Table V. Review of the literature.

Tumor location	No. of patients	Follow-up (years)	Overall survival rate (%)			Prosthesis survival rate (%)			Functional score (%)	Complications rate (%)	Revision rate (%)	Amputation rate (%)
			3-year	5-year	10-year	3-year	5-year	10-year				
Carty *et al* (15)												
[a]DF and [b]PT	20	7.5							83			
Tunn *et al* (21)												
DF	41	6.5							87		31	
PT	27								77			
Myers *et al* (19)												
PT	194										28	18
Biau *et al* (14)												
DF and PT	96	5.1					80	52		58		
Torbert *et al* (20)												
DF	57	4.7				90	84	66				
PT	27					63	63	63				
Bickels *et al* (13)												
DF	110						93	88			14	4
Ilyas *et al* (18)												
PT	15	3.5							61	73	0	13
Grimer *et al* (16)												
PT	151	6.7							77		63% at 10-year	17
Horowitz *et al* (17)												
DF	61	5.5		75	67		59	28				12% at 5-year
PT	16			93	93		54	36				22% at 5-year
Present report												
DF	45	8.0	69	63	63	81	73	73	81	38	24	0
Proximal lower leg	18		100	91	91	84	75	50	82	61	33	0

[a]DF, distal femur; [b]PT, proximal tibia.

survival rate was 62 and 52% in the subjects with tumors of the distal femur and the proximal leg, respectively. The 5-year prosthetic survival rate was 73% in the distal femur and 75% in the proximal lower leg. The mean functional score according to the scoring system of the MSTS score was 81% in the patients with distal femur tumors and 82% in the patients with proximal lower leg tumors. Post-operative complications occurred in 27 patients (43%), but no limb amputation was performed.

Both the distal femur and proximal tibia are common anatomic sites for primary and metastatic bone tumors. These tumors were traditionally treated with arthrodesis or amputation of the affected extremity and resulted in unfavorable functional and psychologic outcomes (9,10). However, the prognosis of the sarcoma patients has recently improved as a result of a better understanding of tumor biology, refined chemotherapeutic protocols, advances in diagnostic imaging and improvements in surgical techniques. Improvements in the survival of sarcoma patients made these drawbacks not only more pronounced, but also promoted the development of new surgical reconstructive procedures which promised more useful limb function. Simon *et al* compared the results of limb-sparing resections to those of amputation in 227 patients with distal femoral osteosarcoma, and concluded that the limb salvage surgery did not shorten the disease-free interval or compromise the long-term survival of these patients compared to amputation (11).

Although amputation still remains one of the important options for patients whose tumors are too advanced to obtain a safe margin, amputation has a negative impact on the patient's psychological well-being. With regard to arthrodesis, it is not suitable for many Asian patients, because squatting is necessary in many components of the Asian lifestyle, such as eating meals while sitting on the floor. Rotationplasty provides good local disease control and good function, but the cosmetic outcome is sometimes not acceptable for patients, especially younger patients. Harris *et al* also evaluated the results after amputation, arthrodesis and endoprosthetic replacement. They found that only patients with prosthetic reconstruction felt themselves to be close to the healthy population (12). We therefore have pursued limb salvage surgery using a prosthesis as the first choice for resectable malignant tumors occurring around the knee.

In the distal femur, the 3-, 5- and 10-year prosthesis survival rates were 85, 73 and 68%, respectively. In the proximal lower leg, the 3-, 5- and 10-year prosthesis survival rates were 92, 75 and 50%, respectively. In previous studies, the prosthesis survival of the proximal tibia has been reported to be lower than that of the distal femur (Table V) (13-21). Poor soft tissue coverage, difficulties with anchoring the patellar tendon and possible injuries to the neurovascular system are the most likely causes for this difference. Horowitz *et al* reported that distal femur prostheses showed 75 and 67% survival, while proximal tibia prostheses showed 54 and 36% survival at 5 and 10 years, thus indicating that there were significant differences in the prosthetic survival between distal femur and proximal tibia prostheses (17). Biau *et al* reported prosthesis 5- and 10-year survival rates of 85 and 55% in the distal femur, respectively, while they were 72 and 45% in the proximal tibia (14). In contrast, Torbert *et al* showed no statistically significant differences in the event-free prosthetic survival rate between the distal femur and proximal tibia prosthesis (20). Distal femur prostheses showed 90, 84 and 66% survival rate at 3-, 5- and 10-year, respectively, while proximal tibia prostheses showed 63% survival rates at 3-, 5- and 10-year. Direct comparisons of the present results to other published reports may be difficult due to the heterogeneity with respect to the patient population and implant used. In our series, log-rank testing showed no significant differences between the prosthetic survival and tumor location. Although the present study consisted of a relatively small number of patients with proximal lower leg tumors, there was no aseptic loosening except for one patient who underwent prosthetic reconstruction before 1987. The HMRS has a porous-coated stem, and permits bone ingrowth at the bone/prosthesis junction. Thus, this prosthetic design might reduce the risk of mechanical failure and loosening, as well as the improvement of the surgical technique.

The rate of infection was 4.8% in our series, and ranged from 2.2-33% in previous studies (13,16,19). Infections required secondary procedures. But the consequences nonetheless were noteworthy, with no limb amputation. Infections are difficult to avoid in prosthetic limb salvage surgery for numerous reasons: multiple surgeries, resection of large amounts of tissue, skin sloughing, adjuvant treatments and poor blood supply to the allograft. Providing adequate soft tissue coverage after reconstruction is one of the most critical factors for reducing infection. Grimer *et al* (16) reported that their initial results for proximal tibia tumor reconstruction were poor because of the high infection rate derived from wound breakdown and an infection rate of 33%. However, this rate later decreased to 12% with the use of a flap to cover the wound. We therefore recommend the use of a musculocutaneal flap for this operation, though infection, remains a common complication in our study.

In our series, the Mann-Whitney U test showed that male patients had significantly better limb function than female patients. We speculate that this result could be due to the residual muscle power in male patients. Patients with peroneal nerve palsy also showed a significantly worse MSTS score than those without peroneal palsy. These results suggest that preservation of the quadriceps femoris muscle and avoidance of peroneal nerve palsy are critical to maintaining a good limb function.

In summary, prosthetic reconstruction for musculoskeletal tumors around the knee provides good oncological and functional results. However, the high complication rate is a major concern for the prosthetic replacement. Future improvements of prostheses are very important. With regard to the surgical techniques, the preservation of the quadriceps femoralis muscle and avoidance of peroneal nerve palsy are important.

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Section Editor: **Terese T. Horlocker**

TECHNICAL COMMUNICATION

Anatomy and Clinical Implications of Ultrasound-Guided Selective Femoral Nerve Block

Shigeo Ishiguro, MD,* Ayumu Yokochi, MD,† Kiyoyuki Yoshioka, MD,† Naoki Asano, MD,‡ Akira Deguchi, MD,§ Yasushi Iwasaki, MD,|| Akihiro Sudo, MD,‡ and Kazuo Maruyama, MD†

In this study we evaluated the anatomic basis and clinical findings of ultrasound-guided femoral nerve block performed close to the distal apex of the femoral triangle. Cadaver studies were conducted in 9 thighs of fresh bodies within 24 hours postmortem. In all cases, during injection of 10 mL of blue dye, the skin proximal to the injection site was compressed to prevent the proximal flow. In the first thigh, from the area just distal to the inguinal ligament, an epidural catheter was advanced distally beneath the fascia iliaca over the femoral nerve. In the remaining cases, 10 mL of blue dye was injected into the femoral nerve at the level of the proximal adductor canal and dye spread was evaluated after local dissection. The clinical study was conducted in 20 patients with severe varus deformities. Ten milliliters of 0.75% ropivacaine was injected as in the cadaveric series. The femoral nerve was successfully dyed in all cases of the cadaver study, whereas the muscular branch to the sartorius muscle and quadriceps muscle, with the exception of the vastus medialis muscle, evaded dyeing. All 20 patients with varus knee deformities reported analgesia; none of them experienced motor block. We conclude that local anesthetic injection at the site where the superficial femoral artery has passed beneath the medial border of the sartorius muscle (8 to 12 cm distal to the inguinal crease), combined with efforts taken to prevent proximal flow may anesthetize the sensation of the anterior-to medial aspect of the knee and motor branch of the vastus medialis muscle, without blocking the sartorius or quadriceps muscles. (Anesth Analg 2012;115:1467–70)

Falling after having received a femoral nerve block for postoperative pain relief is a serious concern in early stage ambulation, although a more diluted anesthetic is being tried with the aim of prevention.[1] In the present study, a dissection was conducted in a series of fresh cadavers within 24 hours postmortem to provide a detailed description of the femoral triangle at the level of the adductor canal, including the emanation of motor branches.[2]

METHODS

Anatomical Study

After approval by the IRB (Mie Prefecture, Japan), nine thighs from 6 adult cadavers within 24 hours postmortem were included in this study. Each cadaver was placed in the supine position. In the first cadaveric investigation, the femoral nerve was identified just distal to the inguinal ligament through a 4-cm transverse skin incision. An epidural catheter was then advanced 10 cm distally along the femoral nerve, beneath the fascia iliaca. Skin 6 cm distal to the inguinal ligament (proximal to the catheter insertion site) was compressed while 10 mL of blue dye was injected.

In the second-to-fifth cases, a linear probe equipped with a portable ultrasound machine (FAZONE CB, Fujifilm Medical Co. Ltd., Tokyo, Japan) was placed perpendicular to the femoral vessels where the superficial femoral artery has just passed beneath the medial border of the sartorius muscle. In short-axis view, using in-plane technique, 10 mL of blue dye was injected into the triangular shape just lateral to the superficial femoral artery with manual measures to prevent proximal flow (Fig. 1).

In the sixth-to-ninth cases, 10 cc of blue dye was injected in the same manner as the second-to-fifth cases (Fig. 1). Without rotating the linear probe, using out-of-plane technique, epidural catheter insertion was attempted 4 cm proximal to the injection point.

In all cases, skin incisions were performed from the midpoint of the inguinal ligament to the adductor tubercle of the femur. The level/site of nerve branches as well as muscular terminations were carefully explored and recorded.

Upon review of our data for the initial 9 cases, it was decided that further sampling would likely yield similar results. For this reason, along with institutional obstacles to obtaining approval for cadaver study in Japan, it was decided to limit our sampling to 9 dissections in 6 cadavers.

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The authors declare no conflicts of interest.

Reprint will not be available from the authors.

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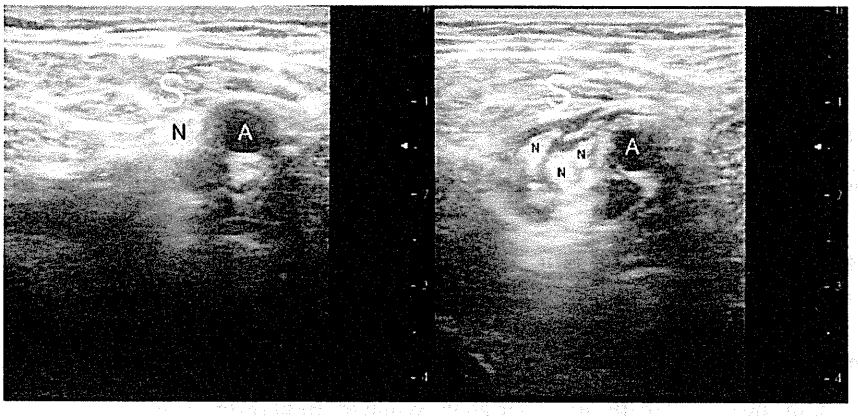

Figure 1. Even at the apex of the femoral triangle or the proximal edge of the adductor canal, the triangular femoral nerve structure can be visualized. After injection, the femoral nerves seemed bloated with their segments dispersed inside a closed cavity. A = superficial femoral artery; S = sartorius muscle; N = femoral nerve.

Case Series

After approval by the ethics committee of the Oyamada Memorial Spa Hospital, an observational prospective study, including 20 varus knees in 18 female outpatients, was conducted. According to the Kellgren and Lawrence classification,[3] all knees were classified as grade 4, the most severe classification. All patients complained of knee pain with a score of 6 or more on the visual analog scale. Patients who suffered from dementia were excluded from this study. Written informed consent was obtained from all patients.

Anatomical structures of the edge of the femoral triangle were visualized using a portable ultrasound machine (FAZONE CB, Fujifilm Medical Co. Ltd., Tokyo, Japan) with a linear 5- to 10-MHz probe. During the procedure, each patient was supine, with her thigh externally rotated at the hip. After sterilization of the thigh, the probe was placed at the same site at which the cadaveric study was conducted (Fig. 1). After skin infiltration with 3 mL of 1% lidocaine, a 21-gauge needle was advanced using the in-plane technique, and 10 cc of 0.75% ropivacaine was introduced into the triangular structure, with the skin surface proximal to the injection site compressed by the practitioner's finger to prevent the proximal flow of the local anesthetic (Fig. 1). Thirty minutes after the injection, sensory block with a pinprick was rated on a 3-point scale: 0, normal sensation; 1, decreased sensory block; and 2, complete sensory block. We considered the sensory block successful if there was a complete absence of sensation to a pinprick in the area from the upper pole of the patella, to the tibial tuberosity and the medial aspect of the knee joint. Patients were contacted the day after the block procedure and, if symptomatic, daily thereafter until their symptoms resolved. Upon review of data from 18 knees of 20 patients, it was decided that further sampling would likely yield similar results; thus it was decided to limit our sampling to 20 clinical cases at this time.

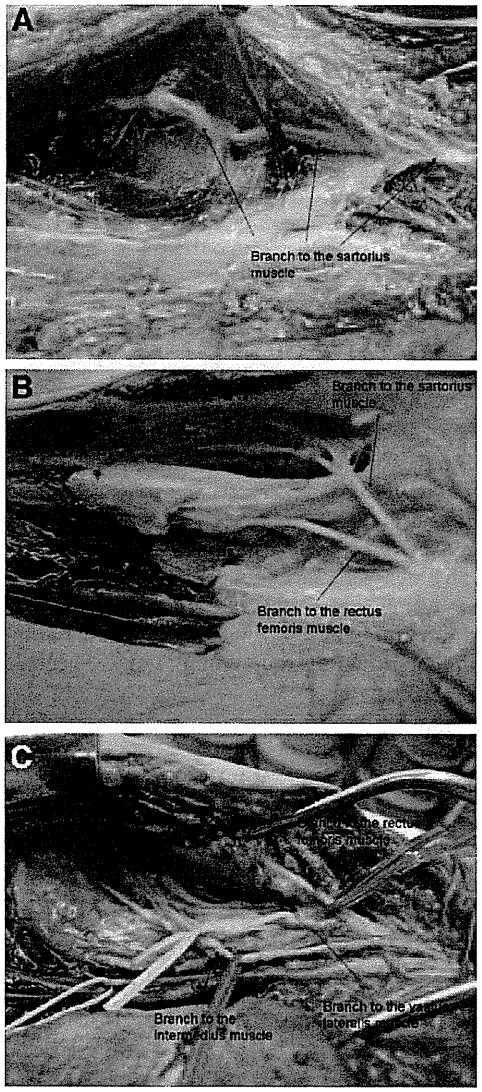

Figure 2. A, Because the fascia iliaca is a membrane or barrier, the branch for the sartorius muscle evaded dyeing after it had penetrated the fascia iliaca. However, it coursed close to the adductor canal. B, Only the fascia iliaca of the dyed area was incised, whereas muscular branches to the rectus femoris and the sartorius muscle had just penetrated the fascia iliaca. C, After the undyed area of the fascia iliaca was incised, it was noted that the muscular branches to the vastus lateralis muscle and the vastus intermedius muscle also evaded dyeing.

RESULTS

Anatomical Study

Nine dissections in 6 cadavers were performed. There were 4 women and 2 men with a mean (SD) age of 83 (11) years, height of 151 (12) cm, and a weight of 37 (10) kg. In all cases, proximal to the apex of the femoral triangle (less than 20 mm distal to the inguinal ligament) and beneath the fascia iliaca, the muscular branches emerged

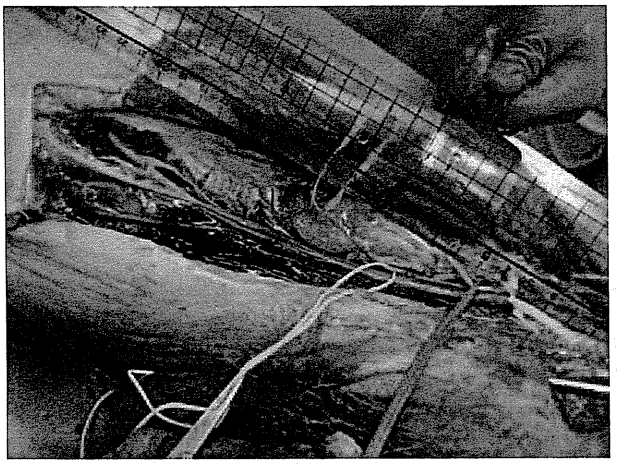

Figure 3. The entire dyed length was approximately 14 to 15 cm.

from the femoral nerve trunk to the sartorius muscle and the quadriceps muscles but not to the vastus medialis muscle.

The muscular branches to the rectus femoris muscle, the sartorius muscle, the vastus lateralis muscle, and the vastus intermedius muscle had pierced the fascia iliaca within 40 mm distal to the inguinal ligament and coursed for a few cm through loose connective tissue until they reached the respective muscle (Fig. 2). In all 9 cases, the injected 10 mL of blue dye colored the inner space from the apex of the femoral triangle to the lower edge of the adductor canal. No specific boundary was observed between the apex of the femoral triangle and the adductor canal, and only the saphenous nerve and femoral nerve trunk, which divides into several motor branches to the vastus medialis, were dyed blue (Fig. 3). In the sixth-to-ninth cases, the tip of the epidural catheter was successfully placed within the dyed area.

Case Series

Twenty varus-knee patients were included in this study, all female, with a mean (SD) age of 82 (6) years, height of 147 (5) cm, and weight of 51 (8) kg. Of the 20 patients, 19 (95%) underwent complete sensory blocks (2/2). One patient experienced decreased sensory block (1/2). None of them experienced motor block. Pain rating on a visual analog scale while walking decreased from 7.8 (1.4) to 0.1 (0.4) immediately after block, and at 2-week follow-up it remained 4.9(2.2). The mean (SD) value of their subjective duration of the paresthesia was 4.8 (3.5) days.

DISCUSSION

The femoral nerve is commonly approached between the level of the inguinal ligament and the inguinal crease and can provide postoperative analgesia to the entire front of the upper thigh down to and including the patella as well as the medial side of the lower leg to approximately the medial malleolus.[2]

In our clinical study, as indicated in the cadaver investigation, local anesthetic injection where the superficial femoral artery has just passed beneath the medial border of the sartorius muscle (8 to 12 cm distal to the inguinal crease), with efforts taken to prevent proximal flow anesthetized only the saphenous nerve and several branches to the vastus medialis. Unlike selective saphenous nerve block, our method may also block the pain or sensation of the vastus medialis. In the cadaver study, we could successfully insert the epidural catheter via the skin point 4 cm proximal to the injection site, and advance the catheter beneath the sartorius muscle.

We acknowledge that it could have been possible that proximal local anesthetic spread occurred, despite our dye study. Hence future clinical studies for knee surgery are required.

In conclusion, in our cadaveric exploration, no boundary was found between the apex of the femoral triangle and the adductor canal. Furthermore, ultrasound-guided injection on our distal blocking site can anesthetize the sensation of the anterior-to medial aspect of the knee and motor branch of the vastus medialis muscle, without blocking the sartorius or quadriceps muscles. The clinical utility of these findings requires additional investigation.

DISCLOSURES

Name: Shigeo Ishiguro, MD.
Contribution: This author helped design the study, conduct the study, analyze the data, and write the manuscript.
Attestation: Shigeo Ishiguro has seen the original study data, reviewed the analysis of the data, approved the final manuscript, and is the author responsible for archiving the study files.
Name: Ayumu Yokochi, MD.
Contribution: This author helped conduct the study.
Attestation: Ayumu Yokochi has seen the original study data, reviewed the analysis of the data, and approved the final manuscript.
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Attestation: Kiyoyuki Yoshioka has seen the original study data, reviewed the analysis of the data, and approved the final manuscript.
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Attestation: Naoki Asano has seen the original study data, reviewed the analysis of the data, and approved the final manuscript.
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Attestation: Yasushi Iwasaki has seen the original study data, reviewed the analysis of the data, and approved the final manuscript.
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Contribution: This author helped analyze the data and write the manuscript.
Attestation: Akihiro Sudo has seen the original study data, reviewed the analysis of the data, and approved the final manuscript.

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Contribution: This author helped analyze the data and write the manuscript.
Attestation: Kazuo Maruyama has seen the original study data, reviewed the analysis of the data, and approved the final manuscript.
This manuscript was handled by: Terese T. Horlocker, MD.

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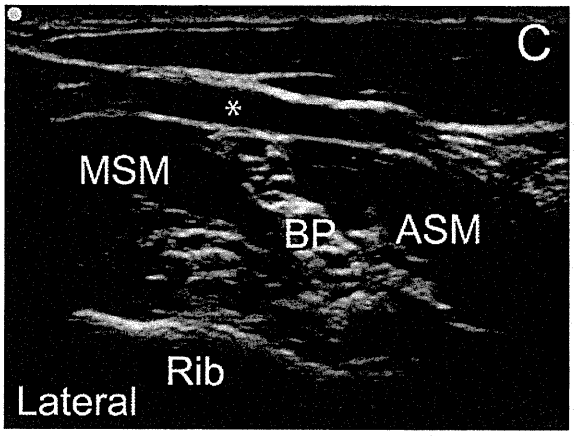
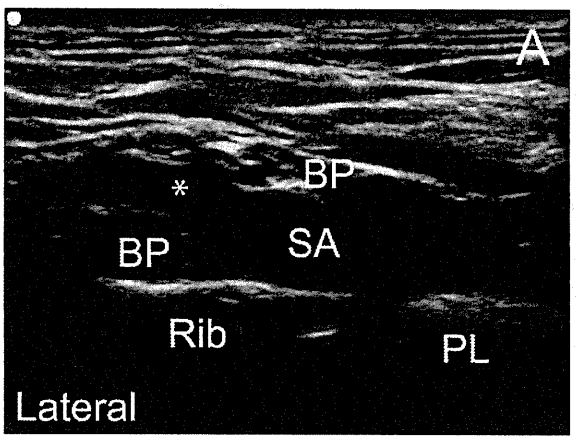
The Presence of Transverse Cervical and Dorsal Scapular Arteries at Three Ultrasound Probe Positions Commonly Used in Supraclavicular Brachial Plexus Blockade: Erratum

In the article by Murata et al. that appeared on page 470 in the August 2012 issue of volume 115 of *Anesthesia & Analgesia*, Figures 2C and 3A were mislabeled in the article. The authors apologize for the labeling error and have provided the correctly labeled figures for publication below.





Reference:

1. Murata H, Sakai A, Hadzic A, Sumikawa K. The presence of transverse cervical and dorsal scapular arteries at three ultrasound probe positions commonly used in supraclavicular brachial plexus blockade. Anesth Analg 2012;115:470–3

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Tenosynovitis of the extensor pollicis longus tendon caused by an intratendinous ganglion: a case report

H. Satonaka, M. Tsujii and A. Sudo

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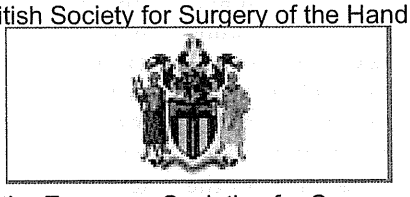
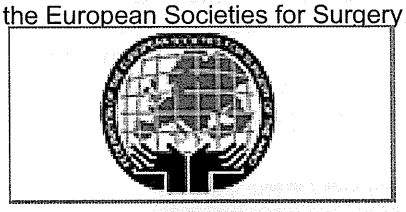


Federation of the European Societies for Surgery of the Hand



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Tenosynovitis of the extensor pollicis longus tendon caused by an intratendinous ganglion: a case report

Dear Sir,

Stenosing tenosynovitis of the first extensor compartment, de Quervain's disease, is common, while tenosynovitis of the other five compartments is rare in non-rheumatoid patients (Huang and Strauch, 2000). Although an extensor tendon ganglion attaches to the dorsal surface of the tendon and may be associated with a stenosing synovitis, an intratendinous ganglion is a rare condition. We report a case of an intratendinous ganglion in the extensor pollicis longus (EPL) tendon that caused stenosing tenosynovitis.

A 45-year-old female presented with a 3 year history of right wrist pain as well as a painful triggering of her thumb with extension. In spite of gradually increasing pain and swelling at the dorsal radial aspect of the right wrist, her thumb triggering had disappeared for a few months. A palpable and mildly tender hard elastic mass, approximately 1.5 × 1 cm in size, was located at the ulnar side of the anatomical snuff box. The mass moved with active excursion of the EPL tendon. MRI revealed a well-circumscribed cystic lesion existed in the EPL tendon at the level of the intercarpal joint. Based on these findings, an intratendinous EPL ganglion was suspected and surgical exploration was performed. A cystic structure, measuring 14 × 8 × 7 mm, was found within the substance of the EPL tendon at the dorsal aspect of the intercarpal joint and this lesion had no connection to the adjacent joint capsule or tendon sheath. The cystic lesion within the tendon impinged on the outlet of the third extensor compartment, and there was synovitis around the EPL tendon and degenerative change of the impinged tendon (Figure 1). The EPL tendon was, therefore, excised

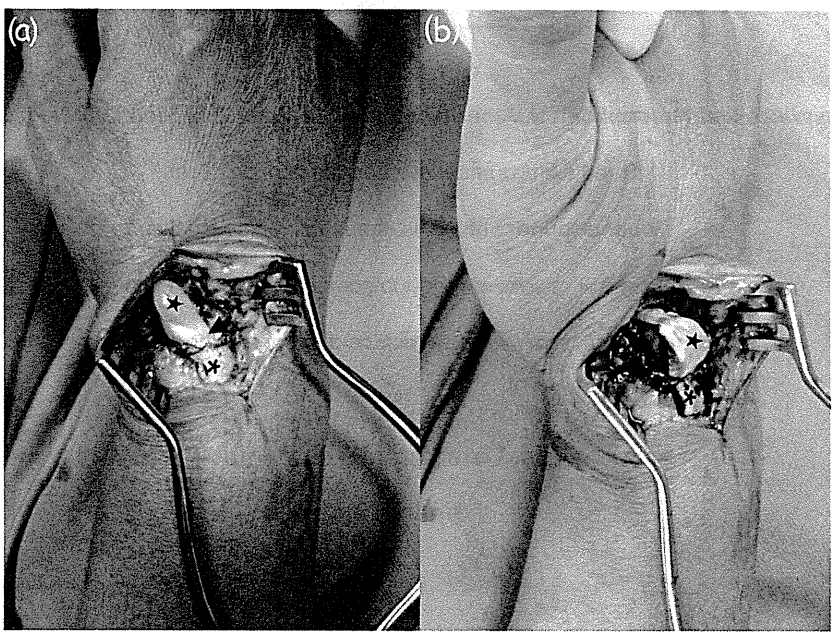

Figure 1. Intraoperative photographs reveal (a) the intratendinous ganglion (star) of the extensor pollicis longus tendon (arrow). (b) The intratendinous ganglion (star) impinges on the outlet of the extensor retinaculum (asterisk) with extension of the thumb.

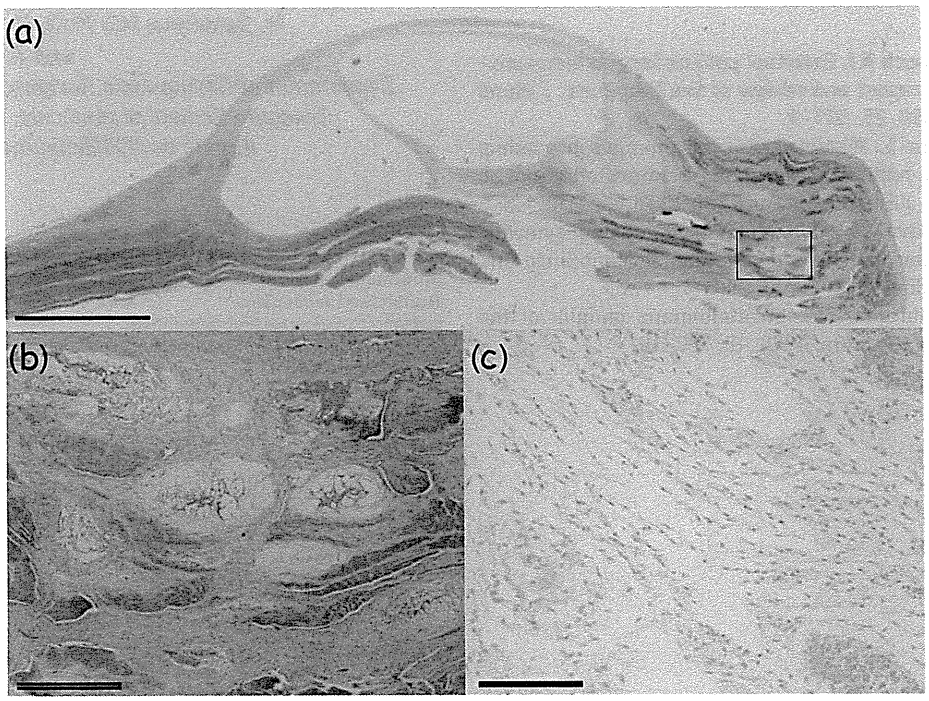

Figure 2. Histopathological findings of the excised EPL tendon in a longitudinal section show (a) the intratendinous cyst contained myxoid areas enclosed by fibrous tissues and lacked an epithelial lining. (b) The proximal tendon has thin and fragile collagen fibres with hyaline degeneration and mucoid degeneration. (c) The synovium exhibits infiltration of inflammatory cells and increased vascularity (bar, 5 mm; double bar, 200 μm) (haematoxylin and eosin stain).

en bloc with the lesion because of macroscopic findings consistent with considerable structural weakness in the EPL tendon adjacent to the proximal edge of the lesion. Next, the transferred extensor indicis proprius tendon was woven into the distal rest of the EPL tendon. The patient recovered uneventfully with normal function of the affected hand after 3 months. Histopathological examination of the excised EPL tendon including the lesion demonstrated that the intratendinous cyst contained myxoid areas enclosed by fibrous tissue and lacked an epithelial lining. In the proximal area of the excised tendon, thin and fragile collagen fibres with hyaline degeneration and mucoid degeneration were visible. In addition, hyperplastic synovial cells had accumulated within the collagen fibres of the tendon. The synovial tissue exhibited infiltration of inflammatory cells and increased vascularity (Figure 2).

It was considered that stenosing tenosynovitis of the third extensor compartment is caused by swelling of the EPL tendon in the tight cross-sectional area of the third compartment at Lister's tubercle (Kardashian et al., 2011). Although our patient initially complained of a painful triggering of the thumb, the thumb triggering disappeared with increasing pain and swelling of the wrist. These findings suggest that her tenosynovitis was caused by impingement of the intratendinous EPL ganglion, which blocked the sliding of the tendon beneath the extensor retinaculum of the third dorsal compartment. Histopathological findings lend support to the hypothesis that intratendinous ganglion impingement caused a secondary tenosynovitis.

Treatment of the intratendinous ganglion should include preserving the tendon, which may be weakened by the ganglion (Kannus and Jozsa, 1991; Seidmen and Margles, 1993). Histopathological findings revealed that the tendon substance weakness was caused by a degenerative change in the tendon adjacent to the proximal edge of the lesion, and hyperplastic synovial cells accumulated into the collagen fibres of the tendon in our case. Therefore, it was suitable for the EPL tendon to be resected en bloc with the ganglion and reconstructed with the tendon transfer.

Conflict of interests

None declared.

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ORIGINAL ARTICLE

Prevalence and characteristics of unilateral knee osteoarthritis in a community sample of elderly Japanese: do fractures around the knee affect the pathogenesis of unilateral knee osteoarthritis?

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Abstract
Background The purpose of this study was to investigate the prevalence and characteristics of unilateral knee osteoarthritis (KOA), to investigate what percent of contralateral healthy knees in patients with unilateral KOA progress to KOA, and to investigate whether knee fractures influence unilateral KOA.
Methods Studies were performed every two years from 1997 to 2009 in Miyagawa village, for a total of seven studies. A total of 1239 village inhabitants aged ≥65 years participated in these studies at least once. KOA was defined as a Kellgren–Lawrence (K/L) grade ≥2. Based on the knee X-ray at the first examination, participants were divided into three groups: no KOA (N group), unilateral KOA (U group), and bilateral KOA (B group). The U group was divided into two subgroups: K/L grade II-I combination (II-I group), and the U group without the II-I combination (G>2 group). To investigate whether knee fractures influence unilateral KOA, the fracture history was considered.
Results The percentages of participants classified into the N, B, and U groups (II-I and G>2 group) were 68.4, 21.6, and 10.0 % (7.8 and 2.1 %), respectively. Most of the U group had the II-I combination (78.7 %). The percentages of knee fractures in the N, B, II-I, and G>2 groups were 3.3, 5.3, 6.3, and 38.5 %, respectively. Overall, 49.2 % of the U group proceeded to bilateral KOA over an average of 5.3 years.
Conclusions The prevalences of definite radiographic bilateral and unilateral KOA were 21.6 and 10.0 %, respectively. Overall, 49.2 % of the participants with unilateral KOA developed KOA in the contralateral knee over an average of 5.3 years. If bilateral KOA advanced simultaneously, the II-I group was considered to represent the midpoint of progression to bilateral KOA. Bilateral KOA advanced simultaneously except in cases with a history of knee trauma, such as fractures.

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Introduction

Osteoarthritis of the knee (KOA) is the most common form of arthritis leading to pain and loss of function in older adults [1]. It is well known that Japan has an aging population, and thus the prevalence of Japanese patients with KOA is increasing [2]. Several studies have described the prevalence of KOA as well as various risk factors and their associations with KOA [3–10]. However, in most previous studies, information on only one knee—the knee with the worst grade—was recorded. There have been only a few studies where each knee grade was recorded, meaning that the natural history of the contralateral knee is poorly understood [11, 12]. In clinical practice, patients with unilateral knee pain are frequently encountered. If only the knee with the worst grade is X-rayed, then the condition of the contralateral knee can only be assumed. For clinicians to treat KOA, it is important to know the prevalences of unilateral and bilateral KOA in order to satisfy both the

physical and psychological needs of the patients. The possibility and predicted speed of KOA progression in the contralateral knee is important, as it may influence therapeutic approaches. A further reason for obtaining the estimated rates of progression is to estimate sample sizes when planning clinical trials. To the best of our knowledge, only one study [11] has estimated the rate of incidence of KOA in the contralateral knee in a population-based cohort study.

The purpose of this study was to investigate the prevalence and characteristics of unilateral KOA, to investigate what percent of the contralateral healthy knees in the unilateral KOA group progressed to KOA, and to investigate whether knee fractures influence unilateral KOA.

Materials and methods

The Miyagawa cohort study, a population-based study, began in 1997 in Miyagawa, a mountain village located in the center of Mie Prefecture, Japan. Participants were self-recruited, community-dwelling volunteers who were ≥65 years old. Studies were performed every two years from 1997 to 2009 at Houtoku Hospital in the village, for a total of seven studies. The population of the village was 4196 in 1997, when 1463 of the residents met the age criterion. The population dropped to 3490 in 2010, at which time 1553 individuals met the age criterion. A total of 1239 inhabitants (786 women and 453 men) participated in these studies at least once. The Committee for the Ethics of Human Research of Mie University approved the study protocol, and all participants provided their written informed consent before study enrollment. Using data from the Miyagawa cohort study, individuals who were found at a baseline screening examination to have unilateral KOA were investigated.

Baseline data obtained from standard questionnaires administered by orthopedic surgeons included information regarding age, sex, medical history, knee fracture history, and knee pain. Knee fractures were defined as including the patella, distal femur, and proximal tibia (such as the tibial plateau). Knee pain was determined from the question, "Have you experienced knee pain lasting for over one month during the past year?" Knee pain was recorded as absent, unilateral, or bilateral. Height and weight were measured, and the body mass index (BMI) was calculated as the weight in kilograms divided by the square of the height in meters.

All participants had anteroposterior (AP) radiographs of both knees in the fully extended position with the same equipment. These radiographs were scored blind to clinical details according to the Kellgren–Lawrence (K/L) grading system [13] that uses the following grades: 0, normal; 1, possible osteophytes only; 2, definite osteophytes and possible joint space narrowing; 3, moderate osteophytes and/or definite joint space narrowing; and 4, large osteophytes, severe joint space narrowing, and/or bony sclerosis. Confirmed radiographic KOA was defined as a K/L grade of ≥2. All knee radiographs were independently evaluated by three orthopedists, and the final score was reached by consensus among two or three of the orthopedists, but the median score was accepted when the opinions of all three differed. Ankylosing spondylitis was not identified in any of the participants. Knees that had undergone total knee replacements (TKA) were defined as having KOA.

The following factors were examined. Firstly, based on the first knee X-ray examination for each participant, the participants were classified into three groups: no OA (N group), unilateral KOA (U group), and bilateral KOA (B group). Moreover, the U group was classified into two subgroups: K/L grade II-I combination (II-I group), and the U group without the II-I combination (G>2 group; participants in this group had one knee grade that differed by two or more grades from that of the other knee). These groups were compared in terms of physical characteristics such as age, sex, height, weight, BMI, and knee pain. Secondly, to investigate whether knee fractures influenced unilateral KOA, the percent of participants with a knee fracture history was examined in each group. Knee fractures were defined as patellar fracture, tibial plateau fracture, and/or distal femur fracture. Thirdly, the natural history of the contralateral (healthy) side knee was examined in the U group. The subjects of this series were participants in the U group who had participated in the examinations at least twice. Changes in the contralateral (healthy) side knee K/L grade U group over 2–12 years were recorded, and we determined whether the KOA had changed (K/L grade ≥2). Moreover, the incidence of KOA was compared between those with and without a history of knee fracture.

Statistical analysis

Mean ± standard deviations (SD) were calculated for variables unless otherwise noted. Associations among the physical characteristics among the groups were determined by the unpaired *t* test. The relationships between KOA and knee fractures were analyzed using age, sex and BMI-adjusted logistic regression analyses with the Bonferroni correction. The change in the contralateral (healthy) side knee of the U group was analyzed by Kaplan–Meier analysis with the log-rank test. The significance level for entry into the model was 0.05. All data were analyzed using the PASW Statistics (version 18) software package (SPSS, Chicago, IL, USA).

Table 1 Characteristics of the participants with no knee osteoarthritis, bilateral knee osteoarthritis, and unilateral knee osteoarthritis

	N group (n = 837)	B group (n = 264)	U group (n = 122)	
			II-I group (n = 96)	G>2 group (n = 26)
Age	71.0 ± 6.6	73.5 ± 7.5*	72.4 ± 6.9	73.8 ± 5.5**
Sex (female/male)	480/357	210/54*	70/26*	15/11††
Height (cm)	152.1 ± 8.3	149.6 ± 8.5*	149.1 ± 8.3*	151.9 ± 9.7
Weight (kg)	53.2 ± 19.2	55.6 ± 10.1**	52.9 ± 9.0††	55.7 ± 11.8
BMI (kg/m^2)	22.9 ± 8.5	24.8 ± 3.6*	23.7 ± 3.2††	23.9 ± 3.2
Knee pain (−/+/++)	589/147/100	79/69/116*	39/32/25*†	11/9/6**

Knee pain defined as: −, absent; +, unilateral; ++, bilateral

N group no knee osteoarthritis group, *B group* bilateral knee osteoarthritis group, *U group* unilateral knee osteoarthritis group, *BMI* body mass index

*$p < 0.01$ versus N group, **$p < 0.05$ versus N group, †$p < 0.01$ versus B group, ††$p < 0.05$ versus B group

Table 2 Distribution of Kellgren–Lawrence grades at baseline in the unilateral knee osteoarthritis group

K/L grade	II-0	II-I	III-0	III-I	IV-0	IV-I	TKA-0	TKA-I
Participants	8	96	2	13	0	2	0	1
%	6.6	78.7	1.6	10.7	0.0	1.6	0.0	0.8

OA osteoarthritis, *K/L grade* Kellgren–Lawrence grade, *TKA* total knee arthroplasty

Results

Of the 1239 participants who attended at least one of the seven examinations associated with this study, 16 patients with rheumatoid arthritis were excluded, and a total of 1223 villagers fulfilled the study criteria.

Table 1 shows the physical characteristics of the four groups. The percentages of participants who were classified into the N, B, and U groups (II-I and G>2 groups) were 68.4, 21.6, and 10.0 % (7.8 and 2.1 %), respectively. The B group differed significantly from the N group in terms of age, sex, height, weight, BMI, and knee pain. The II-I group differed significantly from the N group in terms of sex, height, and knee pain. The G>2 group differed significantly from the N group in terms of age and knee pain. The II-I group differed significantly from the B group in terms of weight, BMI, and knee pain. There was a significant difference in sex between the G>2 and B groups.

Table 2 shows the distribution of K/L grades at baseline for the U group. Most of the U group had the II-I combination (78.7 %), followed by the III-I combination (10.7 %), and then the II-0 combination (6.6 %). Only one participant had TKA (TKA-I combination).

Figure 1 shows the relationship between knee fracture and group. There were 28, 14, 6, and 10 participants with a knee fracture history in the N, B, II-I, and G>2 groups, respectively. The percentages of knee fractures were 3.3, 5.3, 6.3, and 38.5 % in the N, B, II-I, and G>2 groups, respectively. The G>2 group experienced more knee fractures than the N, B, and II-I groups ($p < 0.01$).

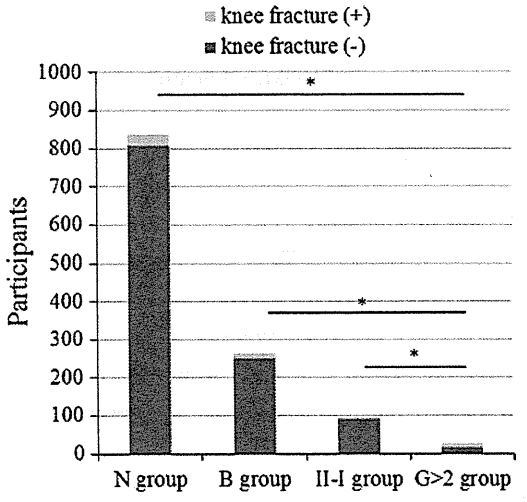

Fig. 1 Relationship between knee fracture and knee osteoarthritis. *N group* no knee osteoarthritis group, *B group* bilateral knee osteoarthritis group, *U group* unilateral knee osteoarthritis group. *$p < 0.01$ versus G>2 group

Table 3 shows the natural K/L grade history of the knees in the U group over 2–12 years (average, 5.3 ± 3.2 years). A total of 65 participants in the U group participated in the examination at least twice. The percentages of participants in whom the contralateral knee had changed to KOA (K/L grade ≥2) were 33.3 % (1/3), 54.7 % (29/53), 0 % (0/2), 40 % (2/5), 0 % (0/1), and 0 % (0/1) in the II-0, II-I, III-0, III-I, IV-0, and TKA-I groups, respectively. Thus, 32 of 65 participants (49.2 %) proceeded to bilateral KOA. Figure 2 shows the change in the contralateral (healthy) knee in the

Table 3 Natural history of bilateral knees in the unilateral knee osteoarthritis group

Baseline K/L grade combination	Follow-up K/L grade combination										Total
	II-0	II-I	II-II	III-I	III-II	III-III	IV-0	IV-I	IV-IV	TKA-I	
II-0	1	1			1						3
	(4.0)	(2.0)			(12.0)						(6.0)
II-I		21	17	3	4	7			1		53
		(4.4)	(5.3)	(6.0)	(5.5)	(8.0)			(12.0)		(5.5)
III-0				1			1				2
				(4.0)			(2.0)				(3.0)
III-I				2	1	1		1			5
				(5.0)	(4.0)	(8.0)		(2.0)			(4.8)
IV-0											0
IV-I								1			1
								(2.0)			(2.0)
TKA-0											0
TKA-I										1	1
										(2.0)	(2.0)

The numbers in parentheses are the average numbers of follow-up years

K/L grade Kellgren–Lawrence grade, *OA* osteoarthritis, *TKA* total knee arthroplasty

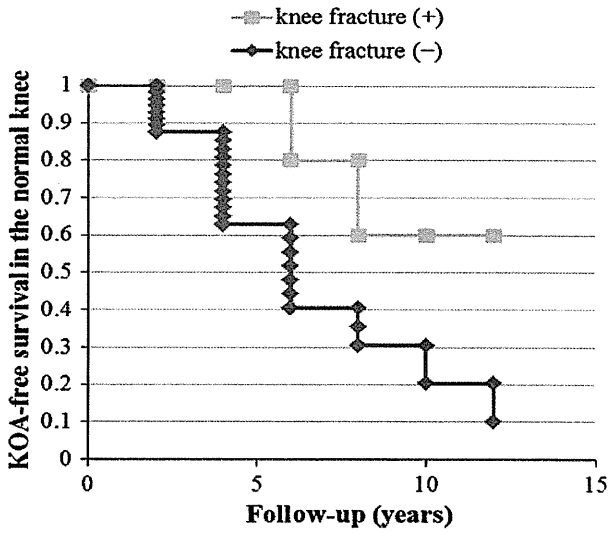

Fig. 2 The prognosis for the opposite healthy side in the unilateral knee group. The censored data in this Kaplan–Meier analysis are the normal knees (Kellgren–Lawrence grade 0 or 1). There was no significant difference between knee fracture (+) and knee fracture (−) based on the log-rank test

U group (normal knee or KOA) on Kaplan–Meier analysis and compares KOA between those with and without a history of knee fracture. There was no significant difference between those with and without a history of knee fracture based on the log-rank test.

Discussion

The present study found that the prevalences of definite radiographic bilateral and unilateral KOA were 21.6 and 10.0 % in older Japanese villagers. The present data also show that, in participants with unilateral KOA, a high percentage (49.2 %) developed OA in the contralateral healthy knee within an average of 5.3 years (range 2–12 years). A history of knee fractures had a stronger influence on the unilateral KOA group (except for those with the II-I combination) than the no KOA and bilateral KOA groups.

Davis et al. [14] reported that bilateral KOA was more than twice as prevalent as unilateral KOA in the National Health and Nutrition Examination Survey I sample. The present data showed that the B group was twice as large as the U group, so the present findings support their data. Spector et al. [11] reported that 34 % of women with unilateral KOA progressed to contralateral OA within two years. Spector et al. [14] also reported that 92 % of patients with unilateral KOA developed bilateral KOA over 11 years. The present data showed that 49.2 % participants with unilateral KOA developed bilateral KOA within an average of 5.3 years (range 2–12 years). This rate is similar to previously reported rates of 34 % for 2 years [11] and 92 % for 11 years [15]. McMahon et al. [16] reported that, in their TKA series, the percentages of bilateral KOA and unilateral KOA were 88.8 and 11.2 %, respectively. They also reported that the ten-year risk of undergoing TKA in their study population was 37.2 %, excluding

patients who underwent a contralateral TKA at the time of index surgery or within six months. Moreover, Hochberg et al. [17] reported that the presence of contralateral KOA was a risk factor for the development of definite KOA in their longitudinal study [adjusted odds ratio (OR) 6.04]. Sayre et al. [18] reported that the OR for having a contralateral knee with K/L >2 was 20.1 compared to a knee without KOA in K/L >2 knees. A contralateral knee joint grade of K/L was strongly associated with a K/L grade in the other knee. These data suggest that, with unilateral KOA, the contralateral healthy knee is at a high risk of developing KOA.

Many cross-sectional studies and a longitudinal study have reported a relationship between knee injury and KOA [14, 19–21]. Rademakers et al. [22] reported that 31 % of patients with a history of tibial plateau fractures developed secondary KOA. Marsh et al. [23] performed a review concerning the importance of anatomic reduction with respect to articular fractures. They also underlined the fact that malalignment after treatment contributes to a poor outcome after tibial plateau fractures. Experimental and observational evidence suggest that knee injuries with irregularity of the articular surface secondary to tibial plateau and distal femoral fractures, as well as angular deformity following fracture of the femoral and tibial shaft, produce increased articular surface stress, thus increasing the risk of subsequent KOA. Davis et al. [14] reported that obesity was a stronger predictor of bilateral osteoarthritis than knee injury was, with an OR of 6.6 for obesity and an OR of 3.5 for right knee injury. However, knee injury was a stronger predictor of unilateral osteoarthritis than obesity was (ORs of 3.4 and 2.4 for obesity in the right and left knee, respectively, and ORs of 16.3 and 10.9 for knee injury in the right and left knee, respectively). They concluded that different pathogenetic processes may exist for unilateral and bilateral KOA.

In the present study, the most common distribution of K/L grades at baseline for the U group was the II-I combination (78.7 %). If bilateral KOA advanced simultaneously, this combination was considered to represent the midpoint of the progression to bilateral KOA. Therefore, the relationship between the U group without the II-I combination (G>2 group) and knee fractures was examined. The G>2 group had far more cases with a knee fracture history than the N, B, and II-I groups. These data suggest that, with no history of knee fracture, the bilateral knee would become worse (and develop KOA) almost simultaneously.

The present study had several potential limitations. Firstly, Miyagawa is a mountain village, and many inhabitants are typically engaged in forestry. Secondly, participants who could attend the hospital were generally healthier than nonparticipants. Thirdly, the knee X-rays were non-weight-bearing, so the K/L grade was underestimated. Therefore, the prevalence of KOA was lower than in other reports from Japan [2, 24]. Fourthly, other traumatic risks for KOA—knee ligament injuries [25, 26] and meniscus injuries [25–27]—were not considered in this study.

Conclusion

The prevalences of definite radiographic bilateral and unilateral KOA were 21.6 and 10.0 %, respectively, in a group of older Japanese villagers. Overall, 49.2 % of the participants with unilateral KOA developed KOA in the contralateral knee over an average of 5.3 years. A very strong association was found between unilateral KOA (except for the K/L grade II-I combination) and knee fracture. The results of the present study indicate that bilateral KOA advanced simultaneously, except in cases with a history of knee injury, such as fractures.

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Conflict of interest The authors state that they have no conflict of interest.

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Oncolytic virotherapy for human bone and soft tissue sarcomas using live attenuated poliovirus

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Abstract. The poliovirus receptor CD155, is essential for poliovirus to infect and induce death in neural cells. Recently, CD155 has been shown to be selectively expressed on certain types of tumor cells originating from the neural crest, including malignant glioma and neuroblastoma. However, the expression pattern of CD155 in soft tissue sarcoma has not been examined. Therefore, we first examined CD155 expression in sarcoma cell lines, and found the expression of both CD155 mRNA and protein in 12 soft and bone tissue sarcoma cell lines. Furthermore, we examined the effect of live attenuated poliovirus (LAPV) on 6 bone and soft tissue sarcoma cell lines *in vitro*, and found that LAPV induced apoptosis by activating caspases 7 and 3 in all of these cell lines. Furthermore, in BALB/c nu/nu mice xenotransplanted with HT1080 fibrosarcoma cells, administration of live attenuated poliovirus caused growth suppression of the tumors. These results suggest that oncolytic therapy using a LAPV may represent a new option for the treatment of bone and soft tissue sarcomas.

Introduction

The treatment of soft tissue and bone sarcoma remains challenging because higher-grade sarcomas are associated with higher local treatment failure rates and increased metastatic potential. An estimated 10,520 cases of soft tissue sarcoma and 2,650 cases of malignancy of the bones and joints were diagnosed in the United States in 2010 (1). There are more than 50 different histological types of soft tissue sarcoma, and the histological diagnosis of the rare sarcoma is confusing. Typically, low-grade sarcoma demonstrates local invasion with a lower propensity to metastasize, while high-grade sarcomas have a greater likelihood of distant spread to the lungs by a hematogenous route (2).

Surgical resection remains the most effective therapeutic approach for the management of soft tissue sarcoma, especially small (<5 cm), superficial high-grade and low-grade sarcoma (3). Although local control of high-grade soft tissue sarcoma can be obtained through the use of surgery and radiotherapy, recurrence was reported to occur in more than 50% of such patients (4). In most patients with soft tissue sarcoma that is of a high histological grade, large size (>5 cm), has invaded deep into the fascia, or is a local recurrence, a combined modality approach comprising preoperative or postoperative chemotherapy is used in addition to the radical surgical procedures (5). However, the role of chemotherapy for adult-type soft tissue sarcoma remains less well defined. According to the results from a meta-analysis of 14 trials performed in 1997 (6), the overall survival at 10 years improved from 50% to 54% with adjuvant chemotherapy, but this difference was not statistically significant. Moreover, according to the results from a randomized clinical trial with neoadjuvant chemotherapy (7), the disease-free survival at 5 years improved from 52% to 56%, but this difference was also not statistically significant. Therefore, the development of a novel therapeutic strategy is required to better treat patients with refractory bone and soft tissue sarcomas.

Oncolytic virotherapy, the selective killing of tumor cells by viruses, is a promising experimental treatment for cancer. The first report of oncolytic virotherapy was due to an unintentional exposure to naturally-occurring viruses or the administration of live attenuated vaccine strains, such as Newcastle disease virus and reovirus (8). Clinical trials of virotherapy for cancer started early in the 20th century. Despite encouraging results in case reports, the overall clinical results have disappointed clinicians because of the weak therapeutic effects if such treatments (9). Recently, a better understanding of the viral tropism for the cells allowed for the development of new strategies to enhance the specificity of viruses for cancer cells and to improve the viral replication in cancer cells (10). In addition to DNA viruses, such as adenovirus and herpes simplex virus, that are molecularly engineered to replicate specifically in tumor cells, RNA viruses with inherent tumor specificity have been developed as oncolytic agents for cancer treatment. This group of viruses includes reovirus (11), Newcastle disease

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virus (12), measles virus (13), vesicular stomatitis virus (14) and poliovirus (15).

The poliovirus is a non-enveloped plus-strand RNA virus belonging to the Picornaviridae, and is the causative agent of paralytic poliomyelitis. The vast majority of poliovirus infections remain asymptomatic, but 1-2% of cases result in neurologic complications (16). The restriction of poliovirus cell tropism to motor neurons resident within the spinal cord and brainstem gives rise to a highly characteristic clinical syndrome dominated by flaccid paralysis. Selective targeting of motor neurons by poliovirus is most likely determined by the distribution of its cellular receptor, the Ig superfamily molecule CD155 (also known as poliovirus receptor: PVR and nectin-like molecule-5: Necl-5). This assumption is supported by the observation that mice transgenic for human *CD155* develop a polio-like syndrome after poliovirus infection (17-19). In addition, intracellular conditions favoring viral replication have also been reported to contribute to poliovirus cell-type specificity (20).

Recently, the biological functions of CD155 have become clearer. It is now known that CD155 plays an important role in cell adhesion, migration, polarization and proliferation (21). CD155 interacts in trans with nectin-3, a member of the Ig-like nectin family, a Ca^{2+}-independent cell-cell adhesion molecule, and cooperatively forms adherence junctions with cadherin. When the cells contact other cells, CD155 is removed from the cell surface by clathrin-dependent endocytosis, due to its trans-interaction with nectin-3. When the cells do not come in contact with other cells, CD155 is upregulated by growth factor-induced signaling, while thus assembling the leading edge of moving cells (22).

Previous reports indicated that malignant tumors originating from the neural crest, including malignant glioma (23) and neuroblastoma (24) could be experimentally treated with various types of live attenuated poliovirus (LAPV), suggested its potential for clinical applications. However, there have been no reports which have so far examined whether bone and soft tissue sarcomas also represent targets of LAPV. In the present study, we first investigated the CD155 expression in bone and soft tissue sarcomas. We next investigated the oncolytic effects of a LAPV on 6 human bone and soft tissue sarcoma cells *in vitro*. Finally, we examined whether LAPV have oncolytic effects on soft tissue sarcomas using a subcutaneous xenograft animal model.

Materials and methods

Cell lines. Twelve human bone and soft tissue sarcoma cell lines were used in this study. HT1080 human fibrosarcoma, HS-SY-II human synovial sarcoma, MFH-ino human malignant fibrous histiocytoma, HS-PSS human malignant peripheral nerve sheath tumors (MPNST), HS-Sch-2 human MPNST, NMS-2 human MPNST, 143B human osteosarcoma, Saos-2 human osteosarcoma, and HOS human osteosarcoma cell lines were obtained from Riken Cell Bank (Ibaraki, Japan). HuO9, HuO9-M112, and HuO9-M132 human osteosarcoma cell lines were a kind gift from Dr Yasuo Beppu (National Cancer Center, Tokyo, Japan). HT1080 and 143B cells were maintained in MEM with 10% fetal bovine serum (FBS) (Invitrogen, Tokyo, Japan). HOS cells were additionally supplemented with 0.1 mM non-essential amino acids (Invitrogen). HS-SY-II, HS-PSS, HS-Sch-2 cells were maintained in DMEM (Invitrogen) with 10% FBS. NMS-2, HuO9, HuO9-M112 and HuO9-M132 cells were maintained in RPMI-1640 (Invitrogen) supplemented with 10% FBS. MFH-ino cells were maintained in DMEM/HamF12 (Invitrogen) with 10% FBS. Saos-2 cells were maintained in McCoy's (Invitrogen) with 10% FBS. HeLa and mouse osteosarcoma cell line LM8 cells were grown in MEM and DMEM, respectively, with 10% FBS.

A live attenuated poliovirus (LAPV). A LAPV vaccine containing the Sabin 1 strain (Japan Poliomyelitis Research Institute, Tokyo, Japan) was used as an oncolytic virus. The virus titer was determined by measuring the 50% tissue culture infectious dose ($TCID_{50}$) in HeLa cells.

One-step viral growth curves. The growth of poliovirus in the fibrosarcoma cell line, HT1080, was measured as previously described (19). Briefly, cell monolayers were washed with medium, and then were treated with medium containing LAPV at a multiplicity of infection of 0.2 and 2 $TCID_{50}$, respectively. After slowly stirring the dish for 30 min at room temperature, the cells were thoroughly washed with medium to remove unbound virus, and then incubated in serum-free medium at 37˚C for different intervals. At 2, 4, 6, 8, 12, 24, and 48 h after virus inoculation, the extracellular virus and the corresponding cell-associated virus were recovered after three consecutive freeze-thaw cycles. The infectivity of the clarified virus suspension was determined by a $TCID_{50}$ assay.

Viability assay and morphological features. The effects of LAPV on the viability of sarcoma cells and the morphological changes induced by viral infection were determined. HT1080, MFH-ino, HS-PSS, HuO9-M112, Saos-2, HOS and LM8 cells were seeded at $1.0x10^4$ per well on 96-well plates and treated with LAPV at a multiplicity of infection (MOI) of 2, 0.2, $2.0x10^{-2}$, $2.0x10^{-3}$ or $2.0x10^{-4}$ $TCID_{50}$/cell, respectively. At different intervals, the cell viability was assessed by the MTS assay (CellTiter 96® AQueous One Solution Cell Proliferation Assay, Promega, Madison, WI, USA). The morphology of cells was evaluated under a phase-contrast microscope.

Apoptosis assay. After 6 h of exposure to LAPV at a MOI of 2 $TCID_{50}$/cell or to vehicle, apoptotic HT1080 cells were detected by the TUNEL (terminal deoxynucleotidyltransferase-mediated dUTP-biotin nick end labeling) assay (ApopTag® Peroxidase *In Situ* Apoptosis Detection Kit, Millipore, Billerica, MA, USA). In addition, the characterization of cell death in HT1080 cells exposed to LAPV was determinated. The HT1080 cells were plated in 96-well plates at $1.0x10^4$ per well. After overnight incubation, the cells were exposed to LAPV at a MOI of 2, 0.2, $2.0x10^{-2}$ $TCID_{50}$/cell, or the vehicle for different intervals. After 0, 6, 12, 24, 36 or 48 h of incubation, 20 μl of Viability/Cytotoxicity Reagent containing GF-AFC Substrate (ApoTox-Glo™ Triplex Assay, Promega) was added. After 30 min of incubation at 37˚C, the fluorescence was recorded at 400 nm excitation/505 nm emission using a microplate reader for fluorescence and luminescence (Promega) to assess the cell viability. After 100 μl of Caspase-Glo 3/7 Regent (ApoTox-Glo Triplex Assay, Promega) was added to the cells and incubated for 30 min at room tempera-

ture, the luminescence was recorded. The evaluation of apoptotic cells was performed by measuring the activity of caspases 7 and 3 using a luminogenic substrate. The induction of apoptosis was defined as a decrease in cell viability with a concomitant increase in the caspase 7 and 3 activity.

Total-RNA extraction and quantitative real-time polymerase chain reaction. RNA was isolated (Isogen, Nippon Gene, Tokyo, Japan), reverse transcribed using the 1st Strand cDNA Synthesis Kit (Roche Applied Science, Mannheim, Germany) and subjected to real-time quantitative PCR with ABI PRISM® 7000 Sequence Detection System (Applied Biosystems, Carlsbad, CA, USA). Primer are purchased from Applied Biosystems. GAPDH was used as an endogenous 'house-keeping' gene for normalization. Standard curves were generated using cDNA samples from HeLa cells. The relative expression levels of each target gene were indicated by calculating the ratio to those in HeLa cells. All assays were performed in triplicate and repeated three times.

Immunofluorescence microscopy. Cells were fixed with methanol, and blocked with 3% BSA in PBS. Cells were stained with primary monoclonal antibody against recognizes the poliovirus binding site of CD155 (mouse D171, Neomarkers, Union City, CA, USA) (25), and secondary antibody Alexa®488-conjugated goat anti-mouse IgG (H+L) (Invitrogen). The nucleus of each sample was detected by bisBenzimie Hoechst 33342 trihydrochloride staining (Sigma-Aldrich, St. Louis, MO, USA). Microscopic signals were observed with an Olympus BX50 epifluorescence microscope (Tokyo, Japan), and the images were captured with an Olympus DP70 digital camera and processed with an Olympus DPController with the DPManager software program.

Western blot analyses. The expression of CD155 protein was determinated by a western blot analysis as previously described (26). The primary antibody was a goat anti-CD155 antibody, sc-27754 (1:200, Santa Cruz Biotechnology, Santa Cruz, CA, USA) (27), and the secondary antibody reaction was performed using a peroxidase-conjugated secondary antibody (Dako, Carpinteria, CA, USA) and visualized using the ECL substrates (GE Healthcare, Piscataway, NJ, USA).

In vivo xenograft model. Four-week-old BALB/c nu/nu mice were maintained in a humidity- and temperature-controlled laminar flow room. For xenografting, $1x10^7$ HT1080 cells in 0.1 ml of PBS were subcutaneously injected into the right flanks of nude mice using a 26-gauge needle. In all the mice, enlargement of the tumors was observed within 1-2 weeks after inoculation. The tumor size was measured with calipers two times a week, and the tumor volume was calculated using the ellipsoid formula: length x width^2 x 0.52 (28). When the tumor volume increased to 0.20-0.25 cm^3, LAPV ($1x10^6$ $TCID_{50}$) or vehicle alone (for control animals), was injected into the right flank tumor once a day for 3 days, and the tumor size was monitored every 3-4 days for 2 weeks.

The mice were sacrificed for histopathological analyses and viral preparation assays. To investigate the histopathological findings, paraffin-embedding samples were observed after hematoxylin and eosin and TUNEL staining. For virus propagation assays, tumor samples were immediately frozen and kept at -80˚C until use. After homogenization of the tumor samples with additional PBS up to 20% weight/volume, the tumor tissue homogenates were centrifuged at 2,000 rpm for 20 min at 4˚C, and the viral titer in the supernatants was determined by the $TCID_{50}$ in HeLa cell culture. All experimental procedures using mice were approved by the Institutional Committees on Animal Welfare.

Statistical analyses. The data were expressed as the means ± SE. The statistical difference between groups was analyzed by Student's t-test or the Mann-Whitney U test. Differences were considered statistically significant for $p<0.05$ or $p<0.01$.

Results

Expression of CD155 in bone and soft tissue tumor cells. Since CD155 is required for the poliovirus to infect cells, we first examined the expression of *CD155* mRNA by quantitative real-time PCR. The expressions level of *CD155* mRNA in HT1080, MFH-ino, HS-Sch-2, HS-SY-II, HuO9-M112 Saos-2, HOS, and 143B cells were, respectively, 2.3-, 6.7-, 2.5-, 1.9-, 1.7-, 4.4-, 2.4- and 5.8-fold higher than those in HeLa cells. In NMS-2, HS-PSS, HuO9 and HuO9-M132 cells, the expression levels of *CD155* were almost equal to that of HeLa cells (Fig. 1A).

Next, we confirmed the CD155 expression using an immunofluorescence technique. In all of the human cell lines, CD155 was definitely identified in the cytoplasm and at the intercellular junctions, although the distribution and signal intensity differed among the cell lines (Fig. 1B).

In addition, we performed a western blot analysis to demonstrate the expression level of the CD155 protein. As shown in Fig. 1C, the expression of CD155 protein was observed in all of the bone and soft tissue sarcoma cell lines (molecular weight ~80 kDa).

LAPV induces apoptosis in bone and soft tissue sarcoma cells in vitro. To examine whether LAPV induces cell death in sarcoma cell lines, we investigated the morphological changes in the HT1080 cells after LAPV exposure. Under a phase-contrast microscope, the infected HT1080 cells showed morphological changes such as rounding, shrinkage, detachment, and floating in the culture medium within 48 h (Fig. 2A).

To measure the cell viability after LAPV exposure, 6 human bone and soft tissue tumor cell lines; HT1080, MFH-ino, HS-PSS, HuO9-M112, Saos-2, HOS and one mouse osteosarcoma cell line, LM8, were incubated in the presence of LAPV at a MOI from 2 to $2.0x10^{-4}$ $TCID_{50}$/cell and their viability was measured by the MTS assay. LAPV strongly induced cell death in a time- and dose-dependent manner in 5 out of the 6 human bone and soft tissue sarcoma cell lines (Fig. 2B).

At first, the HS-PSS cells seemed to be resistant to LAPV exposure. But, because the HS-PSS cells infected by the LAPV showed morphological changes such as rounding, shrinkage, detachment, and floating in the culture medium, we supposed that the slow growth of the cells minimized the effect of the poliovirus exposure in the MTS assay. Thus, we next observed the effect of LAPV exposure for 7 days, and found that the viability of the HS-PSS cells exposed to the poliovirus was significantly lower than that of the control cells at 72 h or more