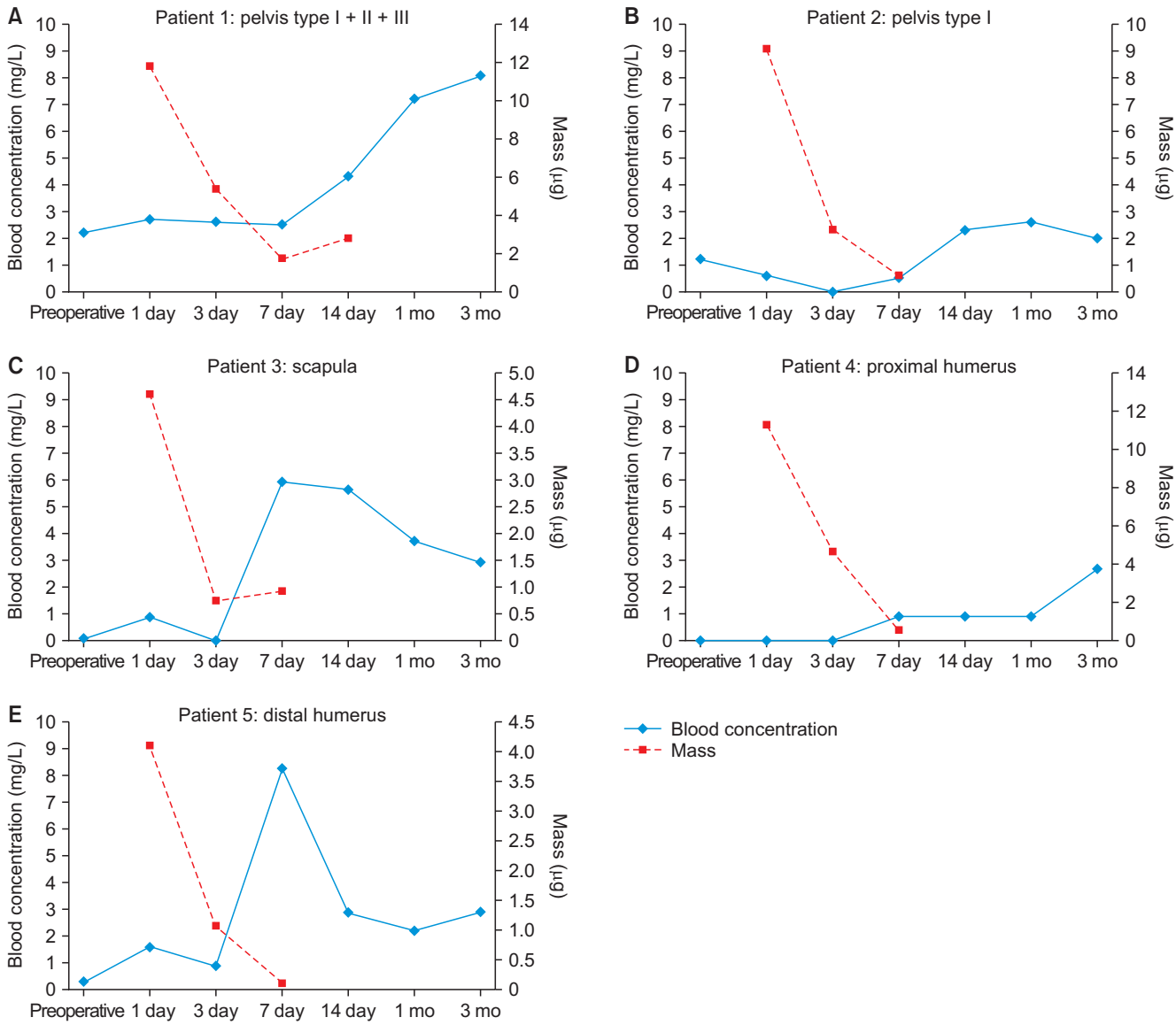

Fig. 2. Blood titanium concentrations (left standard [μg/L]) and the titanium mass within the surgical drain (right standard [μg]) over time of five different anatomical locations for each patient in the serial sample group. (A) Patient 1: pelvis type I + II + III. (B) Patient 2: pelvis type I. (C) Patient 3: scapula. (D) Patient 4: proximal humerus. (E) Patient 5: distal humerus.

RESULTS

In the 5 patients in the SS group, the mean baseline titanium concentration was 0.78 μg/L (range, 0.1–2.2 μg/L). The whole blood titanium concentrations were significantly elevated at 14 days postoperatively compared to baseline (p = 0.04). The mean concentrations were 3.20 μg/L, 3.32 μg/L, and 3.72 μg/L at 14 days, 1 month, and 3 months after surgery, respectively. The highest titanium concentration was 8.1 μg/L in 5.9 μg/L increments from the baseline in patient 1 who underwent a type I + II + III pelvic surgery in which the 3D-printed Ti6Al4V implant was used in combination with conventional total hip arthroplasty. The peak mass was measured at postoperative day 1 and the mean value in the surgical drain was 15.3 μg. The titanium mass showed a washout phenomenon in a week, with a significant uniform decrease (p = 0.04) (Table 3, Fig 2).

In 14 patients of the LT group, the mean titanium concentration in the whole blood was 10.8 μg/L (range, 0.3–36.6 μg/L). The only statistically significant factor was hybridization of the 3D-printed implant and conventional orthopedic implants (p = 0.02). Of 14 patients, 7 underwent limb salvage surgery using 3D-printed implants only, while the remaining 7 patients had surgery using 3D-printed implants with conventional orthopedic implants (5 arthroplasty and 2 intramedullary nails). Among the possible related factors analyzed, there were no significant differences found with respect to age, implant volume, weight, surface area, or preoperative Cr level in laboratory testing (Fig. 3). None of the patients experienced any adverse events, including any allergic reaction of skin or deep soft tissue around the implant, yellow nail syndrome, and elevated erythrocyte sedimentation rate/C-reactive protein or eosinophil count. Of note, the aluminum and vanadium concentrations were both negligible in the LT group.

When comparing titanium concentrations 3 months postoperatively in the SS group and at least 12 months postoperatively in the LT group, a significant increasing trend could be observed (p = 0.03). Of the 5 patients in the SS group, 3 showed a peak concentration before 3 months,

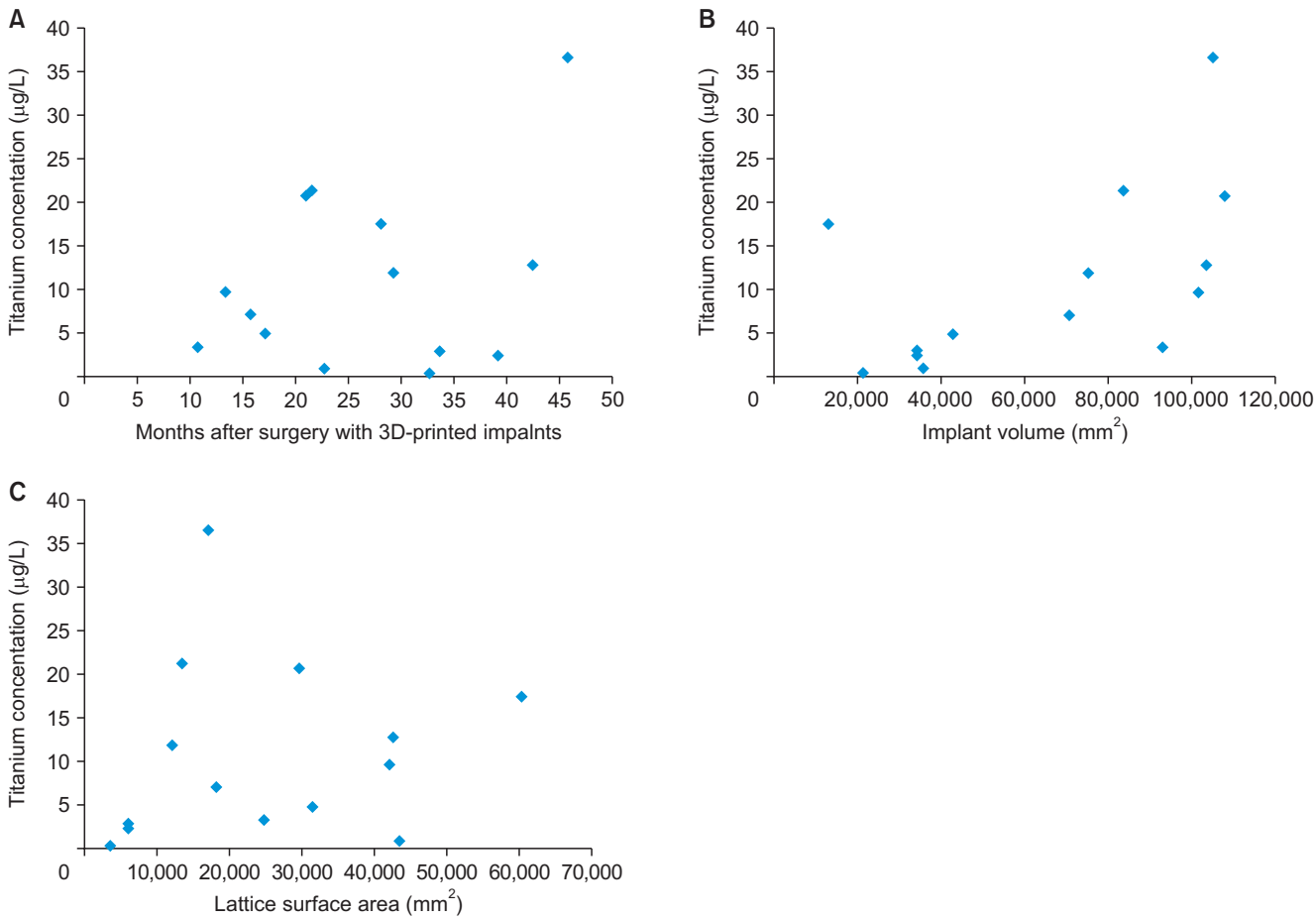

Fig. 3. Scatter plot of blood titanium concentrations by sampling timing after index surgery (A), implant volume (B), and lattice surface area (C). 3D: three-dimensional.

while the remaining 2 patients still showed a continuously increasing pattern at 3 months postoperatively.

DISCUSSION

3D-printed Ti6Al4V implants are utilized in limb salvage surgery in the field of orthopedic oncology. Although there are many advantages to 3D-printed implants, some safety issues still remain. One of these is the possibility that a higher amount of metal debris is eluted from the 3D-implants compared to conventional orthopedic implants. Although Ti6Al4V has a safe toxicological profile, if the leaked metal concentration becomes too high, adverse effects may occur. In addition to the toxicological effect, a high baseline concentration of titanium may mask its function as a marker of implant failure. In this study, the whole blood titanium concentration in patients who underwent 3D-printing implant surgery showed a significant elevation from 2 weeks after surgery and was not saturated even after 3 months postoperatively. The local titanium mass around the 3D implant showed a washout phenomenon from the surgical drains.

Blood titanium concentrations have been well studied in hip arthroplasty and spinal fusion.[5,15-17] Preoperative titanium concentrations of less than 2 μg/L were reported in several studies.[5,15] Postoperative titanium concentrations following well-functioning hip arthroplasty have been reported to range from 1 to 3 μg/L.[5,16,17] There were two studies with elevated titanium concentrations in well-functioning hip arthroplasties.[20,21] Omlor et al.[21] reported that the titanium concentration after bilateral hip arthroplasty was higher than that after unilateral surgery (3.0 μg/L vs. 6.0 μg/L), suggesting a cumulative effect. Bistolfi et al.[20] reported titanium concentrations after hip arthroplasty with EBM-type 3D-printed surface. There was no significant difference between conventional and 3D-printed cups, but the titanium concentration after 3D-printed cup implantation was 13.6 μg/L at 3 months and 11.7 μg/L at 1 year after surgery. In this study, postoperative blood titanium concentration after surgery using 3D-printed custom-made implants was comparable with previous studies, but higher than that using conventional orthopedic implants. The following hypotheses may explain the high blood titanium concentration in the patients with 3D-printed tumor implants. First, tumor prostheses involve large surface area exposure to both the bone and soft tissue. Therefore, a conventional tumor prosthesis is more appropriate as a control group for 3D-printed tumor prosthesis than conventional arthroplasty. Although the cumulative effect of the postoperative titanium concentration on the use of conventional implants has been reported,[21] no previous report has yet examined titanium concentrations following megaprosthesis with large surface area implantation for bone tumors. Second, metal-to-metal assembly using bone cement may cause micromotion between the metals, which results in a high metal concentration. In this study, the only significant factor related to blood titanium level was hybridization of conventional implants and 3D-printed implants. The combining of the two types of implants by bone cement seems to be one of the reasons explaining the increase in the titanium concentration. Third, the debris of 3D-printed Ti6Al4V implants is likely to be high, while the sintered unmelted powder shown to exist microscopically on the surface of the lattice structure in 3D-printed implants could also be a cause.

A high postoperative concentration of titanium in the whole blood indicates wear of a conventional orthopedic implant and has been well-studied in hip arthroplasty and spinal fusion.[5,15-17] When failure of hip or knee implants occurs, the titanium concentration can increase 10- to 100-fold, as analyzed using the HR-ICP-MS method.[21-24] Even in 3D-printed implants, such an increase can indicate implant wear. The titanium concentrations of well-functioning 3D-printed implants in this study were higher than those of well-functioning hip implants. However, according to our results, even in the case of 3D-printed implants, the mean titanium concentration in the whole blood was 10.8 μg/L; thus, the titanium concentration did not increase sufficiently postoperatively to mask the implant failure. There is no strict cutoff value for a titanium concentration suggestive of implant failure for 3D-printed implants. It appears that the cutoff concentration for limb salvage surgery using 3D-printed implants should be set higher than for conventional arthroplasty.

In the LT group, the titanium concentration was higher than in the SS group at 3 months. In addition, the titanium concentration continued to increase at 3 months in 2 of 5 patients in the SS group. Based on this result, it cannot be said that the titanium in the blood had reached saturation at 3 months postoperatively. In a previous study, the postoperative titanium concentration reached a peak at 3 years postoperatively and a tendency to decrease thereafter was reported after conventional total hip arthroplasty.[25] Therefore, a longer follow-up is required for 3D-printed implants to observe titanium clearance.

This study has several limitations. First, the number of patients was small, with 5 in the SS group and 14 in the LT group. Since the follow-up period was relatively short, the saturation period for titanium concentrations could not be accurately confirmed. Second, the size, shape, and

applied anatomical site of the 3D-printed implants were not uniform. Therefore, in this preliminary analysis, hypotheses other than a combination usage of 3D-printed and conventional implants have not been sufficiently proven statistically. Third, it was not possible to distinguish whether the increase in titanium concentration was due to the cumulative effect of the implant size or the 3D-printing method, as there was no proper control group of patients with megaprostheses. Moreover, the titanium source could not be presented in this paper because there were too many uncontrolled variables to suggest the mechanism.

From the surgical drain, the titanium mass showed washout phenomenon in a week; however, for whole blood, titanium concentration equilibrium was not reached until 3 months postoperatively. In previous literature, whole-blood titanium concentrations were higher after surgery using 3D-printed implants than after that using conventional orthopedic implants, but markedly lower than in patients with implant failure. Nevertheless, there were no patients with serious clinical adverse effects.

CONFLICT OF INTEREST

No potential conflict of interest relevant to this article was reported.

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