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Evaluation of the Quantification of Bone Ingrowth and the Influence of Stress Shieldings in Cementless Total Knee Arthroplasty: A Prospective Case \hat{a} \in Control Study

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GJMR-H Classification: NLMC Code: WE312

EVALUATIONOFTHEDUANTIFICATIONOFBONEINGROWTHANDTHEINFLUENCEDFSTRESSSHIELDINGSINCEMENTLESSTOTALKNEEARTHROPLASTYAPROSPECTIVECASECONTROLSTUDY

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Evaluation of the Quantification of Bone Ingrowth and the Influence of Stress Shieldings in Cementless Total Knee Arthroplasty: A Prospective Case †"Control Study

Takao Kaneko ^α, Takahiro Otani ^σ, Takahide Sunakawa ^ρ, Nobuhito Nango ^ω, Hiroyasu Ikegami [¥] & Yoshiro Musha [§]

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Results: There were significantly higher BMC/TV values for PS typethan CR type at ROI.2.4.6 (Lateral, Lateral-Anterior, Lateral-posterior) at 3.18.21 months postoperatively. There were not a significant difference in the relative change in BMC/TV values in ROI.1.3.5 (Materal, Materal-Anterior, Materal-Posterior).

Conclusions: The study indicated that PS type associated with the post-cam mechanism and midflextion instability was caused reactive cancellous stabilized and not occrred the influence of stress shieldings in lateral site under the peg of tibial component than CR typepost-operatively18 months later.

Level of evidence III-

Keywords: porous tantalum modular tibia cementless total knee arthroplasty analysis of three-dimensionally osteomorphometry bone mineral content/total volume (BMC/TV) values stress shielding.

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I. INTRODUCTION

emented total knee arthroplasty has been considered the accepted standard with predictable and durable results [3.4.8.9.10.16. 21.22.25.28.29]. Cementless total knee arthroplasty have induced preservation of bone stock, shoter operating time, ease of revision. Porous tantalum has been introduced as metallic implant material for total knee arthroplasty. The high volumetric porosity (70 \sim 80%), low modulas of elasticity (3~4 MPa), and high friction characteristics of trabecular metal make it conducive for biological fixation [17]. Many groups have reported satisfactory outcomes with cementless total knee arthroplasty using trabecular metal monoblock tibial components that contain porous tantalum as the primary material [5.9.14.18. 24.34]. All reports was used trabecular metal monoblock and radiostereometric results for stastical analysis. No manuscript were evaluated by imaging the postoperative computed tomography. We hypothesized that trabecular metal modular type (posterior stabilized type: PS type) is affected the influence of stress shielding with mid flextion instability. The aim of present study was to compare the bone ingrowth under the peg of trabecular metal modular tibial component between cruciate retaining type (cruciate retaining: CR type) and PS type.

II. MATERIAL AND METHODS

From October 2011 to April 2013, 46 primary total knee arthroplasties were perfomed in 46 patients with porous tantalum modular tibial component (Trabecular Metal; Zimmer, Warsaw, IN). We divided CR and PS type selectively. In all cases, the TKA surgical procedure was performed by one author (T. K.) and was minimally invasive surgery, with a skin incision of 8-11 cm. Patient walking was permitted from the day following the operation. The Knee Society Score (KSS) and The Western Ontario Mcmaster Universities Osteoarthritis Index (WOMAC) were measured preoperarively and up to two years postoperatively by two authors (T.K and T.O). Some case in which

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postoperative simple X-ray examination and, 3D Planning reposition simulation postoperative evaluation (ZedKnee: LEXI. Co., Ltd, Tokyo. Japan) showed malalignment and, cases was excluded from the analysis. We dicided that inclusion criteria was that all cases of tibial component alignment was within 3° varusvalgus to neutral alignment. For determination of the ossification density at 3,6,9,12,15,18,21 months postoperatively, a phantom(Taisho-Toyama Pharm. Co., Ltd., Tokyo. Japan) consisting of a cylinder composed of a material corresponding to cortical bone and filled with a material having a bone density corresponding to cancellous bone was placed under the knee (Fig. 1), and imaging was then performed by multi detector-row computed tomography (MDCT). From the obtained images, the cancellous trabecular structure was visualized three-dimensionally with 3D osteomorphometry software (TRI/3D-BON64; RATOC System Engineering Co., Ltd., Tokyo, Japan), and the structural parameters were calculated (Fig. 2). The structural subjected to analysis parameters were those recommended by the American Society for Bone and Mineral Research [19]: bone mineral content/total volume (BMC/TV) values, representing mineralized bone volume as a percentage of total volume. In the assessment of BMC/TV values by MDCT imaging of the proximal tibia, the joint prosthesis itself generated artifacts, which prevented accurate delineation. Therefore, the overall region was divided into 6 regions of interest (ROIs), consisting of two cylindrical volumes, each 16 mm in diameter and 8 mm in height, with their tops 0.6 mm below the medial or lateral peg, with each further divided into 2 half-cylinders. [ROI. 1 (Medial), ROI. 2 (Lateral), ROI. 3 (Medial-Anterior), ROI. 4 (Medial-Posterior), ROI. 5 (Lateral-Anterior), and ROI. 6 (Lateral-Posterior) (Fig.3). Statistical analysis(SPSS version 17.0 software: SPSS, Chicago, IL, USA) was performed for relative change in ossification density, immediately and after surgery every 3 months in each of the two groups by the Mann-Whitney U test and for comparison between the two groups by the paired t-test. P values of less than 0.05 were considered significant .This study of these patients was approved by the Institutional Review Board and they were informed of the risk of radiation exposure required

III. Results

No significant difference was recognized in age and Body mass index, gender, knee society score, between two groups before the operation (Table. 1).No osteoporosis therapeutic agent was administered in the two groups .There were no significant difference in KSS and WOMAC at 1-year follow-up between the two groups (Table. 2). No prosthetic fracture and prosthetic migration and prosthetic infection were detected during the follow-up periods. At 3.6.9.12.15.18.21 months after operation, the BMC/TV values in ROI. 1 (Medial) was no significant difference in the two groups (Fig .4). The BMC/TV values in ROI. 2 (Lateral) was significant higher in PS type than CR type at 3 and 18. 21 months after operation (p<0.01, p<0.05. Fig .5).

The BMC/TV values in ROI. 3 (medial- anterior) and ROI.4 (Medial- Posterior) was no significant difference in both group at all periods after operation (Fig. 6.7). The BMC/TV values in ROI.5 (Lateral-Anterior), and ROI.6 (Lateral-Posterior) was significant higher in PS type than CR type at 3.18.21 months after operation (p<0.01, p<0.05. Fig. 8.9).

IV. DISCUSSION

There were many manuscripts comparing stemmed cemented versus porous tantalum trabecular metal monoblock tibial component. No significant difference was recognized in KSS score WOMAC, radiographic results, complication and radiostereometric analysis migration between two groups [9.24.27]. Previous studies have shown a decrease in bone mineral density in the proximal part of the tibia after cemented total knee arthroplasty [20.26.31.32.33]. But the decrease in relative bone mineral density in the lateral part of the tibia was significantly less in the group treated with the porous tantalum monoblock tibial component than in the group treated with cemented tibial component up to five years after the operation [23]. Porous tantalum trabecular metal tibial component have been proposed to address looseing due to stress shieldings and breakdown of the cement mantle, in spite of first cementless tibial component includes looseing, particle migration through screw holes, and particle induced ostolysis[1.2.6.7.15.19]. Trabecular metal tibial component exists of monoblock type and modular type. The monoblock type consists of a porous tantalum ingrowth surface compression molded into it and two hexagonal porous tantalum pegs for initial stability. The modular type consists of a titanium alloy modular tray with a porous tantalum layer that also includes two hexagonal pegs and includes a central boss (small circular peg) in the central posterior of the tray that is used with a lock down screw [11]. Early migration for porous tantalum monoblock tibial component was not continue but soon stabilized [12]. Porous tantalum increased initial stability and accerated bone ingrowth and retented of bone stock through reduced stress shielding [20]. The flexibility of porous tantalum modular tibial component plate may produce radiolucencies at higher rate and it exhibited higher bone ingrowth than porous tantalum monoblock tibial component and implantation time was positively correlated with bone ingrowth for monoblock tibial components [13.30].We evaluated the bone mineral content/total volume (BMC/TV) values between CR and PS type of porous tantalum modular tibial component up to twenty one months. In the current study, No manuscript were evaluated the BMC/TV values of CR and PS type by imaging the postoperative computed tomography.

The relationship between CR and PS type had the same factor for postoperative activites and accuracy position of total knee arthroplasty. There were siginificantly higher BMC/TV values for PS type than CR type in ROI.2.4.6 (Lateral, Lateral-Anterior, Lateral-Posterior) at three and eighteen, twenty one months after operation. We did not find a significant difference in the relative change in BMC/TV values in ROI.1.3.5 (Materal, Materal-Anterior, Materal-Posterior) between PS and CR type postoperatively. The presents study suggests that PS type associated with the post-cam mechanism was caused reactively higher BMC/TV values than CR type, associated with bone sclerotic change in medial tibial plateau for medial knee osteoarthritis at 3 months. We discussed that trabecular metal modular tibia (PS type) with midflextion instability was caused reactive cancellous stabilized and not occurred the influence of stress shieldings in lateral site under peg of tibial component than CR type, postoperative 18 months later. The present study had several limitations that should be considered. First, this study was prospective study, but patients could not be randomized. Additional research is required to determine the long-term benefits of porous tantalum modular tibial component for CR and PS type. Second, there are a relatively small size with short term follow up. Thid, computed tomography was not perfomed before operation, furthermore BMC TV values was not measured in view of radiation exposure. In present study we recongnized that trabecular metal modular tibia (PS type) was not affected the influence of stress shieldings.in spite of than CR type, post-operative 18 months later to 21 months.

V. Conclusions

This study revealed that trabecular metal modular tibia (PS type) with midflextion instability was caused reactive cancellous stabilized and not occurred the influence of stress shielding in lateral site under peg of tibial component than CR type postoperative 18 months later.

VI. Acknowledgments

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Conflict of interest

The authors declare to conflict of interest.

References Références Referencias

 Berger RA, Lyon JH, Jacobs JJ, Barden RM, Berkson EM, Sheinkop MB, Rosenberg AG, Galante JO (2001) Problems with cementless total knee arthroplasty at 11years follow up. Clin Orthop Relat Res. 392: 196.

- Bloebaum RD, Bachus, KN. Jensen JW, Hofmann AA (1997) Postmortem analysis of consecutively retrived asymmetric porous-coated tibial components. J Arthroplasty 12 (8): 920.
- 3. Clarke HD, Cushner FD (2008) Monoblock tibial components in total knee arthroplasty. Tec Knee Surg 7: 115-125.
- 4. Diduch DR, Insall JN, Scott WN, Schudeli GR, Font-Rodriguez D (1997) Total knee replacement in young, active patients: long-term follow-up and functional outcome. J Bone Joint Am 79: 575-582.
- Dunbar MJ, Wilson DA, Hennigar AW (2009) Fixation of a trabecular metal knee arthroplasty component. A prospective randomized study. J Bone Joint Surg Am 91 : 1578-1586.
- Engh GA, Parks NL, Ammen DJ (1994) Tibial osteolysis in cementless total knee arthroplasty. A review of 25 cases treated with and without tibial component revision. Clin Orthop Relat Res 309: 33.
- Engh GA, Bobyn JD, Peterson TL (1998) Radiographic and histologic study of porous coated tibial component fixation in cementless total knee arthroplasty. Orthopedics 11(5); 725.
- 8. Forster MC (2003) Survival analysis of primary cemented total knee arthroplasty :which designs last ? J Arthroplasty 18: 265-279.
- 9. Ghalayini SR, Helm AT, McLauchlan GJ (2012) : Minimum 6year results of an uncemented trabecular metal tibial component in total knee arthroplasty. Knee 19 : 872-874.
- 10. Gill GS, Joshi AB (2001) Long-term results of Kinematic Condylar knee replacement :an analysis of 404 knees. Bone Joint Surg Br 83:355-358.
- 11. Henricson AK, Linder L, Niosson KG (2008)A trabecular metal tibial component in total knee replacement in patients younger than 60 years: a two-year radiostereophotogrammetric analysis.J Bone Joint Surg Br 90: 1585-1593.
- 12. Henricson AK, Gioe TJ, Simoneli C, Tatman PJ, Schoeller MC (2010) Do porous tantalum implants help preserve bone: Evaluation of tibial bone density surrounding tantalum tibial implants in TKA. Clin Orthop Relat Res 468. 2739-2745.
- Josa A, H, Judd S, D, Clare MR, Steven MK (2015) Ingrowth retrieval study group. Is there a difference in bone ingrowth in Modular versus monoblock porous tantalum tibial tray J Arthroplasty 30: 1073-1078.
- Kamath AF, Lee GC, Sheth NP, Nelson CL, Garino JP, Israelite CL (2011)Prospective results of uncemented tantalum monoblock tibia in total knee arthroplasty: minimum 5-year follow-up in patient younger than 55 years. J Arthroplasty 26: 1390-1395.

- Kim YH, Oh JH, Oh SH (1995) Osteolysis around cementless porous-coated anatomic knee knee prostheses. J Bone Joint Surg Br 77 (2); 236.
- Laskin RS. The Genesis total knee prosthesis (2001) a 10-year follow up study. Clin Orthop Relat Res 388: 95-102.
- 17. Levine B, Della Valle CJ, Jacobs JJ (2006) Applications of porous tantalum in total hip arthroplasty. J Am Acad Orthop Surg 14; 646.
- Levine BR, Sporer S, Poggie RA, Della Valle CL, Jacobs JJ (2006)Experimental and clinical performance of porous tantalum in orthopaedic surgery. Biomaterials 27: 4671-4681.
- Levitz CL. Lotke PA, Karp JS (1995) Long-term changes in bone mineral density following tibia after total knee arthroplasty. Clin Orthop Relat Res 321: 68.
- 20. Li MG, Nilsson KG (2000) Changes in bone mineral density at the proximal tibia after total knee arthroplasty. A 2-year follow-up of 28 knees using energy X-ray absorptiometry. J Orthop Res 18:40
- 21. Lombardi AV Jr, Berasi CC, Berend KR (2007) Evolution of tibial fixation in total knee arthroplasty. J Arthroplasty 22 (4 suppl 1): 25-29.
- 22. Milchteim C, Unger AS (2011) Cementless fixation in high performance knee design. Tec Knee surg 10: 136-142.
- Minoda Y, Kobayashi A, Ikebuchi M, Iwaki H, Inori F, Nakamura H (2013) Porous Tantalum tibial component prevents periprosthic loss of bone mineral density after total knee arthroplasty for five years-A matched cofort stydy. J Arthroplasty 28 : 1760-1764.
- 24. Niemelainen M, Skytta ET, Remes V (2014) Total knee arthroplasty with an uncemented trabecular metal tibial component: a registry-based analysis. J Arthroplasty 29(1):57.
- 25. Pavone V, Boettner F, Fickert S, Sculco TP (2001) Total condylar knee arthroplasty:a long-term follow up. Clin Orthop Relat Res 388: 18-25
- 26. Petersen MM, Nielsen PT, Lauritzen JB, Lund B (1995) Changes in bone mineral density of the proximal tibia after uncemented total knee

arthroplasty. A 3-year follow-up of 25 knees.Acta Orthop Scand 66; 513.

- 27. Pulido L, Abdel MP, Lewallen DG (2014). The Mark Coventry Award: Trabecular Metal Tibial Components Were Durable and Reliable in Primary Total Knee Arthroplasty: A Randomized Clinical Trial. Clin Orthop Relat Res [Epub ahead of print]. SYMPOSIUM : 2014 KNEE SOCIETY PROCEEDING.
- 28. Pavone V, Boettner F, Fickert S, Sculco TP (2001) Total condylar knee arthroplasty:a long-term follow up. Clin Orthop Relat Res 388:18-25.
- 29. Rodriguez JA, Bhende H, Renawat CS (2001) Total condylar knee replacement A 20-year followup study. Clin Orthop Relat Res 388:10:17
- Stilling M, Madsen F (2011) Superior fixation of pegged trabecular metal over screw-fixed pegged porous titanium fiber mesh: A randomized clinical RSA study on cementless tibialcomponents. Acta Orthop 82 (2): 177-186.
- 31. Wang CJ, Wang JW, Ko JY, Weng LH, Huang CC (2006) Three-year changes in bone mineral density around the knee after a six-month course of oral alendronate following total knee arthroplasty. A prospective randomized study. J Bone Joint Surg Am; 88; 267.
- 32. Wang CJ, Wang JW, Weng LH, Hsu CC, Huang CC, Chen HS (2003)The effect of alendronate on bone mineral density in the part of the femur and proximal part of the tibia after total knee arthroplasty. J Bone Joint Surg Am 85; 2121.
- Wegzyn J. Roux JP, Arlot ME, Boutroy S, Vilayphiou N, Guyen O, Delmas PD, Chapurlat R, Bouxsein L (2011) Determinants of Mechanical behavior of human lumbar vertebrae after simulated mild fracture. J Bone Miner Res 26(4):739-746.
- Wilson DA, Richardson G, Hennigar AW, Dunbar MJ (2012) Continued stabilization of trabecular metal tibial monoblock total knee arthroplasty components at 5 years-measured with radiostereometric analysis. Acta Orthop 83 : 36-40.

Subject preoperative data	CR type (n=23)	PS type (n=23)	P value
Age.mean \pm SD years	75.4 ± 5.2	76.1 ± 4.8	0.328
Sex (women/men)	22 / 1	22 / 1	>0.999
BMI,mean \pm SD kg/m ²	26.3 ± 2.2	25.2 ± 3.1	0.253
Knee Society Score Knee ± SD points Function ± SD points	51.2 ± 9.6 44.2± 7.2	48.6 ± 10.2 45.9 ± 8.4	0.321 0.289
Femoro-tibial angle ± SD degree	192.1 ± 9.2	190.9 ± 8.1	0.271

Table 1 : Pre-operative clinical data

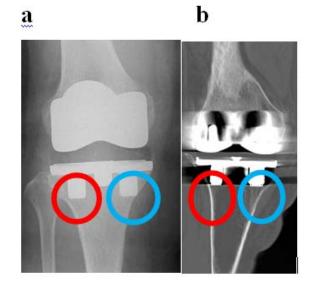
Subject preoperative data	CR type (n=23)	PS type (n=23)	P value
Knee Society Score Symptoms(25) Patient satisfaction(40) Patient expectation(15) Functional activities(100) ± SD points	$\begin{array}{c} 18.3 \pm 4.3 \\ 23.3 \pm 8.8 \\ 9.24 \pm 3.2 \\ 61.4 \pm 14.2 \end{array}$	$\begin{array}{c} 20.1 \pm 4.4 \\ 24.1 \pm 8.1 \\ 10.1 \pm 2.8 \\ 64.1 \pm 16.9 \end{array}$	0.328 0.420 0.364 0.348
WOMAC Score Pain(20) Stiffness(8) Daily activities ±SD points	$\begin{array}{c} 12.3 \pm 5.8 \\ 6.71 \pm 1.2 \\ 48.7 \pm 14.2 \end{array}$	11.6 ± 6.0 5.89 ± 1.8 47.3 ± 13.9	0.410 0.483 0.332
Hip-Knee- Ankle angle ±SD angle	178.2 ± 2.3	177.9 ± 1.7	0.509
Condylar-twist angle(CTA) ±SD angle	3.47 ± 1.9	3.59 ± 1.1	0.441

Table 2 : Post-operative clinical data at 1-year follow up



Fig. 1: The phantom(Taisho-Toyama Pharm. Co., Ltd, Tokyo. Japan) consisting of a cylinder composed of a material corresponding to cortical bone and filled with a material having a bone density corresponding to cancellous bone was placed under the knee.

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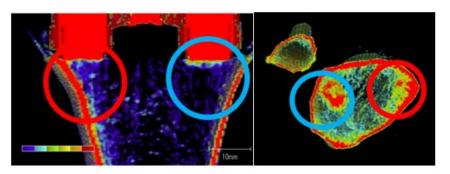


Fig. 2 : The measurement of bone mineral content/total volume (BMC/TV)v alues, representing mineralized bone volume as a percentage of total volume in ROI.1 (medial), ROI.2 (lateral)

- a. Postoperative radiograph using trabecular metal modulartibial component (CR type)
- b. Coronal plain CT image demonstrating
- c. Coronal 2D-MDCT image
- d. Axial 3D-MDCT image

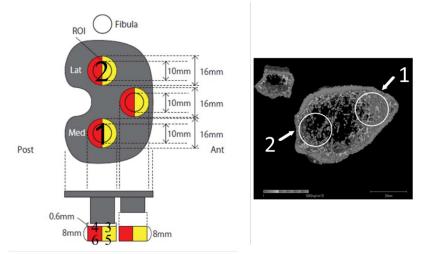


Fig. 3 : 6 Regions of Interest (ROI) under the peg of the tibial component. : Regions 1 (Medial) and 2 (Lateral), and 3 (Medial-Anterior), 4 (Medial-Posterior), 5 (Lateral-Anterior), and 6 (Lateral-Posterior)

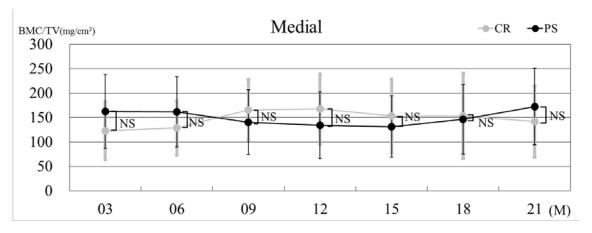


Fig. 4 : The relative change in bone mineral contents/total volume (BMC/TV)values in ROI .1 (Medial) was no significant difference in the two groups.

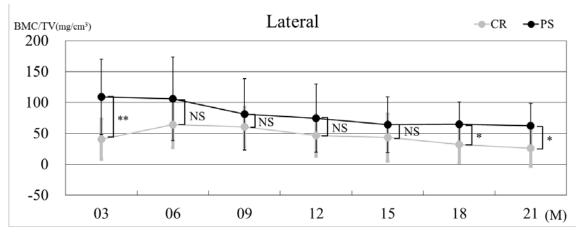


Fig. 5 : The relative change in BMC/TV values in ROI. 2 (Lateral) was significant higher in PS type than CR type at 3.18.21 months after operation (p<0.01**, p<0.05*).

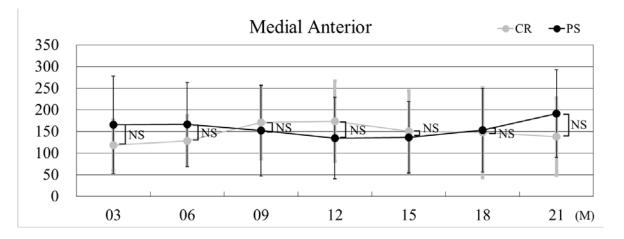
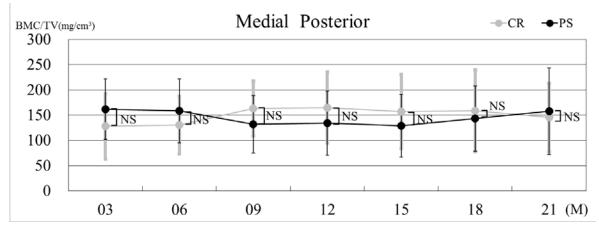
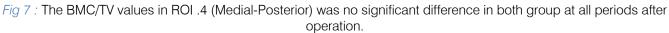


Fig 6 : The BMC/TV values in ROI .3 (Medial-Anterior) was no significant difference in both group at all periods after operation.





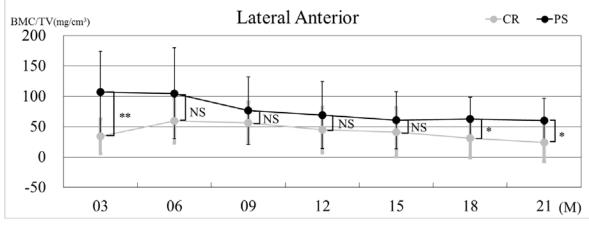


Fig. 8 : The relative change in BMC/TV values in ROI. 5 (Lateral-Anterior) was significant higher in PS type than CR type at 3.18.21 months after operation ($p<0.01^{**}$, $p<0.05^{*}$).

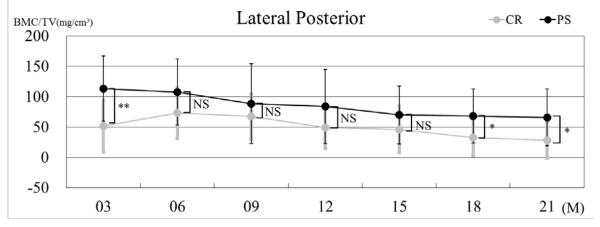


Fig. 9 : The relative change in BMC/TV values in ROI. 6 (Lateral-Posterior) was significant higher in PS type than CR type at 3.18.21 months after operation ($p<0.01^{**}$, $p<0.05^{*}$).