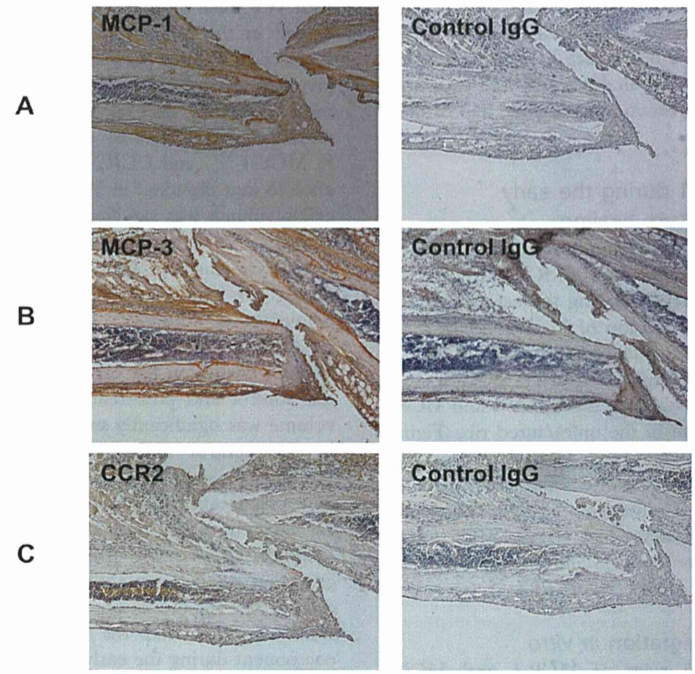


**Figure 2. Immunohistochemical analysis of WT rib fracture on day 3.** A, B, Protein expression levels of MCP-1 (**A**) and MCP-3 (**B**) were identified at the periosteum and endosteum on day 3. **C**, CCR2-positive cells were predominantly found within the bone marrow and surrounding tissues on day 3. Original magnification, 40×. Middle panel, high-magnification views (original magnification, 200×). The result is representative of three separate experiments.
doi:10.1371/journal.pone.0104954.g002

## Discussion

Our data highlight crucial roles of the MCP-1/CCR2 axis in the early phase of fracture healing. Compared with no fracture, the expression levels of many inflammatory chemokines increased on day 3 after fracture. In particular, *MCP-1* and *MCP-3* expression were temporarily up-regulated in the early phase of fracture healing (Figure 1). Then, we found that deletion of either MCP-1 or CCR2 caused delayed fracture healing (Figure 4), and that, blockade of CCR2 only in the early phase of healing caused delayed fracture healing (Figure 5). Taken together, these results

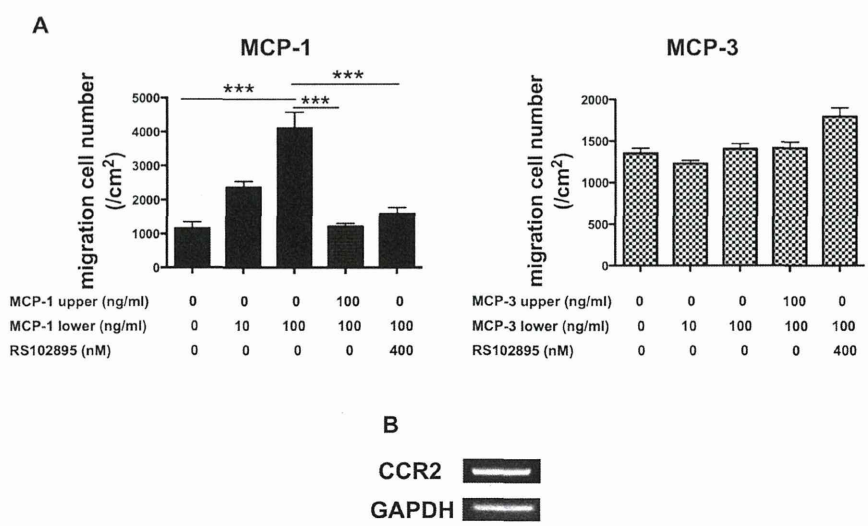


**Figure 3. Effect of MCP-1 on cell migration. A**: *In vitro* migration assay. mBMSCs were stimulated by MCP-1 or MCP-3 at indicated doses and RS102895 at 400 nM. Cells that migrated to the undersurface of the membrane were counted. Numbers of cells are represented as cell number per $cm^2$. Values are means ± SEM ($n=5$, respectively). **B**: Expression of *CCR2* mRNA in mBMSCs. Data are shown as means ± SEM. *$P<0.05$.
doi:10.1371/journal.pone.0104954.g003

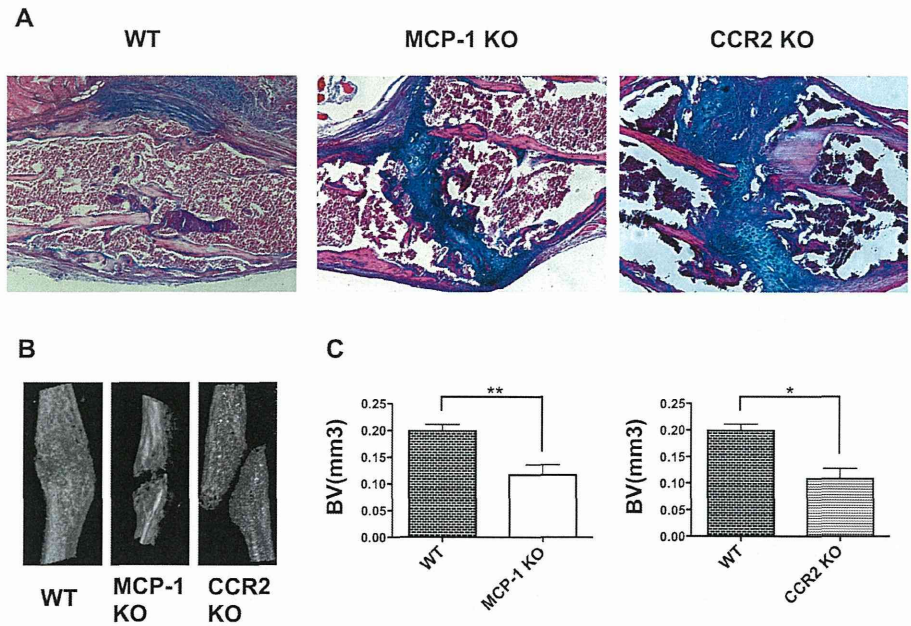


**Figure 4. MCP-1$^{-/-}$ and CCR2$^{-/-}$ mice displayed delayed fracture healing *in vivo*.** **A**: Histology of the fracture callus stained by hematoxylin-eosin/alcian-blue staining on day 21. **B**: Representative 3D micro-CT image of a fractured rib on day 21. **C**: Newly formed callus volume in the MCP-1$^{-/-}$ and CCR2$^{-/-}$ mice on day 21 was quantified using micro-CT.
doi:10.1371/journal.pone.0104954.g004

suggest that the temporary increase of MCP-1, MCP-3 and CCR2 expression in the early inflammatory phase may play a pivotal role for successful fracture healing. A recent study reported that deletion of CCR2 induces delayed fracture healing because of a decreased ability to resorb bone by osteoclasts in the remodeling phase [28]. The persistent cartilage fracture healing phenotype

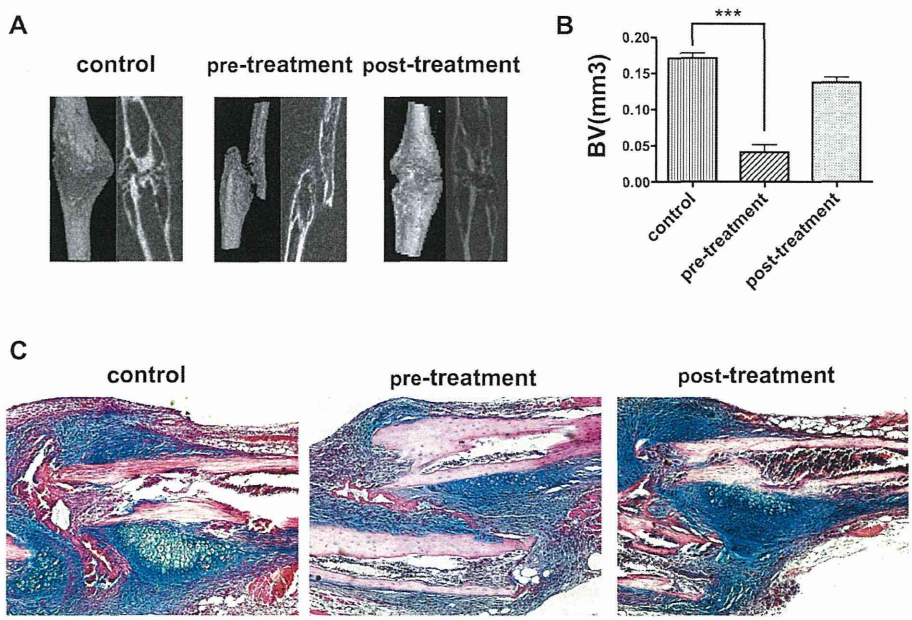


**Figure 5. Blockade of CCR2 in the early phase displayed delayed fracture healing *in vivo*.** **A**: WT mice received continuous administration of CCR2 antagonist, RS102895, or DMSO (controls) until day 12, beginning 2 days before or 4 days after rib fracture. In the control group, DMSO was administered as a control for 14 days. Representative micro-CT image of a fractured rib on day 21. **B**: Newly formed callus volume on day 21 in the pre-treatment or post-treatment group was quantified using micro-CT. **C**: Histology of the fracture callus stained by hematoxylin-eosin/alcian-blue staining on day 7.
doi:10.1371/journal.pone.0104954.g005

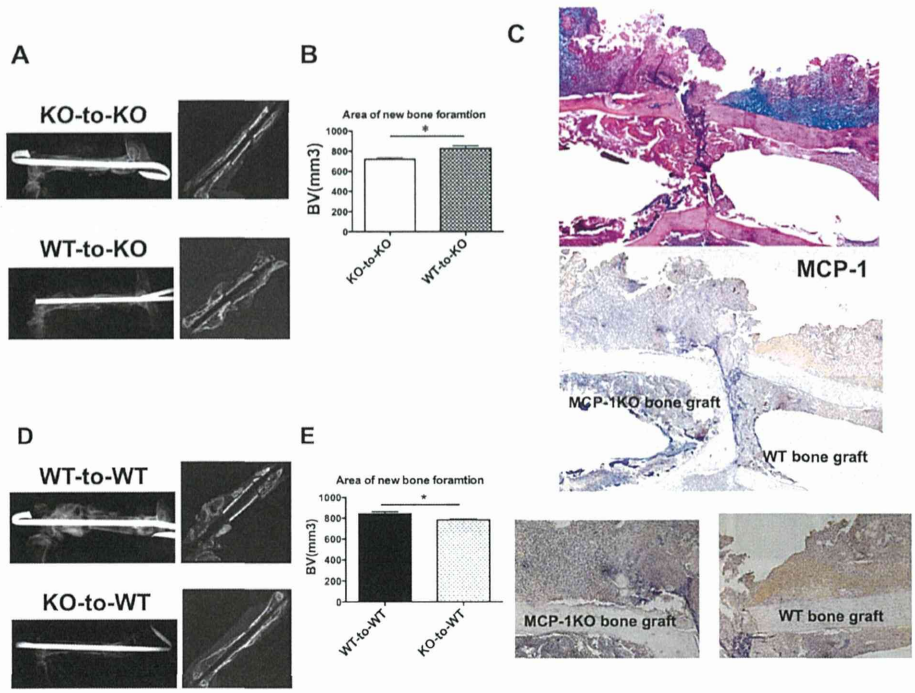


**Figure 6. Femoral segmental bone graft exchanging model.** Bone exchange surgeries were performed between WT and MCP-1$^{-/-}$ mice as described in the Methods section. Samples were harvested on day 21 for micro-CT and histological analyses. **A and B**, Representative micro-CT images and quantitative analyses demonstrate that WT mouse-derived bone graft caused a significant increase of new bone formation compared with KO mouse-derived bone graft. **C**, Immunohistochemical staining for MCP-1 in MCP-1$^{-/-}$ mice is shown. Pronounced MCP-1 expression at the periosteum and endosteum in the WT graft was observed in the host MCP-1$^{-/-}$ mouse. **D**, Representative micro-CT images and quantitative analyses demonstrate that MCP-1$^{-/-}$ mouse-derived bone graft caused a significant decrease of new bone formation compared with WT mouse-derived bone graft. Values are means ± SEM of more than three separate experiments. *$P<0.05$.
doi:10.1371/journal.pone.0104954.g006

could be caused by defects in chondroclast/osteoclast chemotaxis that delays vascular invasion, calcification and/or remodeling. However, MCP-1 expression is known to induce the early inflammatory phase [29], when osteoclasts do not play major roles in fracture healing. Consistent with the findings of previous studies, our data show that *MCP-1* and *MCP-3* mRNA were up-regulated on day 3 (Figure 1) and that localized MCP-1 and MCP-3 expression were increased in the periosteum and endosteum in the early phase of fracture healing (Figure 2A). This suggests that increased MCP-1 and MCP-3 expression in the early inflammatory phase may be essential for normal fracture healing.

MCP-1 and its receptor CCR2 are involved in recruitment of various cells, including leukocytes, BMSCs and hematopoietic stem cells [30–33], and in the regeneration of damaged tissues [34,35]. As established in earlier developmental studies, CCR2 is necessary for organ-specific homing of bone marrow-derived pluripotent mesenchymal stem cells into damaged tissues [36,37]. Consistent with this finding, our data showed that *CCR2* mRNA was induced in the mBMSCs derived from WT mice (Figure 3B). We also found that MCP-1, but not MCP-3, induced the migration of mBMSCs in a dose-dependent manner and that *in vitro* migration was markedly inhibited by a CCR2 antagonist (Figure 3A). Therefore, this axis may be a potent candidate in the development of stem/progenitor cell-based therapy for improving fracture healing.

We have previously demonstrated that SDF-1 is induced in the periosteum during bone injury and promotes endochondral bone repair by recruiting mesenchymal stem/progenitor cells to the site of injury. In the PCR array, an increased level of SDF-1 was not observed during fracture healing in this current study, especially in the early inflammatory phase. This inconsistency may be explained partly by the differences between the presence of the unimpaired bone marrow in simple fracture healing and bone graft healing with an intramedullary nail. Moreover, the previous study investigated allograft healing, in which the surgical site is greatly avascular, and under hypoxia this induces hypoxia-inducible factor-1 activation and subsequent SDF-1 up-regulation. Hence, we consider that the MCP-1/CCR2 axis is a crucial signaling pathway during the normal fracture healing process.

Previous studies have demonstrated that damage to the periosteum and bone marrow leads to impaired osteogenesis and chondrogenesis, and delays bone healing [38,39]. Thus, the periosteum and bone marrow seem to be important sources for recruiting mesenchymal stem/progenitor cells or osteogenic progenitor cells for promoting osteogenesis and chondrogenesis. In this study, we found that the expression of CCR2 increased transiently in the bone marrow in the early inflammatory phase and that the expression of MCP-1 also increased transiently in the periosteum and endosteum during the same period (Figure 2). We also found that WT mouse-derived bone graft markedly increased new bone formation and promoted successful fracture healing, whereas the MCP-1$^{-/-}$ mouse-derived bone graft caused less new bone formation and delayed fracture healing (Figure 6). Importantly, although other osteogenic factors were present at the

fracture site, they could not compensate for the lack of MCP-1. Collectively, these findings indicate clearly that increased MCP-1 expression in the periosteum and endosteum recruits CCR2-expressing cells and is essential for successful fracture healing.

This study has several limitations. First, we did not fully analyze the functions of other ligands for CCR2, such as MCP-3 and MCP-5, which may have roles different from those of MCP-1 in fracture healing. However, CCR2 KO mice showed similar impairment of bone healing compared with MCP-$1^{-/-}$ mice. Therefore, it is reasonable to consider that other ligands may also have similar functions. Second, the MCP-1/CCR2 axis may have a function other than the recruitment of progenitor cells in the early phase of fracture healing, such as promoting angiogenesis. Several studies report the role of the MCP-1/CCR2 axis in angiogenesis, but not in fracture healing. This point should be clarified in the future. Lastly, we did not elucidate the cell source(s) of mesenchymal stem/progenitor cells for fracture healing in this study. Recent reports, including ours, indicate the periosteum is the key source of potent cells [19,40], but this requires further investigation.

In conclusion, we have shown that increased expression of MCP-1 in the early phase plays a pivotal role in fracture healing by recruiting CCR2-expressing cells derived from surrounding tissues. The MCP-1/CCR2 axis is a potential target for achieving successful fracture healing. Further studies are needed to understand the functional relevance of the MCP-1/CCR2 axis in fracture healing.

## Supporting Information

**Figure S1 Immunohistochemical analysis of WT unfractured rib. A, B, C,** Low expression levels of MCP-1, MCP-3 and CCR2 were observed at the periosteum in the unfractured rib.
(TIFF)

**Figure S2 Effects of MCP-1 on osteogenesis, and chondrogenesis. A**: mBMSCs were cultured in osteoinduction media with or without MCP-1 for 14 days and stained with alizarin red S. The expression of each gene was analyzed by quantitative RT-PCR. ($n = 5$, respectively). **B**: ATDC5 cells were induced chondrocyte differentiation. MCP-1 (0, 20, 100 or 200 ng/ml) was simultaneously added every 2 days with the medium change. On days 28 after plating, cells were harvested, and the expression of each gene was analyzed by quantitative RT-PCR. ($n = 6$, respectively).
(TIFF)

**Figure S3 A, C: Histology of the fracture callus stained by hematoxylin-eosin/alcian-blue staining on day 7 (A) or day 21(C). B**: Histology of the fracture in MCP-1 or CCR2 KO stained by hematoxylin-eosin on day 25 (left panel) or 23 (right panel).
(TIFF)

## Acknowledgments

We are grateful to Drs. R. Tsutsumi, S. Tanida and K. Nishitani (Kyoto University Graduate School of Medicine) for their valuable technical assistance.

## Author Contributions

Conceived and designed the experiments: MI HI SM. Performed the experiments: MI TK KM HS KY TF. Analyzed the data: MI HI HY MF. Contributed reagents/materials/analysis tools: MI TK. Contributed to the writing of the manuscript: MI HI TK.

## References

1. Tsuboi M, Hasegawa Y, Suzuki S, Wingstrand H, Thorngren KG (2007) Mortality and mobility after hip fracture in Japan: a ten-year follow-up. J Bone Joint Surg Br 89: 461–466.
2. Bliuc D, Nguyen ND, Milch VE, Nguyen TV, Eisman JA, et al. (2009) Mortality risk associated with low-trauma osteoporotic fracture and subsequent fracture in men and women. JAMA 301: 513–521.
3. Griffin M, Iqbal SA, Bayat A (2011) Exploring the application of mesenchymal stem cells in bone repair and regeneration. J Bone Joint Surg Br 93: 427–434.
4. Hernigou P, Poignard A, Manicom O, Mathieu G, Rouard H (2005) The use of percutaneous autologous bone marrow transplantation in nonunion and avascular necrosis of bone. J Bone Joint Surg Br 87: 896–902.
5. Claes L, Recknagel S, Ignatius A (2012) Fracture healing under healthy and inflammatory conditions. Nat Rev Rheumatol 8: 133–143.
6. Vortkamp A, Pathi S, Peretti GM, Caruso EM, Zaleske DJ, et al. (1998) Recapitulation of signals regulating embryonic bone formation during postnatal growth and in fracture repair. Mech Dev 71: 65–76.
7. Glass GE, Chan JK, Freidin A, Feldmann M, Horwood NJ, et al. (2011) TNF-alpha promotes fracture repair by augmenting the recruitment and differentiation of muscle-derived stromal cells. Proc Natl Acad Sci U S A 108: 1585–1590.
8. Lange J, Sapozhnikova A, Lu C, Hu D, Li X, et al. (2010) Action of IL-1 beta during fracture healing. J Orthop Res 28: 778–784.
9. Dimitriou R, Tsiridis E, Giannoudis PV (2005) Current concepts of molecular aspects of bone healing. Injury 36: 1392–1404.
10. Mountziaris PM, Mikos AG (2008) Modulation of the inflammatory response for enhanced bone tissue regeneration. Tissue Eng Part B Rev 14: 179–186.
11. Sasaki M, Abe R, Fujita Y (2008) Mesenchymal stem cells are recruited into wounded skin and contribute to wound repair by transdifferentiation into multiple skin cell type. J Immunol 180: 2581–2587.
12. Schenk S, Mal N, Finan A, Zhang M, Kiedrowski M, et al. (2007) Monocyte chemotactic protein-3 is a myocardial mesenchymal stem cell homing factor. Stem Cells 25: 245–251.
13. Si Y, Tsou C, Croft K, Charo I (2010) CCR2 mediates hematopoietic stem and progenitor cell trafficking to sites of inflammation in mice. J Clin Invest 120: 1192–1203.
14. Docheva D, Haasters F, Schieker M (2008) Mesenchymal stem cells and their cell surface receptors. Curr Rheumatol Rev 4: 155–160.
15. Bielby R, Jones E, McGonagle D (2007) The role of mesenchymal stem cells in maintenance and repair of bone. Injury 38 Suppl 1: S26–32.
16. Augello A, Kurth TB, De Bari C (2010) Mesenchymal stem cells: a perspective from in vitro cultures to in vivo migration and niches. Eur Cell Mater 20: 121–133.
17. Caplan A (2009) Why are MSCs therapeutic? New data: new insight. J Pathol 217(2): 318–324.
18. Jones E, Yang X (2011) Mesenchymal stem cells and bone regeneration: current status. Injury 42: 562–568.
19. Ito H (2011) Chemokines in mesenchymal stem cell therapy for bone repair: a novel concept of recruiting mesenchymal stem cells and the possible cell sources. Mod Rheumatol 21: 113–121.
20. Granero-Moltó F, Weis JA, Miga MI, Landis B, Myers TJ, et al. (2009) Regenerative effects of transplanted mesenchymal stem cells in fracture healing. Stem Cells 27: 1887–1898.
21. Khosla S, Westendorf JJ, Mödder UI (2010) Concise review: Insights from normal bone remodeling and stem cell-based therapies for bone repair. Stem Cells 28: 2124–2128.
22. Kumar S, Wan C, Ramaswamy G, Clemens TL, Ponnazhagan S (2010) Mesenchymal stem cells expressing osteogenic and angiogenic factors synergistically enhance bone formation in a mouse model of segmental bone defect. Mol Ther 18: 1026–1034.
23. Kitaori T, Ito H, Schwarz EM, Tsutsumi R, Yoshitomi H, et al. (2009) Stromal cell-derived factor 1/CXCR4 signaling is critical for the recruitment of mesenchymal stem cells to the fracture site during skeletal repair in a mouse model. Arthritis Rheum 60: 813–823.
24. Ito H, Akiyama H, Shigeno C, Iyama K, Matsuoka H, et al. (1999) Hedgehog signaling molecules in bone marrow cells at the initial stage of fracture repair. Biochem Biophys Res Commun 262: 443–451.
25. Ito H, Koefoed M, Tiyapatanaputi P, Gromov K, Goater JJ, et al. (2005) Remodeling of cortical bone allografts mediated by adherent rAAV-RANKL and VEGF gene therapy. Nat Med 11: 291–297.
26. Murata K, Kitaori T, Oishi S, Watanabe N, Yoshitomi H, et al. (2012) Stromal cell-derived factor 1 regulates the actin organization of chondrocytes and chondrocyte hypertrophy. PLoS One 7: e37163.
27. Murata K, Ito H, Yoshitomi H, Yamamoto K, Fukuda A, et al. (2014) Inhibition of miR-92a enhances fracture healing via promoting angiogenesis in a model of stabilized fracture in young mice. J Bone Miner Res 29: 316–326.

28. Xing Z, Lu C, Hu D, Yu Y, Wang X, et al. (2010) Multiple roles for CCR2 during fracture healing. Dis Model Mech 3: 451–458.
29. Shireman PK, Contreras-Shannon V, Reyes-Reyna SM, Robinson SC, McManus LM (2006) MCP-1 parallels inflammatory and regenerative responses in ischemic muscle. J Surg Res 134: 145–157.
30. Qian BZ, Li J, Zhang H, Kitamura T, Zhang J, et al. (2011) CCL2 recruits inflammatory monocytes to facilitate breast-tumour metastasis. Nature 475: 222–225.
31. Zhang F, Tsai S, Kato K, Yamanouchi D, Wang C, et al. (2009) Transforming growth factor-beta promotes recruitment of bone marrow cells and bone marrow-derived mesenchymal stem cells through stimulation of MCP-1 production in vascular smooth muscle cells. J Biol Chem 284: 17564–17574.
32. Tsou C, Peters W, Si Y (2007) Critical roles for CCR2 and MCP-3 in monocyte mobilization from bone marrow and recruitment to inflammatory sites. J Clin Inv 117: 2–9.
33. Serbina NV, Pamer EG (2006) Monocyte emigration from bone marrow during bacterial infection requires signals mediated by chemokine receptor CCR2. Nat Immunol 7: 311–317.
34. Lu H, Huang D, Saederup N, Charo IF, Ransohoff RM, et al. (2011) Macrophages recruited via CCR2 produce insulin-like growth factor-1 to repair acute skeletal muscle injury. FASEB J 25: 358–369.
35. Shireman PK, Contreras-Shannon V, Ochoa O, Karia BP, Michalek JE, et al. (2007) MCP-1 deficiency causes altered inflammation with impaired skeletal muscle regeneration. J Leukoc Biol 81: 775–785.
36. Belema-Bedada F, Uchida S, Martire A, Kostin S, Braun T (2008) Efficient homing of multipotent adult mesenchymal stem cells depends on FROUNT-mediated clustering of CCR2. Cell Stem Cell 2: 566–575.
37. Van Linthout S, Stamm C, Schultheiss H-P, Tschöpe C (2011) Mesenchymal stem cells and inflammatory cardiomyopathy: cardiac homing and beyond. Cardiol Res Pract: ID 757154. DOI:10.4061/2011/757154
38. Colnot C (2009) Skeletal cell fate decisions within periosteum and bone marrow during bone regeneration. J Bone Miner Res 24: 274–282.
39. Utvåg SE, Grundnes O, Reikeraos O (1996) Effects of periosteal stripping on healing of segmental fractures in rats. J Orthop Trauma 10: 279–284.
40. Murao H, Yamamoto K, Matsuda S, Akiyama H (2013) Periosteal cells are a major source of soft callus in bone fracture. J Bone Miner Metab 31: 390–398.

Japan College of Rheumatology
MODERN RHEUMATOLOGY

http://informahealthcare.com/mor
ISSN 1439-7595 (print), 1439-7609 (online)

Mod Rheumatol, 2014; Early Online: 1–6
© 2014 Japan College of Rheumatology
DOI: 10.3109/14397595.2014.966975

informa
healthcare

ORIGINAL ARTICLE

# Delayed wound healing after forefoot surgery in patients with rheumatoid arthritis

Shinichiro Ishie[1], Hiromu Ito[1], Masayuki Azukizawa[1], Moritoshi Furu[1,2], Masahiro Ishikawa[1,2], Hiroko Ogino[1], Yosuke Hamamoto[1], and Shuichi Matsuda[1]

[1]*Department of Orthopadeic Surgery, Kyoto University Graduate School of Medicine, Sakyo, Kyoto, Japan and* [2]*Department of the Control for Rheumatic Diseases, Kyoto University Graduate School of Medicine, Sakyo, Kyoto, Japan*

**Abstract**

*Objective.* To elucidate the systemic and local risk factors and the effect of surgical procedures for delayed wound healing after forefoot surgery in patients with rheumatoid arthritis (RA).
*Methods.* Fifty forefoot surgeries were performed in 39 patients using resection arthroplasty or a joint-preserving procedure (25 feet for each procedure). The associations between the occurrence of delayed wound healing and clinical variables, radiological assessment, or surgical procedures were analyzed.
*Results.* Delayed wound healing was recorded in nine feet of eight patients. The duration of RA was significantly longer in the delayed healing group than that in the healed group. Age, sex, smoking history, concomitant diabetes, and RA medication did not differ between the groups. Radiological evaluation showed significant differences between groups in metatarsophalangeal dorsal flexion angle. The shortened length of the fourth and the fifth metatarsal bones affected the occurrence of the complication. The joint-preserving procedure had significantly less delayed wound healing compared with resection arthroplasty.
*Conclusions.* Preoperative dorsoplantar deformity and perioperative tissue damage can cause delayed wound healing after forefoot surgery in RA patients.

**Keywords**
Delayed wound healing, Forefoot surgery, Operative complication, Rheumatoid arthritis

**History**
Received 26 June 2014
Accepted 13 September 2014
Published online 3 November 2014



## Introduction

Rheumatoid arthritis (RA) is a systemic inflammatory disease that causes destruction of multiple joints. The combination of disease-modifying anti-rheumatic drugs and biological agents has markedly improved the disease status of patients with RA in the past two decades, but many patients experience continuous pain, severe deformity, and impaired activities of daily living.

There is a high prevalence of forefoot deformity of up to 90% in RA patients [1]. Synovitis and inflammation affect the metatarsophalangeal (MTP) joints and interphalangeal joints, leaving them with subluxation and dislocation deformities such as hallux valgus, hammer toes and claw toes. Rojas-Villarraga et al. showed that foot deformities are significantly associated with disability and disease activity risk. As the deformities progress, patients experience pain and find it difficult to walk; operative procedures are often required [2].

Numerous variations of many procedures to correct forefoot deformities have been reported, including resection of the metatarsal head (resection arthroplasty), shortening osteotomy (joint-preserving procedure), arthrodesis, and prosthetic joint replacement, all of which produce sufficient outcomes [3–6]. However, complications can occur after forefoot procedures. In a retrospective study, Bibbo et al. reported an overall complication rate of 32% and that wound-healing problems were most common followed by superficial infection, non-union, and delayed union [7]. Other studies have reported that wound-healing problems occur in 4–24% of patients [8–10]. However, Bibbo et al. found no significant differences between delayed healing and healed groups in the use of RA medications, age, sex, number of procedures per patient, or the presence of rheumatoid nodules [7]. To our knowledge, there is little information on the systemic risk factors for complications after forefoot procedures or how these procedures might cause such complications.

Based on our observed outcomes after forefoot procedures and an extensive search of the literature, we hypothesized that severe deformity would lead to wound-healing complications and that a joint-preserving procedure would lessen the risk. The aim of the present study was to identify the systemic, local, and surgical risk factors for wound-healing complications in forefoot surgery in patients with RA. We asked the following questions: 1) Does the patient's background or medication affect the risk of delayed wound healing? 2) Does the severity of the deformity affect the risk of delayed wound healing and, if so, which deformities increase the risk? 3) Does any perioperative change affect the risk? 4) Does a joint-preserving procedure lessen the risk?

## Materials and methods

Institutional review approval was obtained before starting the study. This retrospective cohort study included a consecutive series of patients with RA who underwent primary forefoot surgery between December 2005 and February 2013. Reoperations were excluded. A total of 50 forefoot surgeries in 39 patients were included. In this series of patients, no patient died or was lost to

Correspondence to: Hiromu Ito, MD, PhD, Department of Orthopaedic Surgery, Kyoto University Graduate School of Medicine, 54 Kawahara-cho, Shogoin, Sakyo, Kyoto 606-8507, Japan. E-mail: hiromu@kuhp.kyoto-u.ac.jp

follow-up at the last visit of evaluation. There were 37 women and 2 men, with a mean age of 61.4 ± 9.6 (43–78) years. All patients with RA fulfilled the 1987 diagnostic criteria of the American College of Rheumatology or the 2010 differentiation criteria of the European League Against Rheumatism/the American College of Rheumatology [11,12].

The operative indications were unresolved pain and/or intolerable disturbance in daily life caused by the forefoot deformity and radiological evidence of joint destruction and/or dislocation in any MTP joint after receiving a sufficient conservative treatment such as foot orthosis and/or physiotherapy. We explained both operative procedures described below, possible complications, and postoperative courses; each patient selected one of the two operations. We did not perform different operations in different toes in a single foot. Resection arthroplasty of the metatarsal heads was performed in 25 feet, and joint-preserving surgery was performed in 25 feet. In 8 feet given the joint-preserving surgery, only the hallux was involved; in the other 42 feet, surgery involved both the hallux and lesser toes. Resection arthroplasty was not performed solely on the hallux.

### Operative techniques

All operations were performed with the patient in the supine position with a thigh tourniquet under general anesthesia. In both the resection arthroplasty and joint-preserving surgery, we preoperatively evaluated the overlapping length between the metatarsal bone and the middle phalanx in the most dislocated toe using a dorsoplantar weight-bearing radiograph to decide on the amount of bone to be removed using a method described previously [6]. We then decided on the amount of resection on the other toes to make a smooth curve from the second to the fifth toe.

Resection arthroplasty was performed as described previously [13]. Briefly, in the resection arthroplasty of the hallux, we made a 3-cm longitudinal incision on the medial side of the first MTP joint, released the soft tissues, and opened the medial capsule in a V-shaped incision to reach the MTP joint. We then resected the metatarsal head to the predetermined cutting length to correct the deformity. In the surgery on the lesser toes, we made two 3-cm dorsal longitudinal incisions in the second and fourth intermetatarsal spaces, released the soft tissues (in cases of severe deformity, we cut the extensor tendon to correct the dislocation), and opened the dorsal capsule in a longitudinal incision to reach the MTP joint. We exposed the metatarsal head completely to correct the dislocation and then resected the metatarsal head to the predetermined cutting length.

The joint-preserving procedure was performed as described previously with some modifications [6,14]. Briefly, we made a 1.5-cm longitudinal incision on the dorsolateral side of the first MTP joint, released the soft tissues, and cut the transverse ligament, the insertion of the adductor hallucis, and the lateral side of the capsule to release the contracture. Then, we made a 3-cm longitudinal incision on the medial side of the first MTP joint, released the soft tissues, and opened the medial capsule using a longitudinal incision to reach the MTP joint and correct the hallux valgus deformity by releasing the contracture. We made another 5-cm longitudinal incision on the dorsolateral side of the first metatarsal bone and performed an oblique shortening osteotomy to the predetermined cutting length at the base in severe cases, or at the metatarsal neck in mild cases. On the lesser toes, we made two 3-cm dorsal longitudinal incisions in the second and the fourth intermetatarsal spaces as done in the resection arthroplasty. We released the soft tissues and performed an oblique shortening osteotomy of the stem of the metatarsals to the predetermined cutting length. For both the hallux and lesser toes, we used screws or Kirschner wire to fix the bones, according to the bone fragility, severity of the deformity, and the need for concomitant temporal fixation of the reduced MTP joint. Temporal Kirschner wire fixation (from the top of the toe to the metatarsal bone) and/or digit procedures (cutting the extensor tendon and corrective osteotomy in the PIP joint) were performed for severe deformities.

### Postoperative wound care and rehabilitation

Postoperatively, the operated foot was secured with a splint or an orthosis for 2–4 weeks. Physiotherapy was started the day after the operation, and the patient was allowed weight bearing at 1–4 weeks after the operation, dependent on the bone fragility, the operative procedure, and the patient's ability.

### Clinical outcome assessment

Data were collected retrospectively from medical records and radiographs. Two authors (SI and MA) who did not perform the operation recorded the patients' complete history from their medical charts and evaluated radiographs. Operation-related complications were also recorded, and we defined "delayed wound healing" as a wound that did not appear to follow the normal healing process over 21 days. The patients were divided into two groups: those whose wound healed well by 21 days after the operation (healed group; 31 patients, 41 feet) and those whose wound had not healed by 21 days after the operation (delayed healing group; 8 patients, 9 feet). The detailed clinical data include age at the time of operation, sex, duration of the disease, smoking history, comorbidities (diabetes mellitus and severe circulation disturbance in the feet), medication use (steroids, methotrexate, and/or biological agents at the time of the operation), radiological assessment, operative procedure (resection arthroplasty or joint-preserving procedure), and the number of toes processed (Table 1). The minimum length of follow-up was 1 year with a mean of 4.0 ± 2.0 (1.0–8.3) years.

### Radiological assessment

Standard dorsoplantar and lateral weight-bearing radiographs were assessed. All radiographs were analyzed by the two authors (SI and MA) who did not perform the operation. In the pre- and postoperative radiographs, we evaluated the Larsen grade [15], hallux valgus angle (HV angle), the first-to-the-second intermetatarsal angle (M1M2 angle), the first-to-the-fifth metatarsal angle (M1M5 angle), the length from the base of the second metatarsal bone to the tip of the second toe (forefoot length, Figure 1A), the length of overlap between the metatarsal and the proximal phalangeal bones (overlap length, Figure 1A), the length change of the metatarsal bone calculated by subtracting the postoperative length from the preoperative length (shortened length, Figure 1A and B) and MTP dorsal flexion angle (Figure 1C).

### Statistical analysis

Differences between groups were identified using the chi-square test or Fisher's exact test for dichotomous measures and the Wilcoxon signed-rank test for continuous measures. The differences in ratios between groups were evaluated using Fisher's exact test with Yate's correction. Significance was set at $p$ value of less than 0.05.

## Results

Of the 50 feet, delayed wound healing was recorded in 9 feet (18.0%). All of the complications occurred at the dorsal intermetatarsal incision: one occurred at the second intermetatarsal space only, one at the fourth intermetatarsal space only, and seven at both the second and fourth intermetatarsal spaces. Two of these had severe wound necrosis that required several months to heal.

Table 1. Clinical assessment of healed and delayed groups.

| Characteristic | Healed group, $N=41$ | Delayed group, $N=9$ | $P$ value |
|---|---|---|---|
| Age (SD) | 60.2 (9.5) | 67.1 (9.0) | 0.065 |
| Female ratio (%, n) | 97.6 (40) | 88.9 (8) | 0.330 |
| Disease duration in yr (SD) | 13.4 (7.3) | 21.3 (6.6) | 0.008† |
| Duration of follow-up in yr (SD) | 4.1 (2.1) | 3.1 (1.1) | 0.130 |
| Ratio of smoking history (%, n) | 12.2 (5) | 22.2 (2) | 0.595 |
| Ratio of diabetes (%, n) | 2.4 (1) | 0 (0) | 1.000 |
| Ratio of steroid intake (%, n) | 58.5 (24) | 55.6 (5) | 1.000 |
| The amount of steroids (SD) | 4.3 (2.3) | 6.5 (2.9) | 0.086 |
| Ratio of methotrexate intake (%, n) | 75.6 (31) | 44.4 (4) | 0.106 |
| The amount of methotrexate (SD) | 6.3 (2.1) | 6.5 (1.0) | 0.665 |
| Ratio of the use of biological agent (%, n) | 26.8 (11) | 22.2 (2) | 0.775 |
| The number of toes processed (SD) | 3.5 (1.7) | 4.8 (0.6) | 0.034† |

Values are given as mean (SD). $P$ values for the comparison between Healed group and Delayed group were calculated with the use of the chi-square test or Fisher's exact test for dichotomous measures and Wilcoxon signed-rank test for continuous measures.
†$P<0.05$ versus Healed group.



Non-union and severe circulatory disturbance occurred in one patient from each group. We found no deep infection, neurological deficit, or thromboembolism in the postoperative period in this series.

Clinical variables were compared between the two groups (Table 1). The duration of the disease was significantly longer in the delayed healing group ($p=0.008$). Patients in the delayed healing group tended to be older at the time of the operation, although this difference was not significant ($p=0.065$). The two groups did not differ significantly in the sex distribution ($p=0.330$), smoking history ($p=0.595$), presence of diabetes mellitus ($p=1.000$), or steroid intake ($p=1.000$), although there was a non-significant trend for steroid intake to be greater in the delayed healing group ($p=0.086$). The use of methotrexate ($p=0.106$) and its dosage ($p=0.665$), and the use of a biological agent ($p=0.775$) did not differ significantly between groups. Non-union was found in the second metatarsal bone in only one patient in the healed group. We found no severe circulatory disturbances in the feet of any patient. The number of processed toes was significantly higher in the delayed healing group than in the healed group ($p=0.034$).

Radiographic findings showed no difference in the preoperative HV ($p=0.428$), M1M2 ($p=0.569$), M1M5 angles ($p=0.135$), and Larsen grade ($p=0.179$, Table 2a). The preoperative MTP dorsal flexion angle was significantly smaller in the healed group ($p=0.016$, Table 2a).

There was no or little difference in the perioperative changes in the forefoot length ($p=0.919$) and MTP dislocation ratios ($p=0.699$) between the two groups (Table 2a). We then analyzed the shortened length of each metatarsal bone and compared these between the groups (Table 2b). The shortened length of the metatarsal bone was shorter in the healed group, especially for the fourth ($p=0.005$) and the fifth toes ($p=0.002$). In contrast, the preoperative overlap length of the dislocated MTP joint did not differ significantly between the two groups for any of the toes ($p=0.422$, Table 2a).

Finally, we assessed the effect of the two procedures on wound healing. Of the 25 feet that underwent the joint-preserving procedure, only 1 foot had delayed wound healing, whereas 8 of the 25 feet that underwent resection arthroplasty had delayed wound healing. This difference was significant ($p=0.002$, Figure 2), while the baseline characteristics were similar between the two groups except for the disease duration and the number of toes processed (Table 3).

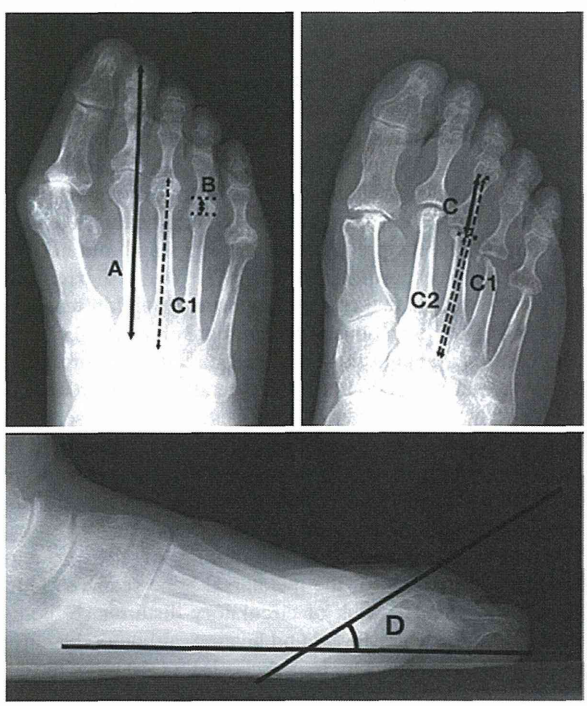


Figure 1. Pre- and postoperative radiological evaluation: a) Preoperative dorsoplantar weight-bearing radiograph. A; forefoot length, B; overlap length of the dislocated MTP joint, C1; preoperative length of the metatarsal bone. b) Postoperative dorsoplantar weight-bearing radiograph. C2; postoperative length of the metatarsal bone. C = C1–C2. c) Preoperative lateral weight-bearing radiograph. D; MTP dorsal flexion angle.

## Discussion

Wound-healing problems are more or less inevitable in all operative procedures. However, delayed wound healing is much more common in forefoot surgery for patients with RA than that in other surgeries or other disorders based on our experience and a thorough literature review [6–10,16]. Many factors can affect wound healing: local factors such as oxygenation and infection, and system factors such as age, sex, stress, medication, obesity, nutrition, alcohol, and smoking [17]. Patients with RA tend to have several risk factors, most notably steroid intake; however, we found that in addition to background factors, the presence of a preoperative deformity and surgical factors contributed to the development of delay in wound healing.

Because of RA itself and steroid intake, patients with RA have fragile skin subcutaneous tissues, and wound-healing problems occur more frequently in patients with RA compared with non-RA patients. This study showed that patients with a longer duration of the disease tend to have wound-healing complications after

Table 2a. Radiological assessment.

| | Healed group, N = 41 | Delayed group, N = 9 | P value |
|---|---|---|---|
| Larsen grade (I/II/III/IV/V) | 0/7/7/21/6 | 0/0/1/6/2 | 0.514 |
| HV angle (°) | 44.7 (14.6) | 42.1 (14.2) | 0.428 |
| M1M2 angle (°) | 16.1 (4.9) | 15.1 (4.2) | 0.569 |
| M1M5 angle (°) | 34.5 (5.80 | 31.1 (4.2) | 0.135 |
| MTP dorsal flexion angle (°) | 30.3 (17.4) | 45.4 (17.6) | 0.016† |
| Forefoot length (mm) | 101.4 (9.5) | 101.0 (10.7) | 0.919 |
| MTP dislocation rate (%, n) | 63.4 (26) | 77.8 (7) | 0.699 |
| Overlap length of the dislocated joint (mm) | 8.4 (4.4) | 9.4 (4.7) | 0.422 |

Values are given as mean ± SD. *P* values for the comparison between Healed group and Delayed group were calculated using Wilcoxon signed-rank test for continuous measures.
M1M2 angle: angle between the first and second metatarsals.
M1M5 angle: angle between the first and fifth metatarsals.
MTP: metatarsophalangeal
†$P < 0.05$ versus Healed group.

Table 2b. Shortened length of the metatarsal bone.

| | | Healed group | | Delayed group | | *P* value |
|---|---|---|---|---|---|---|
| | No. | No. | Shortened length | No. | Shortened length | Shortened length |
| 1st toe | 47 | 47 | 13.1 (6.4) | 0 | — | — |
| 2nd toe | 39 | 31 | 11.3 (5.0) | 8 | 13.6 (8.2) | 0.286 |
| 3rd toe | 37 | 29 | 11.8 (5.3) | 8 | 14.3 (6.6) | 0.183 |
| 4th toe | 32 | 25 | 12.4 (5.7) | 7 | 19.6 (4.8) | 0.005† |
| 5th toe | 32 | 25 | 12.0 (4.2) | 7 | 18.4 (3.0) | 0.002† |

Values are given as mean (SD). *P* values for the comparison between Healed group and Delayed group were calculated using the chi-square test or Fisher's exact test for dichotomous measures and Wilcoxon signed-rank test for continuous measures.
†$P < 0.05$ versus Healed group.



surgery. In addition, age and the amount of steroid intake were non-significantly higher in the delayed healing group than in the healed group. Use of methotrexate and biological agents did not differ between groups, indicating that these agents may be beneficial by improving both local and systemic conditions of RA patients. In a prospective comparative study, Bibbo and Goldberg showed that tumor necrosis factor-α inhibitory agents may be used safely in the perioperative period without increasing the risk of healing or infectious complications [18].

Another important finding in this study is that having a specific preoperative deformity contributes to delayed wound healing. Our results show that the HV, M1M2, and M1M5 angles did not influence wound healing. By contrast, the MTP dorsal flexion angle was significantly smaller in the healed group, suggesting that this may be a key variable for predicting wound-healing complications. These findings indicate that deformities in the dorsoplantar plane are more important than those in the mediolateral plane. This study also found that wound-healing complications are more likely to occur as the number of toes processed increases. This fact can be explained by the assumption that fewer toe deformities occur in the dorsoplantar plane and that more severe dorsoplantar deformities usually involve more toes. Taken together, these results suggest that preoperative dorsoplantar deformities should be monitored to prevent postoperative wound-healing complications. Interestingly, the preoperative overlap length did not differ significantly between groups, but the MTP dorsal flexion angle did. This observation suggests the importance of skin tension on wound-healing complications after forefoot surgery. This is probably because overlapping does not lead directly to shortening of the skin, whereas correction of hyperextended toes increases skin tension directly. The severity of joint destruction (Larsen grade) was not significantly associated with delayed wound healing; thus morphological changes, especially in the dorsoplantar plane, seem to contribute more to wound-healing complications via changes in skin tension rather than joint destruction.

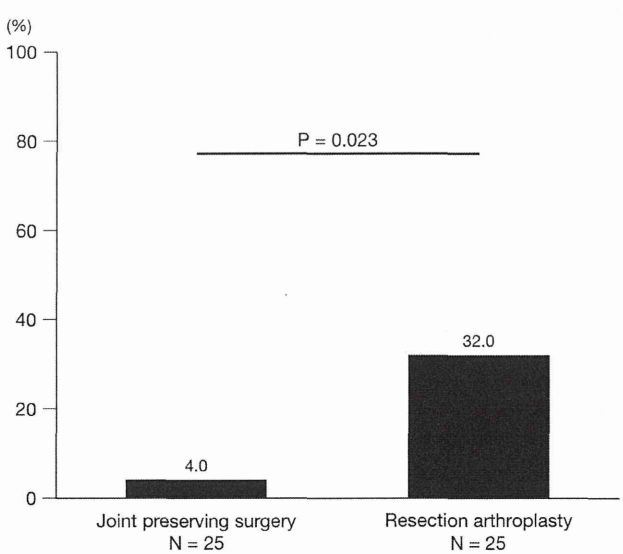


Figure 2. Assessment of the two procedures. The ratios of wound-healing complications are shown. $P = 0.002$ versus joint-preserving surgery.

Why does the perioperative shortening of the metatarsal bone matter? Shortening of the metatarsal bone seems to reduce the skin tension and the risk of circulatory disturbance, both of which appear to be better for wound healing, although the current results suggest otherwise. Our hypothesis is that the more the bone is shortened, the more tissue damage occurs. Shortening of the bone does not necessarily lead to shortening of the foot length or reducing skin tension. In our study, the forefeet length was similar pre- and postoperatively in the two groups. Conversely, a longer resection of the metatarsal head may be accompanied by tissue damage, which hinders the circulation because of postoperative swelling and may even damage blood vessels directly. Bibbo et al.

Table 3. Clinical assessment of Joint-preserving procedure and Resection arthroplasty groups.

| Characteristic | Joint-preserving procedure, $N = 25$ | Resection arthroplasty, $N = 25$ | $P$ value |
|---|---|---|---|
| Age (SD) | 59.3 (9.8) | 63.5 (9.2) | 0.145 |
| Female ratio (%, n) | 100.0 (25) | 92.0 (23) | 0.490 |
| Disease duration in yr (SD) | 11.5 (5.0) | 18.2 (8.5) | 0.006† |
| Duration of follow-up in yr (SD) | 3.7 (2.4) | 3.7 (1.5) | 0.786 |
| Ratio of smoking history (%, n) | 12.0 (3) | 16.0 (4) | 0.684 |
| Ratio of diabetes (%, n) | 4.0 (1) | 0 (0) | 1.000 |
| Ratio of steroid intake (%, n) | 52.0 (13) | 64.0 (16) | 0.567 |
| The amount of steroids (SD) | 4.2 (2.8) | 5.1 (2.3) | 0.342 |
| Ratio of methotrexate intake (%, n) | 68.0 (17) | 72.0 (18) | 1.000 |
| The amount of methotrexate (SD) | 5.8 (1.9) | 6.8 (2.1) | 0.138 |
| Ratio of the use of biological agent (%, n) | 28.0 (7) | 24.0 (6) | 1.000 |
| The number of toes processed (SD) | 3.0 (1.7) | 4.5 (1.3) | $<0.001$† |

Values are given as mean (SD). $P$ values for the comparison between Healed group and Delayed group were calculated using the chi-square test or Fisher's exact test for dichotomous measures and Wilcoxon signed-rank test for continuous measures.

†$P < 0.05$ versus joint-preserving procedure group.



reported that acute correction of shortened digits may stretch or induce trauma in the digital vessels, narrowing the lumen and causing local ischemia [9].

The same may be true of operative procedures. Resection arthroplasty involving resection of all of the cut bone may cause more damage to the circulation and may sacrifice surrounding structures. Joint-preserving procedure may be less likely to be accompanied with wound-healing complication, but it is not clear whether this complication would be dependent on procedures, surgical techniques, or background characteristics. Indeed, the significant differences in the background characteristics seen between Healed and Delayed groups (Table 1) were also observed in the comparison between joint-preserving procedure and resection arthroplasty groups (Table 3). At least, the current data suggest that resection arthroplasty should be done with the utmost caution to avoid damaging the surrounding tissues and thus to preserve the local circulation.

This study has several limitations. First, the number of patients was not sufficient for logistic multivariate statistics to identify and control for any critical confounding factors. For example, the duration of the disease may be related to the severity of deformity and more severe deformity may be associated with worse conditions such as circulatory disturbance. Second, the operative technique and perioperative care may affect the complication rate. A less-invasive procedure of resection arthroplasty may lessen the risk of delayed wound healing, as noted above. Wound care and postoperative physiotherapy also have certain effects on the risk of complications. The results obtained in this study may not be applied to the plantar approach. Third, radiological measurement may be evaluator dependent, although most of the measurements used in this study are well established in the literature. Finally, the operative decision depends on many other factors. In some cases, it may be impossible to apply joint-preserving procedures in some patients such as those with an extremely severe deformity or with a concomitant skin ulcer or local infection.

In summary, the current results show that preoperative and surgical factors affect wound healing after forefoot surgery in patients with RA. To avoid worrisome and time-consuming necrotic events, it is important to plan using a procedure as minimally invasive as possible to correct the deformity and to pay strict attention to avoid damaging the circulation surrounding the site during and after the operation.

## Acknowledgments

We thank Dr Hiroyuki Yoshitomi, the Center for Innovation in Immunoregulative Technology and Therapeutics, Kyoto University Graduate School of Medicine, for his valuable help. We also thank Professor Emeritus Takashi Nakamura, Department of Orthopaedic Surgery, Kyoto University Graduate School of Medicine, for his tremendous support for this manuscript.

## Conflict of interest

M.F. and M.I. are affiliated with a department that is supported financially by four pharmaceutical companies (Mitsubishi Tanabe Pharma Co., Bristol-Myers K.K., Chugai Pharmaceutical Co., Ltd., Eisai Co., Ltd.). H.I. has received grant and research support from Takeda Pharmaceutical Co., Ltd., Mitsubishi Tanabe Pharma Co., Chugai Pharmaceutical Co., Ltd., Pfizer Japan Inc., Astellas Pharma Inc., and Daiichi Sankyo Co., Ltd. M.F. has received grant and research support from Mitsubishi Tanabe Pharma Co., Astellas Pharma Inc., and Pfizer Japan Inc. S. M. has received grant and research support from Kyocera Medical Co., Ltd., Ishihara Sangyo Kaisha. Ltd., Asahi Kasei Parma Co. Ltd., Taisho Toyama Pharmaceutical Co. Ltd., Hisamitsu Pharmaceutical Co. Inc., Kaken Pharmaceutical Co. Ltd., Astellas Pharma Inc., MSD K.K., Eisai Co., Ltd., Pfizer Japan Inc., Teijin Pharma Ltd., Smith & Nephew Orthopaedics KK, Eli Lilly Japan K.K., Daiichi Sankyo Co., Ltd., and Zimmer Inc.; The sponsors were not involved in the study design; in the collection, analysis, interpretation of data; in the writing of this manuscript; or in the decision to submit the article for publication. The authors, their immediate families, and any research foundations with which they are affiliated have not received any financial payments or other benefits from any commercial entity related to the subject of this article.

## References

1. Trieb K. Management of the foot in rheumatoid arthritis. J Bone Joint Surg Br. 2005;87(9):1171–7.
2. Rojas-Villarrage A, Bayona J, Zuluaga N, Mejia S, Hincapie ME, Anaya JM. The impact of rheumatoid foot on disability in Colombian patients with rheumatoid arthritis. BMC Musculoskelet Disord. 2009;10:67.
3. Hulse N, Thomas AM. Metatarsal head resection in the rheumatoid foot: 5-year follow-up with and without resection of the first metatarsal head. J Foot Ankle Surg. 2006;45(2):107–12.
4. Coughlin MJ. Rheumatoid forefoot reconstruction. A long-term follow-up study. J Bone Joint Surg Am. 2000;82(3):322–41.
5. Hanyu T, Yamazaki K, Ishikawa H, Arai K, Tohyama CT, Nakazono K, Murasawa A. Flexible hinge toe implant arthroplasty for rheumatoid arthritis of the first metatarsophalangeal joint: long-term results. J Orthop Sci. 2001;6(2):141–7.
6. Niki H, Hirano T, Okada H, Beppu M. Combination joint-preserving surgery for forefoot deformity in patients with rheumatoid arthritis. J Bone Joint Surg Br. 2010;92(3):380–6.
7. Bibbo C, Anderson RB, Davis WH, Norton J. The influence of rheumatoid chemotherapy, age, and presence of rheumatoid nodules on postoperative complications in rheumatoid foot and ankle surgery: analysis of 725 procedures in 104 patient. Foot Ankle Int 2003;24(1):40–4.