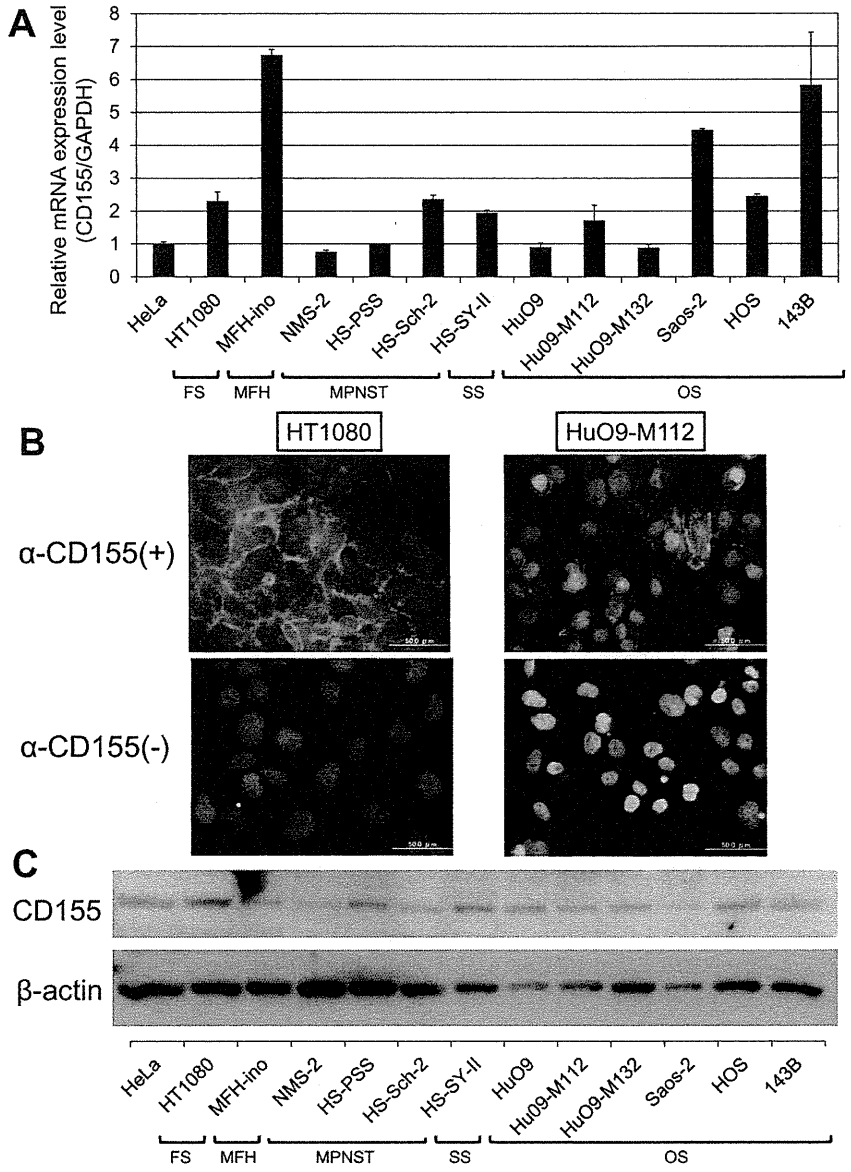

Figure 1. The expression of CD155 in human bone and soft tissue sarcoma cells. (A) The expression of CD155 mRNA in human bone and soft tissue sarcoma cells as determined by quantitative real-time PCR. The relative levels of *CD155* mRNA were calculated after normalization with reference to the expression of GAPDH mRNA. The expression levels of *CD155* mRNA in HT1080, MFH-ino, HS-Sch-2, HS-SY-II, HuO9-M112 Saos-2, HOS and 143B cells were higher than those in HeLa cells. In the NMS-2, HS-PSS, HuO9 and HuO9-M132 cells, the expression levels of *CD155* were almost equal to that of HeLa cells. (B) The immunofluorescence of HT1080 (left) and HuO9-M112 cells (right). CD155 was observed on the cell membrane of these cells. (C) The results of the western blot analysis of CD155 protein expression in human bone and soft tissue sarcoma cells lines. The expression of CD155 protein was observed in all of the bone and soft tissue sarcoma lines that were examined. FS, fibrosarcoma; MFH, malignant fibrous histiocytoma; MPNST, malignant peripheral nerve sheath tumor; SS, synovial sarconma; OS, osteosarcoma.

after infection (data not shown). The viability of the mouse osteosarcoma cell line, LM8, did not differ significantly at any of the time points, and did not exhibit any morphological changes. We considered that the LM8 cell line was completely resistant to LAPV, because mouse cells have no human CD155, which is necessary for the establishment of a poliovirus infection, as was previously known (29).

Our next question was whether the cell growth suppression observed in various sarcoma cell lines was due to apoptotic cell death induced by LAPV. First, we examined the occurrence of apoptotic cell death using the TUNEL assay. We found that the HT1080 cells treated with LAPV had positive staining for the TUNEL reagent (Fig. 3A).

To confirm whether LAPV actually induced apoptotic cell death, the caspase 3/7 activity was measured (Fig. 3C). Our results showed that the poliovirus induced the activation of caspases 3 and 7 in a time- and dose-dependent manner that was consistent with the decrease in cell viability. Hence, we concluded that LAPV induces apoptosis in bone and soft tissue sarcoma cells *in vitro*.

Propagation of live attenuated poliovirus in soft tissue sarcoma cells. Since LAPV was found to induce apoptosis in the bone and soft tissue sarcoma cells, we next examined the propagation of the poliovirus in sarcoma cells. After exposure to LAPV for 30 min at a titer of 0.2 and 2 $TCID_{50}$/cell, the cell-associated

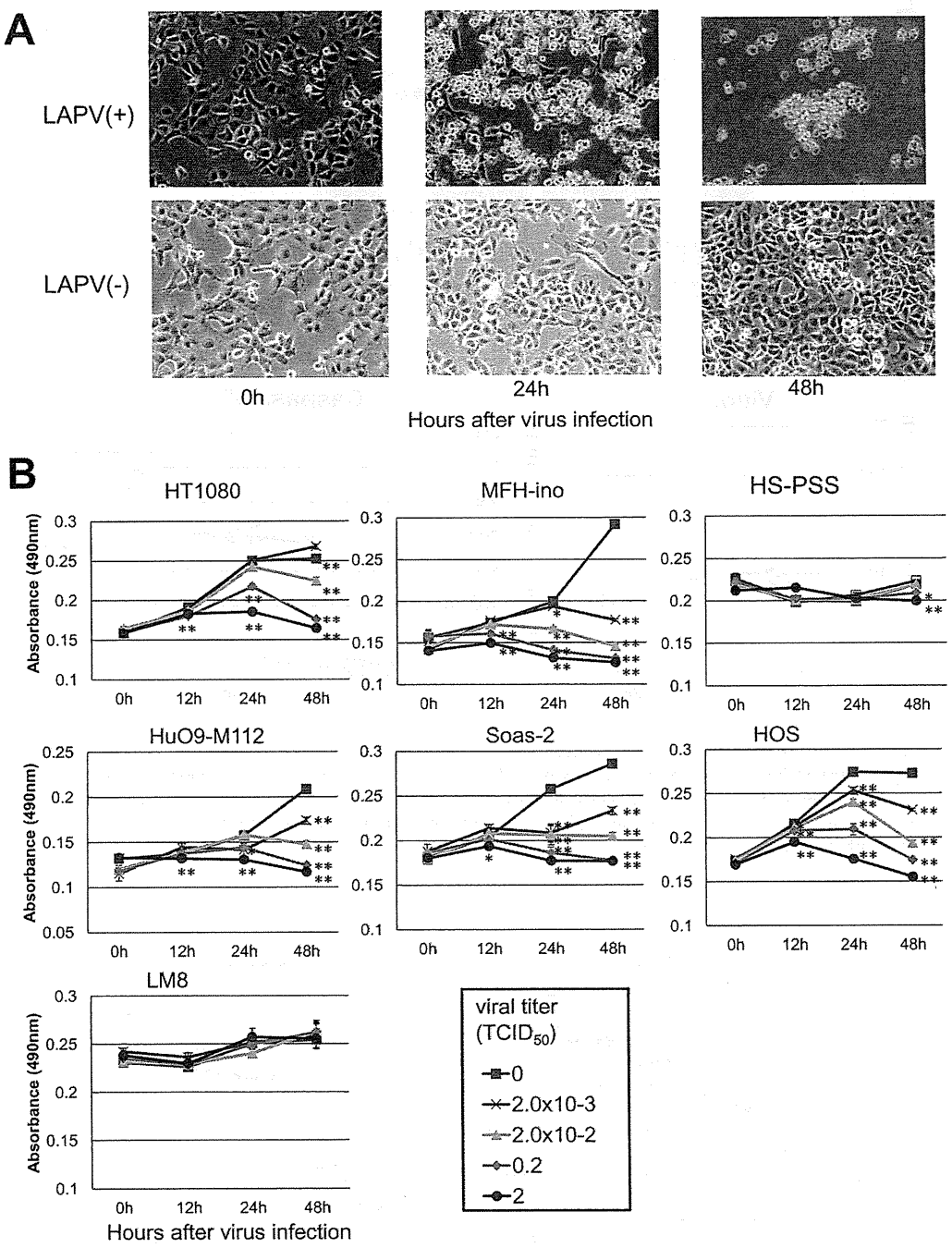

Figure 2. The effect of LAPV. (A) HT1080 cells were photographed before, 24 and 48 h after LAPV infection. (B) The human fibloblastoma cell line, HT1080, human malignant fibrous histiocytoma cell line, MFH-ino, human MPNST cell line, HS-PSS, human osteosarcoma cell lines, HuO9-M112, Saos-2 and HOS, and the mouse osteosarcoma cell line, LM8, were incubated with LAPV for 12, 24 or 48 h at the indicated MOI (■, 0 $TCID_{50}$; x, $2x10^{-3}$ $TCID_{50}$; ▲, $2x10^{-2}$ $TCID_{50}$; ◆, 0.2 $TCID_{50}$; ●, 2 $TCID_{50}$). At different intervals, the cell viability was assessed 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. The viability of mouse osteosarcoma LM8 cells did not differ significantly at any of the time points. The results are expressed as the means ± SE. *P<0.05 and **P<0.01 vs. the control (vehicle, 0 $TCID_{50}$) group as determined by Student's t-test.

and extracellular viral yields were determined by the $TICD_{50}$ assay. We found that one-step viral growth curves showed the propagation of LAPV in monolayer cultures (Fig. 4).

Live attenuated poliovirus kills soft tissue sarcoma cells in vivo. The inherent capacity for LAPV to kill bone and soft tissue sarcoma cells *in vitro* suggested that the virus might be therapeutically useful. To test the oncolytic properties of LAPV *in vivo*, mice bearing subcutaneous HT1080 xenograft tumors were treated with an intratumoral inoculation of LAPV ($1x10^6$ $TICD_{50}$) once a day for 3 days. The size of the xenotransplants in control mice increased by 16.9-fold two weeks after treatment. However, three injections of LAPV inhibited the tumor growth by nearly 40% ($p<0.05$ at 7 days or more, Mann-Whitney U test) (Fig. 5). Histopathologically, many TUNEL positive cells were scattered in the xenotransplants on day 1 after treatment with

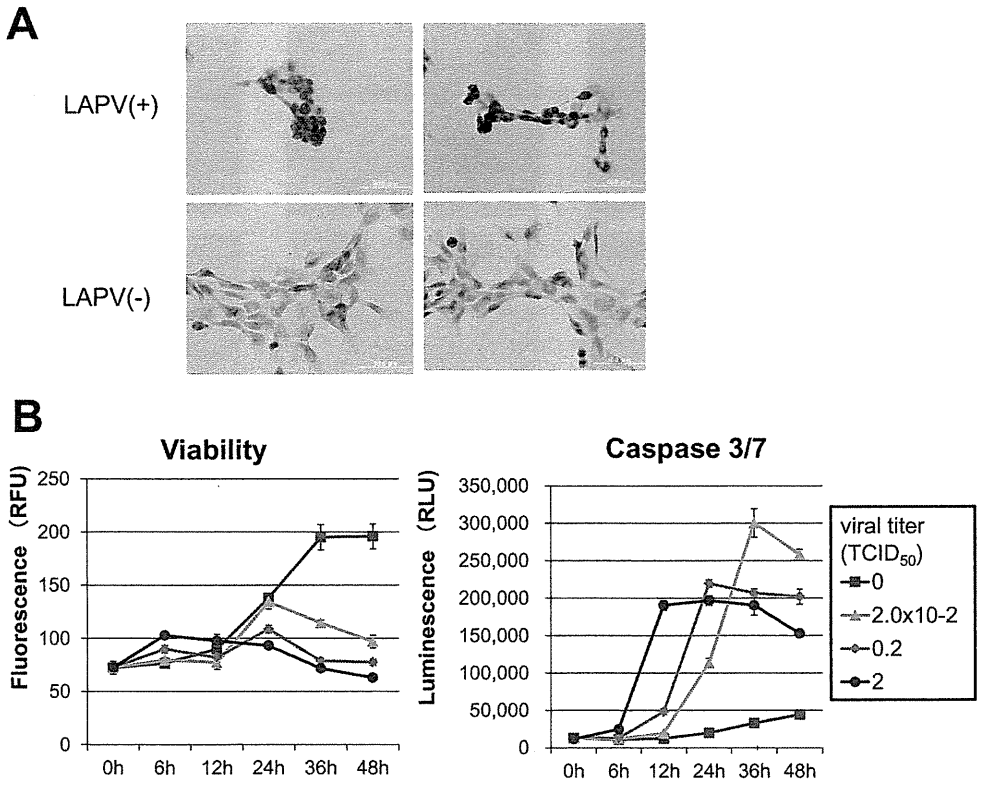

Figure 3. The results of the apoptosis assay in HT1080 cells infected by LAPV. (A) HT1080 cells were incubated with LAPV at a MOI of 2 $TCID_{50}$. Apoptotic HT1080 cells were detected by the TUNEL assay. Positive staining of HT1080 cells was observed after LAPV inoculation (upper panel). (B) The caspase 3/7 activity (right graph) and viability (left graph) of HT1080 cells incubated with LAPV for 6, 12, 24, 36 or 48 h at the indicated MOI (■, 0 $TCID_{50}$; ▲, $2x10^{-2}$ $TCID_{50}$; ◆, 0.2 $TCID_{50}$; ●, 2 $TCID_{50}$) was measured by the ApoTox-Glo Triplex Assay. The poliovirus induced the activation of caspase 3/7 in a time- and dose-dependent manner that was consistent with the decrease in cell viability.

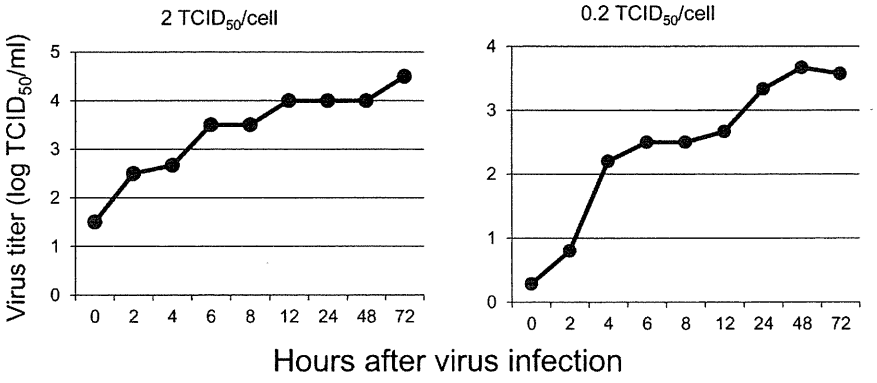

Figure 4. The propagation of LAPV in the HT1080 human fiblosarcoma cell line. One-step growth curves for LAPV were obtained after synchronized infection of monolayer cultures at a MOI of 2 (left graph) and 0.2 $TCID_{50}$/cell (right graph). LAPV was propagated in HT1080 cells.

LAP, and the areas of TUNEL positive degenerated tissue were obviously increased on day 3 (Fig. 6). TUNEL-positive cells were rarely observed in the xenotransplants treated with the vehicle.

To investigate whether the LAPV is propagated in the xenotransplants, the viral titer was determined using the supernatants of tumor tissue homogenates by measuring the $TCID_{50}$ in HeLa cell culture. The maximum virus titer was observed on day 3 after inoculation into the tumor (Fig. 7). This indicated that intratumoral replication of LAPV induced the release of a number of infectious viral particles, which lead to the apoptotic cell death of the xenografts.

Discussion

The clinical outcome of patients with advanced bone and soft tissue sarcomas is still unsatisfactory, despite the use of multidisciplinary treatments including surgery, chemotherapy, and radiotherapy (5,30). Therefore, the development of a novel therapeutic agent is necessary to improve the prognosis of

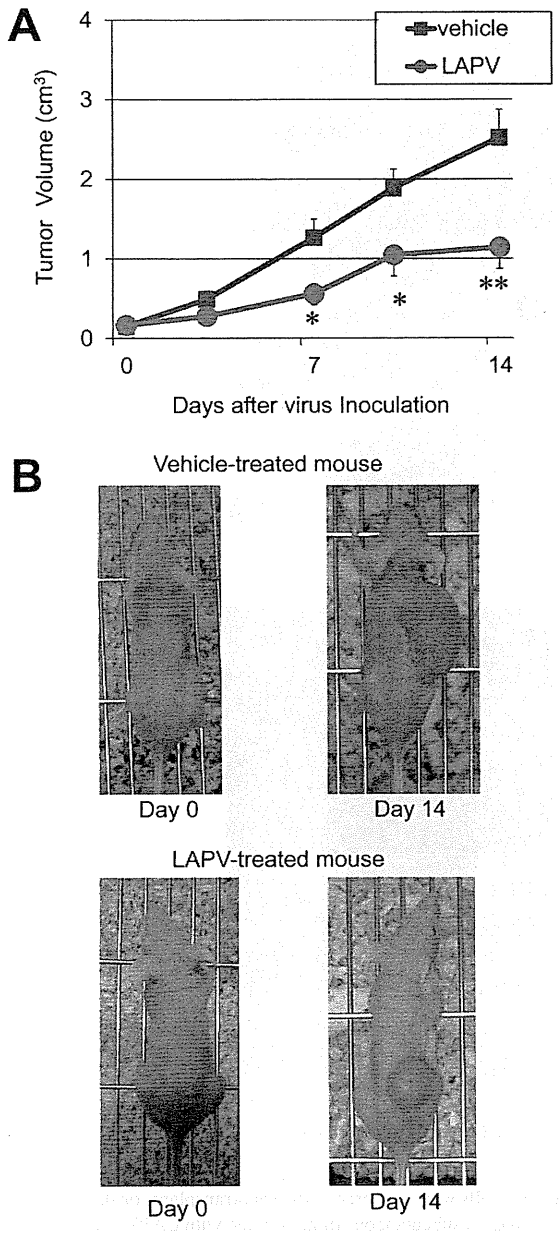

Figure 5. The effects of the LAPV on xenografted HT1080 tumors. HT1080 cells were implanted subcutaneously into the right flank of nude mice to form solid tumors. LAPV (n=14) or vehicle (n=15) was injected into right flank tumor once a day for 3 days. (A) The mean volumes of tumors inoculated with vehicle (■) and LAPV (●). Tumor volumes were caluculated as: tumor volume (cm^3) = 0.52 x a x b^2 (a and b being the longest and shortest diameters of the tumor, respectively). LAPV injection significantly inhibited the tumor growth compared with vehicle injection from day 7 [data are expressed as the means ± SE. $^*P<0.05$ and $^{**}P<0.01$ vs. the control (vehicle) group as determined by the Mann-Whitney U test]. (B) The growth of the tumors treated with LAPV (upper panel) was significantly suppressed compared to the tumors treated with vehicle (lower panel).

these patients. In this study, we showed that LAPV possess an inherent capacity to induce apoptosis in bone and soft tissue sarcoma cells *in vitro* and to suppress the growth of implanted fibrosarcoma cells *in vivo*.

The poliovirus receptor CD155, is a key molecule for oncolytic virotherapy using LAPV, because the expression of CD155 is essential for poliovirus binding and infection (29). We initially supposed that CD155 might be expressed in neurogenic sarcomas, such as MPNST, because the upregulation of CD155 expression was observed in neuroectodermal malignancies (e.g. glioblastoma multiforme, medulloblastoma, or neuroblastoma) (31-33). Surprisingly, however, CD155 expression was observed in all 12 bone and soft tissue sarcoma cell lines examined. This is the first report to show that CD155 is widely expressed in various types of bone and soft tissue sarcoma cell lines. Solecki *et al* previously showed that the expression of the *CD155* gene is transcriptionally activated through the Sonic Hedgehog (SHH) signaling pathway (34). Recently, activation of the hedgehog signaling pathway was reported in certain sarcoma types (35,36). Upregulated expression of CD155 was demonstrated in NIH3T3 cells transformed by the V12-Ki-Ras oncogene, and it contributes to the loss of contact inhibition in transformed cells (37). Further investigation will be needed to clarify the mechanism of upregulation of *CD155* gene expression in sarcoma cells.

We showed that LAPV can kill various types of sarcoma cells, and that there is viral propagation. Gromeier *et al* have exploited this feature of LAPV to kill glioma cells (15). Poliovirus uniquely depends on CD155 for host cell binding and entry. Based on all available empirical evidence, CD155 is sufficient for all binding and entry functions leading to uncoating of the viral genome (29,38). At first, the HS-PSS cells seemed to be resistant to LAPV exposure. However, the HS-PSS cells infected with the LAPV showed morphological changes such as rounding, shrinkage, detachment, and floating. Since HS-PSS cells have a long cell cycle, we observed the effect of LAPV exposure for 7 days, and confirmed that the HS-PSS cells were also susceptible to LAPV infection. Because of the lack of human type of CD155 expression, the poliovirus could not infect mouse LM8 cells. We concluded that all human bone and soft tissue sarcoma cell lines were susceptible to LAPV infection, and that this was dependent on their CD155 expression.

We showed that the poliovirus infection triggered apoptosis in bone and soft tissue sarcoma cells expressing CD155 by TUNEL staining and the ApoTox-Glo Triplex assay. The ApoTox-Glo Triplex assay showed that the poliovirus induced cell death due to activation of the apoptosis pathway in a time- and dose-dependent manner. In addition, LAPV was propagated in HT1080 cells, as indicated by the one-step viral growth curves analysis. These results suggest that sarcoma cells are targets of the poliovirus. In previous studies, poliovirus infection triggered apoptosis in neuroblastoma cells expressing CD155, as shown by DNA fragmentation, activation of effector caspase activity, and mitochondrial dysfunction (24,39). The replication of poliovirus is restricted to neurons in the spinal cord and brainstem, although CD155 expression is observed in both the target and non-target tissues in humans. This tissue tropism results in a distinct disease pattern unique for poliovirus (40). Ida-Hosonuma *et al* showed that α/β interferon (IFN) determine the tissue tropism by comparing the pathogenesis of the virulent Mahoney strain in CD155-transgenic mice and CD155-transgenic mice deficient in the α/β IFN receptor gene (CD155-transgenic/*Ifnar* knockout mice) (41). CD155-transgenic/*Ifnar* knockout mice showed increased susceptibility to poliovirus. They subsequently examined the expression of IFN and IFN-stimulated genes (ISGs) in the CD155-transgenic mice. In the non-target tissues, ISGs were expressed even in the non-infected state, and the expression level increased soon after poliovirus infection. On the contrary,

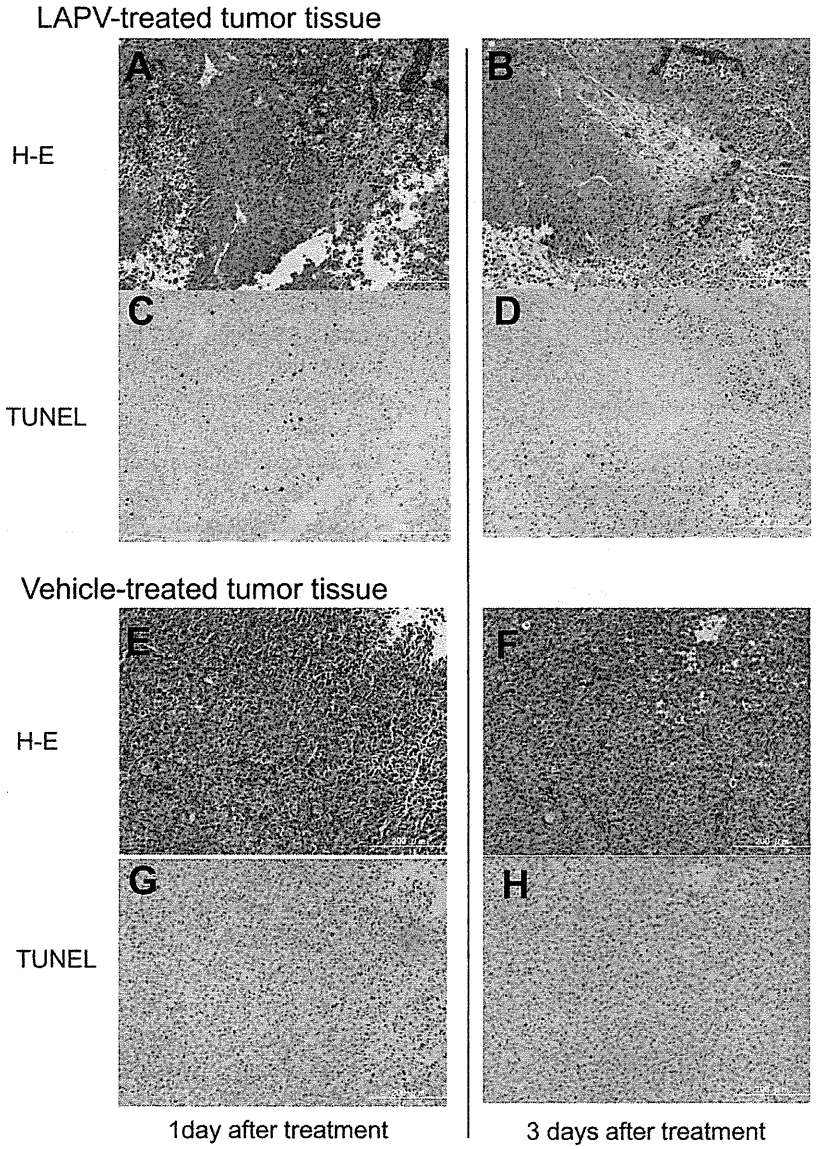

Figure 6. The histopatological evaluation of xenografts after LAPV treatment. TUNEL-positive cells were scattered in the xenotransplants on day 1 after treatment with LAPV, and the areas of TUNEL positive tissue were apparently increased on day 3. (A and C) A subcutaneous tumor treated with LAPV 1 day after treatment. (B and D) A tumor treated with LAPV 3 days after the first treatment. (E and G) A subcutaneous tumor treated with vehicle 1 day after treatment. (F and H) A tumor treated with vehicle 3 days after the first treatment. Sections were stained with hematoxylin and eosin (A-B and E-F) or TUNEL (C-D and G-H). Original magnification, x100.

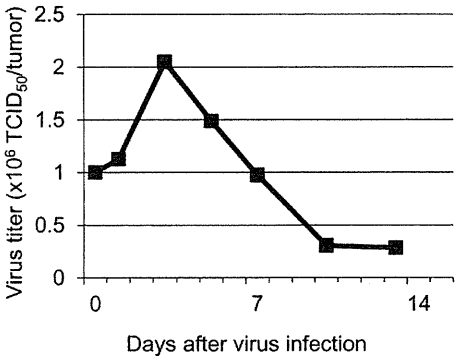

Figure 7. The results of the growth curve analysis of poliovirus in the LAPV-treated tumors. Nude mice bearing subcutaneous HT1080 xenografts were treated with LAPV, euthanized at the indicated intervals, and the viral load in the tumors was measured by the $TCID_{50}$. The maximum virus titer was observed on day 3 after inoculation of the tumor.

in the target tissues, ISG expression was low in the non-infected state and a sufficient response after poliovirus infection was not observed. Although we did not observe the expression of IFN and ISGs in the present study, the low IFN response to the poliovirus may be one of the important determinants of the good susceptibility of sarcoma cells.

We demonstrated that there is efficient oncolysis of subcutaneous xenografted sarcoma in nude mice after intratumoral injection of LAPV. The intratumoral replication of LAPV induced the release of a number of infectious viral particles, which lead to the apoptotic cell death of the xenografts. Therefore, LAPV could be useful for the treatment of bone and soft tissue sarcoma.

The inherent neuropathogenicity of poliovirus is a point of major concern with regard to its potential therapeutic applications. Typically, poliovirus infects the gastrointestinal tract,

causing mild symptoms or no symptoms at all. In 1 to 2% of infections, the poliovirus invades the central nervous system, where it uniquely targets motor neurons for destruction, resulting in flaccid paralysis (42). Paralytic poliomyelitis is considered to result from an invasion by circulating poliovirus into the central nervous system, probably via the blood-brain barrier. This notion is supported by a previous study using a mouse model (43). However, an accumulation of patients with vaccine-associated paralytic poliomyelitis following vaccination with orally administered, attenuated poliovirus (OPV) in Romania has been linked to multiple intramuscular (i.m.) injections of various therapeutic or preventive agents administered to OPV recipients (a phenomenon known as 'provocation' poliomyelitis) (44). Recently, the mechanism of provocation poliomyelitis has been proven using poliovirus-sensitive transgenic mice produced by introducing the human *CD155* gene into the mouse genome. Poliovirus particles exist on vesicle structures in the nerve terminals of neuromuscular junctions (45). Skeletal muscle injury induces retrograde axonal transport of poliovirus, and thereby facilitates the viral invasion of the central nervous system and the progression of spinal cord damage (46). In addition, the direct interaction between the cytoplasmic domain of CD155 and Tctex-1 is essential for the efficient retrograde transport of PV-containing vesicles along microtubules *in vivo* (45).

In our study, LAPV was injected into the right flank tumor once a day for 3 days. It remains unclear whether the intratumoral injection of the LAPV through the muscle surrounding the tumor would be a safe procedure in human subjects. To treat patients with bone and soft tissue sarcomas, it will be necessary to use a highly attenuated phenotype of poliovirus which shows exceedingly poor infectivity in normal neuronal cells while retaining its oncolytic effects against bone and soft tissue sarcomas.

In infected cells, the translation of the plus-strand poliovirus RNA genome is directed by the internal ribosome entry site (IRES), a cis-acting RNA element that facilitates the cap-independent binding of ribosomes to an internal site of the viral RNA. In each Sabin vaccine strain, a single point mutation in the IRES secondary-structure domain V is a major determinant of the neurovirulence attenuation. This point mutation is an A-to-G exchange in the Sabin 1 vaccine strain (A480G according to the Sabin 1 nucleotide numbering) (47). However, because of insufficient genetic stability, the Sabin strain may mutate and convert to a virulent virus during replication in bone and soft tissue sarcomas. To eliminate the neuropathogenic potential, many types of intergeneric poliovirus recombinants were constructed and utilized for the treatment of malignant glioma (14,15,23) and neuroblastoma (48). To treat patients with bone and soft tissue sarcomas, it will be necessary to develop a recombinant poliovirus with a highly attenuated phenotype which minimizes the genetic instability as much as possible.

In the present study, LAPV was injected into the right flank tumor of 4-week-old BALB/c nu/nu mice with an inhibited immune system due to their greatly reduced number of T cells, which leads to impaired antibody formation that requires $CD4^+$ helper T cells. If this treatment strategy is applied to human subjects, the treatment efficacy may be reduced by the neutralizing antibodies against poliovirus which were generated in response to a previous vaccination. However, Toyoda *et al* (48) showed that neuroblastoma tumors xenografted into CD155 transgenic mice that had previously been immunized with poliovirus still remarkably regressed after intratumoral injection of poliovirus without any side effects. Other previous investigations have also shown that treatment with oncolytic viruses can result in enhancement of the antitumor immune response *in vivo* (49,50). Thus, we presume that previous poliovirus vaccination might not inhibit the oncolytic effect of LAPV, but actually intensify the oncolysis.

Another potential pitfall is that during a persistent poliovirus infection in a previous study, specific mutations were selected in the first extracellular domain of the *CD155* of human neuroblastoma cells, and these mutations increased the resistance of cells to poliovirus-induced lysis (51). The same phenomenon may be observed during the clinical application for bone and soft tissue sarcoma.

In brief, we demonstrated the expression of both CD155 mRNA and protein in bone and soft tissue sarcoma cell lines, and LAPV has oncolytic effects on bone and soft tissue sarcoma cells *in vitro* and *in vivo*. The results of our study suggest that LAPV has potential for the clinical treatment of bone and soft tissue sarcomas, but further *in vitro* and *in vivo* studies will be required to evaluate the safety of this strategy.

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The use of muscle relaxants during induction may result in a situation where the anesthetist can neither manually inflate the lungs nor intubate[5] Maintaining spontaneous ventilation retains a degree of muscle tone and allows time to view structures and use the fiberoptic bronchoscope to intubate (Fig. 1). The use of the bronchoscope helps confirm tracheal tube placement and avoid spinal movement during intubation.[4,5]

In a pediatric patient with torticollis of any cause, fiberoptic bronchoscopic intubation under inhalational anesthetic as an inducing agent is the procedure of choice.

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Head Positioning for Reduction and Stabilization of the Cervical Spine During Anesthetic Induction in a Patient With Subaxial Subluxation

To JNA Readers:

Airway management in patients with severe displacement of the cervical spine with or without neurological deficits is a demanding task. Intubating devices, such as the Airway Scope (AWS), can be used to facilitate airway management in this patient population. Laryngoscopy with the AWS has been shown to produce less cervical spine motion than conventional laryngoscopy,[1] and thus, may be considered as an alternative technique for securing the airway. Nevertheless, anesthetic induction in these patients still remains a cause for concern. Despite numerous observations about cervical spine motion during tracheal intubation, there is little knowledge about changes in the cervical spine during anesthetic induction in patients with severe subluxation. We present a preferable head position before induction, and successful tracheal intubation using AWS, in a patient with subaxial subluxation accompanied by severe neurological deficits.

A 63-year-old woman with developmental subaxial subluxation with anterior displacement of the C4 vertebra due to rheumatoid arthritis was scheduled to undergo C2 to C6 laminectomy and posterolateral internal fusion. She suffered from severe neck pain, a spastic gait, and deficient fine motor skills in her fingers. The patient's cervical spine was observed under C-arm fluoroscopy during voluntary flexion and extension of the neck, with the patient in the supine position with a cushion under her back at the scapular level. Only a slight reduction in the subluxation, with little canal widening, was seen even on maximal voluntary extension (Figs. 1A, B).

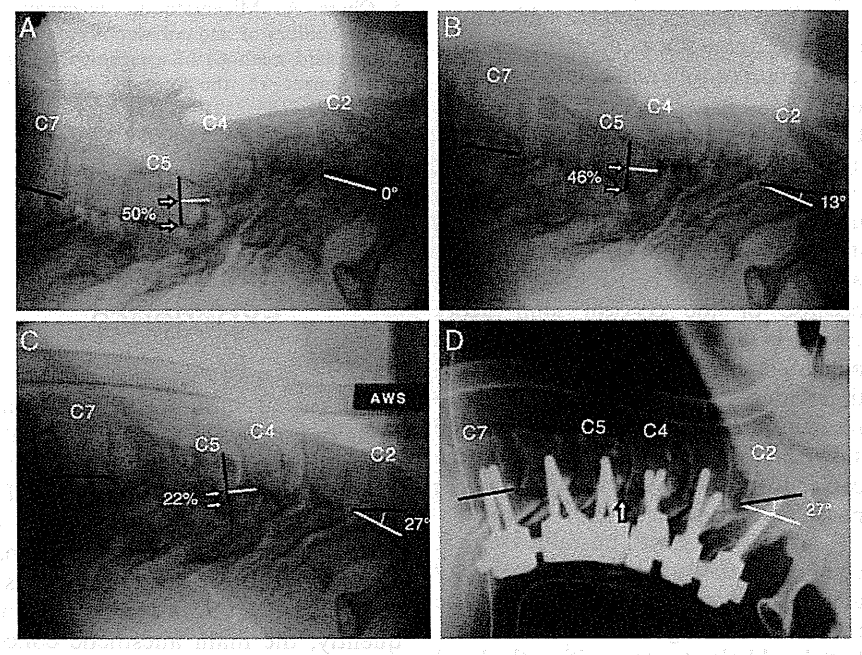

FIGURE 1. A, With maximal voluntary flexion, the slip percentage at the C4 to C5 level was 50% [*A* is the length of C5 vertebra endplate (red line), B is the distance between the upper and the lower arrows; the upper arrow indicates the point at the intersection of a red line with a yellow line that is drawn at a right angle to a red line through the posteroinferior corner of C4. The lower arrow indicates the posterior end of the C5 vertebra endplate; slip percentage = $(B/A) \times 100$]. The absolute rotation angle (ARA; sagittal tangent method), defined as the angle between the posterior vertebral margins of C2 (white line) and C7 (black line), respectively (black line on C2 is a parallel shift of C7), was almost 0 degrees.[5] B, With maximal voluntary extension, the percentage of slip decreased to 46%. ARA increased to 13 degrees. C, With anesthetic induction, the slip percentage showed a significant improvement to 22%. ARA was 27 degrees, which is close to the physiological cervical lordosis. This indicated that the cervical spine was adequately stabilized. No exacerbation of subluxation was seen in the process of Airway Scope (AWS) intubation. D, The operation was performed successfully, and alignment of the cervical spine was achieved completely (arrow; ⇧). ARA was 27 degrees.

The authors have no funding or conflicts of interest to disclose.

For anesthetic induction, remifentanil at a dose of 0.5 μg/kg/min (total, 120 μg), 40 mg propofol, and 30 mg rocuronium were administered, with the patient positioned with her neck in maximum extension. During mask ventilation, application of airway maneuvers was avoided to prevent motion of the neck. With increasing anesthetic depth, reduction in the subluxation and widening of the spinal canal accompanied by correct alignment of the curve of the cervical spine were seen under C-arm fluoroscopy. Tracheal intubation [with a 7.0-mm Parker Flex-Tip PFRC (Parker Medical; Englewood, CO)] was easily achieved with the aid of the AWS, without exacerbation of the subluxation (Figs. 1C, D). Postoperatively, there was no worsening of her neurological condition (Figs. 1D).

Reduction of unstable subluxation of the cervical spine often requires the application of firm and continuous axial traction using a Halo or Gardner-Wells tongs.[2–4] In this case, the reduction and alignment correction obtained during voluntary flexion and extension was minimal. However, AWS-aided tracheal intubation could be safely performed with a suitable cervical spine position because of the cervical joint laxity caused by the anesthetic agents.[3] In this patient, it is likely that anesthetic induction with her neck in flexion could have resulted in anterior displacement of the subluxated vertebra, exacerbating the cervical canal stenosis and resulting in neurological deterioration.

In patients with unstable cervical spines, anesthetists should not only pay attention to minimizing cervical spine movement during tracheal intubation, but should also try to position the head in a way that would align the cervical spine, allowing reduction and stabilization during induction. When necessary, C-arm fluoroscopy should be used to determine the best possible head positioning for induction and intubation of the patient.

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Awake Craniotomy Under Xenon Anesthesia: First Experience

To JNA Readers:

Awake craniotomy has traditionally been used for the removal of epileptic foci or intracranial tumors adjacent to the eloquent cortex. This method provides an opportunity for intraoperative awake brain mapping and prevents functional injury, especially aphasia, after surgery.[1] Consequently, the main anesthetic concern for awake craniotomy is rapid reversibility and smooth awakening during the operation. We report the first case of the use of xenon as a sole anesthetic agent for awake craniotomy.

A 35-year-old male high school lecturer (74 kg) was admitted with low-grade glioma with a cystic component in the left frontotemporal region. The patient suffered from partial seizures in his right hand and leg 3 times a day. He also noted memory and speech impairments for the past month. He was treated with dexamethasone and valproate, but his symptoms worsened. One day before surgery, he had lowered consciousness—he could only count from 1 to 10 and name months of the year.

On arrival in the operating room, the patient was monitored with a pulse oximeter, an electrocardiogram, and a noninvasive blood pressure cuff. Anesthesia was induced with propofol 200 mg and fentanyl 100 mcg IV. A laryngeal mask airway (LMA) was then positioned. Ventilation was started in control mechanical ventilation mode so that it led to mild hyperventilation with ET_{CO_2} 32 ± 2 mm Hg. A Taema Felix Dual anesthesia machine (ALMS, France) designed for anesthesia with xenon was used. During a 10-minute denitrogenation, regional anesthesia of scalp nerves and local anesthesia of the skin incision line was performed with 25 mL ropivacaine 0.75%. During this period, anesthesia was maintained with a propofol infusion of 4 mg/kg/h. The radial artery was cannulated for blood gas sampling and direct blood pressure monitoring. Simultaneously, bispectral index (BIS) monitoring was started. After that, the machine was switched to the xenon mode with FiO_2 30% and xenon concentration as high as 54% to 65%. As soon as BIS lowered to 40, the propofol infusion was stopped and the operation began.

From the beginning of the operation and before the awakening (approximately 120 min), anesthesia was maintained only with xenon inhalation titrated to BIS values 35 to 50. The blood gases were normal. The hemodynamic status was stable with arterial blood pressure 130 to 140/80 to 90 mm Hg and heart rate 55 to 60 min^{-1}. According to the preoperative clinical signs, we expected low intracranial compliance due to brain swelling, but the surgeon described the tissue tension as satisfactory. When the surgeons and the neurophysiologist were ready for the stimulation, the xenon was switched off and washed out with an oxygen flow of 8 L/min. Six minutes later, the patient was awakened and the LMA was removed. The stimulation

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Cobalt and Chromium Ion Release After Large-Diameter Metal-on-Metal Total Hip Arthroplasty

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Abstract: Seventy-five patients underwent unilateral metal-on-metal total hip arthroplasty using a large-diameter head. Serum levels of cobalt and chromium were determined. Significant increases in both cobalt and chromium were observed at 3 months (cobalt, 1.4 μg/L; chromium, 1.4 μg/L) compared with preoperative values ($P < .001$). At 1 year, the median cobalt and chromium levels were 2.3 and 2.1 μg/L, respectively, and the levels had increased significantly compared with 3 months ($P < .001$). There were no significant differences between levels of either metal at 1 or 2 years (cobalt, 2.3 μg/L; chromium, 1.6 μg/L). Pseudotumor occurred in 2 hips. Patients with large-diameter metal-on-metal total hip arthroplasty had higher circulating metal ion levels at 3 months and 1 year, with no additional significant increases at 2 years. **Keywords:** total hip arthroplasty, metal on metal, metal ion, lymphocyte, pseudotumor.

Metal-on-metal bearings for total hip arthroplasty (THA) and hip resurfacing have gained popularity. It is estimated that 35% of THA procedures performed in the United States between October 2005 and December 2006 involved metal-on-metal bearings [1]. Increasing femoral head size in hip arthroplasty is beneficial in many aspects, especially for joint stability and avoidance of component impingement [2]. Limited data on systemic metal concentrations in patients with THA using a large-diameter head are available [3,4]. However, possible complications resulting from the dissemination of metal particles and ions throughout the body are a cause for concern. The particles are nanometers in size and high in number [5], and their dissolution results in measurable increases in cobalt and chromium ions in serum, urine, and red blood cells of patients with a metal-on-metal bearing [6]. Many concerns still remain regarding the effects of prolonged exposure to increased metal ion levels, such as hypersensitivity, carcinogenicity, and fetal exposure to metallic ions in pregnant women [7,8]. In addition, recent studies showed increased metal-related problems, including aseptic lymphocyte-dominated vasculitis-associated lesion and the formation of pseudotumor [9-11].

Our main purpose was to determine whether metal concentrations continue to increase after metal-on-metal THA. We investigated serum levels of cobalt and chromium ions in patients with large-diameter metal-on-metal THA.

Methods

From May 2008 to September 2009, 113 consecutive primary THA procedures were performed in our department. Study exclusion criteria included the presence of other metallic implants. This resulted in 75 patients with unilateral metal-on-metal THA being included in the study (11 men and 64 women). The mean age of participants was 65 years (range, 40-84 years), and the mean body mass index was 23.6 kg/m^2 (range, 18.3-34.9 kg/m^2). The preoperative diagnoses were osteoarthritis in 73 patients and idiopathic osteonecrosis of the hip in 2 patients.

All patients underwent primary cementless THA using a large-diameter head (40, 44, 48, or 52 mm) with a Cormet cup and CTi II stem (Corin, Cirencester, UK) with a metal-on-metal articulation. The Cormet cup and large-diameter head were both made of a cast, high-carbon content cobalt-chromium alloy (0.35% C), which was subject to hot-isostatic pressing and solution annealing (double-heat treatment) before the machining

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process. The Cormet cup was coated with titanium plasma spray for bone ingrowth and implanted by the press-fit technique. The cup can be used with either hip resurfacing or THA systems. The large-diameter head had an open design. Between the head and the stem, no modular adapter was needed. The CTi II stem, which was made of a titanium alloy, had a proximally plasma spray coating. Articular surface roughness was less than 0.05 μm, articulation surface sphericity deviation was less than 8 μm, and component radial clearance was 100 μm, according to the manufacturer. The mean diameter of the acetabular component was 51 mm (range, 46-58 mm). The most used size in percentage was 52 mm (24%). The acetabular component inclination angle was measured on anteroposterior pelvic radiographs. Inclination angle was defined as the angle between the line joining the inferior teardrop points and the axis of opening of the acetabular component. Acetabular anteversion was measured with computer software (Advanced CasePlan Digital Templating Planning Software; Stryker Orthopedics, Mahwah, NJ). The mean inclination angle was 42° (range, 27°-55°), and anteversion angle was 15° (range, 1°-28°).

Clinical evaluation was performed using the Merle d'Aubigne and Postel scoring system, assigning a maximum of 6 points to pain, mobility, and ability of walk, respectively. A score of 18 points is a maximum score [12]. We assessed the score before primary arthroplasty and at 3 months and 1 and 2 years after surgery. This study was approved by the ethics committee of our institution, and all patients gave informed consent.

Blood samples (8 mL) were taken preoperatively and at 3 months and 1 and 2 years after surgery using cobalt-free needles and glass tubes for trace metal analysis without additives for blood collection to avoid metal contamination. Collected blood samples were allowed to clot for 20 minutes and then were centrifuged at 3000 rpm for 15 minutes. Serum samples were submitted for analysis by Mitsubishi Chemistry Medience Co, Ltd (Tokyo, Japan). Serum samples were stored at −20°C in inert polystyrene tubes until assayed. Cobalt levels were assayed at Mayo Medical Laboratories (Rochester, Minn), whereas chromium was assayed at Mitsubishi Chemistry Medience Co, Ltd. Detection limits for each ion were 0.2 μg/L. Cobalt levels were assayed using inductively coupled plasma mass spectrometry (Perkin-Elmer SCIEX Elan 6100 DRC ICP-MS system; Perkin-Elmer Instruments, Norwalk, Conn). Chromium levels were assayed using a graphite furnace atomic absorption spectrometer (Z-5700; Hitachi Ltd, Tokyo, Japan) with polarization Zeeman absorption. All concentrations below that limit were defined as 0.2 μg/L for both cobalt and chromium to allow for statistical calculations.

Complications occurred in 2 patients (Table 1). Both patients developed pain and swelling in their hips. Radiographs revealed no evidence of loosening. Magnetic resonance images and computed tomography scans revealed pseudotumor. The patients were switched to a metal-on-polyethylene articulation 1.9 and 2.5 years postoperatively. All samples of pseudotumor were fixed in 10% neutral-buffered formalin before processing and embedding in paraffin wax. Five-micrometer-thick sample sections were stained with hematoxylin and eosin and examined by light microscopy. Sections of the pseudotumor were also analyzed by immunohistochemistry using antibodies to T lymphocytes (CD3; DAKO, Glostrup, Denmark), B lymphocytes (CD20; DAKO), and macrophages (CD68; DAKO) to characterize the immunophenotype. One month postrevision, a lymphocyte transformation test was performed in these patients.

Revised components underwent macroscopic and microscopic examinations using scanning electron microscopy.

Statistical analysis

For each time point, the median and the 25th and 75th percentiles of cobalt and chromium concentrations were calculated. The Wilcoxon signed rank test was used to compare median concentrations of cobalt and chromium over time. Differences in metal concentrations and head diameters between genders were examined using the Mann-Whitney *U* test. Preoperative and postoperative Merle d'Aubigne and Postel scores were compared using the Wilcoxon signed rank test. The acetabular component inclination and anteversion, the head diameter, the age of the patient, body mass index, and Merle d'Aubigne and Postel score were correlated with serum concentrations of cobalt and chromium using the Spearman rank correlation test, and a multiple regression test was also used. Statistical significance was set at $P < .05$.

Results

Preoperative and postoperative serum concentrations of cobalt and chromium are shown in Fig. 1. The median

Table 1. Detail of the 2 Patients With Pseudotumor

Patients	Age (y)	Gender	Body Mass Index (kg/m^2)	Years in Situ	Cup Size (mm)	Head Diameter (mm)	Cup Inclination (°)	Cup Anteversion (°)
Case 1	58	M	31.7	1.9	54	48	51	6
Case 2	69	F	24.8	2.5	50	44	35	18
All	65*	11 M, 64 F	23.6*	2.5*	51*	44*	42*	15*

M indicates male; F, female.
* Values are given as mean.

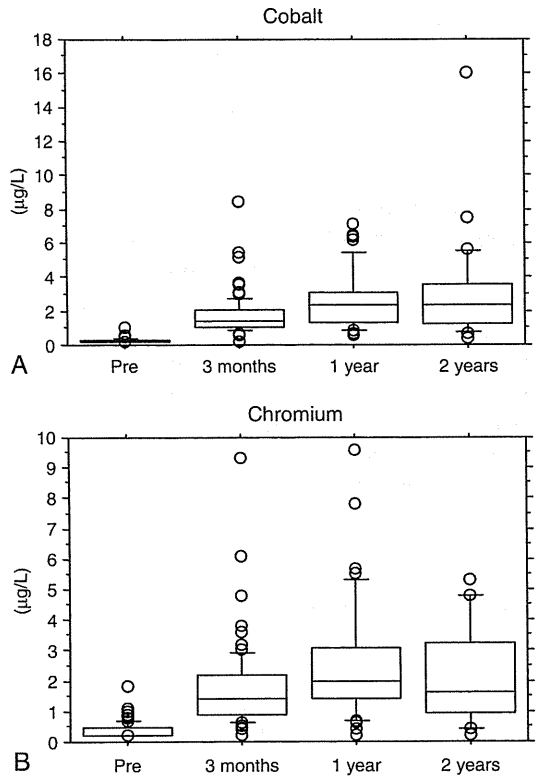

Fig. 1. Serum levels of cobalt (A) and chromium (B) in all patients. The top of the box represents the 75th percentile; the bottom, the 25th percentile; and the line in the middle, the 50th percentile. Bars indicate the range of the 10th and 90th percentiles. Circles represent outliers.

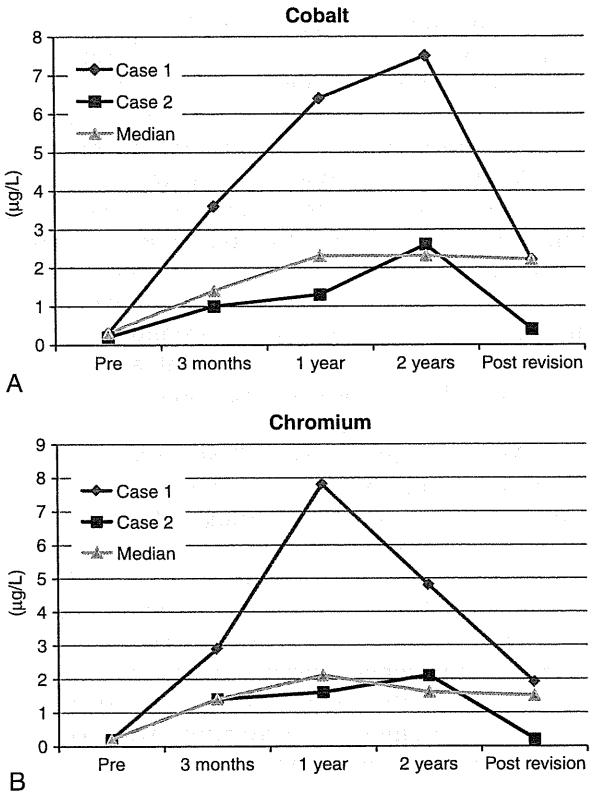

Fig. 2. Serum levels of cobalt (A) and chromium (B) in the patients with pseudotumor. Case 1 showed elevated metal levels.

preoperative serum cobalt and chromium concentrations were 0.3 and 0.2 μg/L, respectively. Significant increases in both cobalt and chromium were observed at 3 months (1.4 μg/L for both cobalt and chromium) compared with preoperative values ($P < .001$). At 1 year, levels of both cobalt (2.3 μg/L) and chromium (2.1 μg/L) had increased significantly compared with levels at 3 months ($P < .001$). There were no significant differences between levels of either metal at 1 and 2 years (2.3 μg/L for cobalt [$P = .234$]and 1.6 μg/L for chromium [$P = .303$]).

In the patients with pseudotumor, serum levels of cobalt and chromium are shown in Fig. 2. Histologic examination showed extensive necrosis and lymphocytic infiltration in periprosthetic tissues of the hips in both cases. We identified perivascular lymphocytes and diffusely distributed lymphocytes. The immunohistochemical methods suggested more CD3-positive T lymphocytes (Fig. 3) than CD20-positive B lymphocytes. CD68-positive macrophage infiltration was also found. Lymphocyte transformation test showed no reactivity to nickel, cobalt, or chromium in both patients. Postrevision, serum cobalt and chromium levels dropped (Fig. 2).

In both retrieved hips, black marking and deposits were visible at the taper and modular head interface. Stripe wear was found on the articular surfaces of the head and cup by macroscopic and microscopic examinations. Modular head interface showed loss of machine mark with fretting by scanning electron microscopy.

Both cobalt and chromium levels at 3 months correlated weakly with the Merle d'Aubigne and Postel score ($R = 0.351$ [$P = .003$] for cobalt and $R = 0.353$ [$P =$

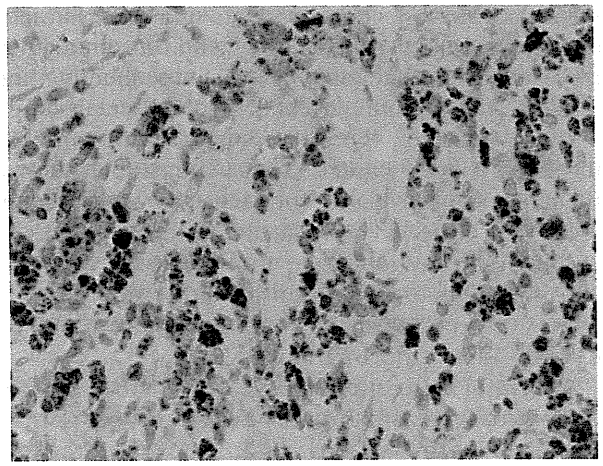

Fig. 3. Immunohistochemistry showing numerous CD3-positive T-lymphocyte infiltration in periprosthetic tissue (original magnification ×400).

.002] for chromium). Cobalt levels at 1 and 2 years as well as chromium levels at 2 years were significantly higher in men than in women (cobalt: $P = .034$ at 1 year and $P = .019$ at 2 years, chromium: $P = .030$ at 2 years). Men were associated with large-head diameter ($P < .001$). Multivariate analyses showed that the correlations between cobalt and chromium levels and the clinical score at 3 months remained statistically significant ($R^2 = 0.123$ [$P = .014$] for cobalt and $R^2 = 0.060$ [$P = .048$] for chromium); however, we found no differences between cobalt and chromium levels and gender ($R^2 = 0.081$ [$P = .140$] for cobalt at 1 year, $R^2 = 0.123$ [$P = .135$] for cobalt at 2 years, and $R^2 = 0.221$ [$P = .073$] for chromium at 2 years).

The Merle d'Aubigne and Postel score improved significantly from 9.4 points (range, 3-15 points) preoperatively to 14.1 points (range, 10-18 points) at 3 months ($P < .001$ compared with preoperatively), 15.3 points (range, 10-18 points) at 1 year ($P < .001$ compared with 3 months), and 15.8 points (range, 10-18 points) at 2 years ($P = .856$ compared with 1 year). Acetabular component inclination, anteversion, head diameter, age of the patient, and body mass index did not show a significant correlation with cobalt or chromium serum concentrations.

Discussion

The present study measured cobalt and chromium ion levels during a 2-year period; levels reached a steady state at 1 year. Vendittoli et al [13] measured metal ion levels during a 2-year period and showed that steady state was reached at 1 year in patients with hip resurfacing and at 3 months in patients with THA. Allan et al [14] reported serum metal ion levels after hip resurfacing with the Cormet cup up to 3 years. Peak levels were observed at 1 year, and levels at 3 years were showing a downward trend, but this decrease was not statistically significant. De Souza et al [15] reported serum metal ion levels in patients with hip resurfacing during a 10-year period. The cobalt and chromium levels rose steeply over the initial 2 years. Subsequently, there was a steady overall decline for both metals up to 5 years. However, there then appeared to be another increase between 5 and 10 years. Other studies showed that metal ion levels did not increase again after the levels fell or reached a plateau [16]. The cobalt and chromium levels observed in the present study are consistent with levels previously reported for resurfacing with Cormet as well as other resurfacing and THA devices (Table 2) [3,14,16-23].

Several in vitro studies have shown that the high-carbon cobalt-chromium alloy has more wear resistance than the low-carbon alloy [24,25]. However, the use of wrought or cast materials, both with and without heat treatment, requires further study to determine the best way to minimize the accumulation of wear debris in the body. A hip-joint simulator study showed little difference in the running-in wear volumes generated by high-carbon wrought or cast materials [26]. Another simulator study compared the wear generation of double-heat–treated (hot-isostatic pressing and solution annealing) and as-cast (no heat treatment) large-diameter metal-on-metal bearings and demonstrated no differences in running-in and steady-state wear [27]. However, a recent clinical study indicated that double-heat treatments could lead to an increased incidence of metal wear–induced osteolysis [28].

Women typically have higher circulating metal ion levels than do men [16,20,29]; however, some studies showed no difference in metal ion levels between men and women [4,15]. In the present study, univariate analyses showed that men had a higher cobalt levels at 1 and 2 years and chromium levels at 2 years. In addition, men were associated with large-head diameter. After adjusting metal ion levels for head size and clinical score, we found no differences between metal ion levels and gender. It is still controversial if larger femoral head diameters decrease wear rates in patients with metal-on-metal THA. Antoniou et al [22] showed that cobalt and chromium levels 6 months postoperatively were significantly lower in patients with 36-mm metal-on-metal THA compared with patients with 28-mm metal-on-metal THA. Heads with a diameter of 28 mm work in boundary lubrication, whereas larger metal-on-metal articulations like Cormet are designed to work in fluid-film lubrication. Smaller femoral head diameters and an acetabular abduction angle of 55° can increase the risk of rim contact, impingement, and edge loading. Ideal fluid-film lubrication occurs in articulation with identical femoral head and acetabular diameters, minimal clearance, and polar contact [30]. The present study demonstrated no correlation between metal ion levels and the head size. This is consistent with previous studies [28,30].

In terms of acetabular component inclination and anteversion, we found no significant correlation between cobalt and chromium serum concentrations. In the present study, we measured acetabular anteversion using Advanced CasePlan Digital Templating Planning Software (Stryker Orthopedics). The accuracy of this software was validated by Levine et al [31]; however, we have no data about interobserver and intraobserver reproducibility. Acetabular anteversion angle corresponds to rotation around an artificial axis, which projects onto a radiograph as the major axis of the cup ellipse. Using this software, we measured anteversion angle from a radiograph according to Lewinnek et al [32] ($\sin^{-1}$ minor axis of an ellipse/major axis of an ellipse). Vendittoli et al [19] was unable to draw any conclusions regarding the acetabular inclination and levels of cobalt. Bernstein et al [30] showed no effect of acetabular inclination and anteversion on the metal ion

Table 2. Serum Cobalt and Chromium Levels in Metal-on-Metal Hip Arthroplasty Procedures

Type of Operation	Authors	Follow-Up (mo)	Implant	Material	Cobalt Median (μg/L)	Chromium Median (μg/L)
Resurfacing	Allan et al [14]	6	Cormet	Cast, heat	2.6	3.7
		12			3.3	4.4
		24			2.1	3.4
		36			2.1	3.6
	Moroni et al [16]	24	BHR	As-cast	1.2*	2.2*
		60			1.1*	2.3*
	Skipor et al [17]	3	Conserve Plus	As-cast	1.3*	1.9*
		12			1.1*	1.8*
	Kim et al [18]	12	Conserve Plus	As-cast	1.0	2.0
		24			1.1	1.6
	Vendittoli et al [19]	3	Durom	Wrought	0.9*	2*
		12			0.7*	1.6*
		24			0.6*	1.4*
	Garbuz et al [3]	12	Durom	Wrought	0.5	0.8
		24			0.5	0.8
THA	Lavigne et al [20]	3	BHR	As-cast	1.0	1.2
		12			1.3	1.4
		24			1.9	1.9
	Witzleb et al [21]	3	Metasul	Wrought	2.2	0.8
	Moroni et al [16]	24	Metasul	Wrought	1.4*	1.8*
		60			1.4*	2*
	Antoniou et al [22]	6	Ultamet	Wrought	1.8	0.3
		12			2.3	0.4
	Imanishi et al [23]	3	Ultamet	Wrought	0.7	0.6
		12			1.1	0.8
	Lavigne et al [20]	3	M2a-Magnum	As-cast	0.5	0.9
		12			0.7	0.8
		24			0.7	1.1
	Lavigne et al [20]	3	ASR XL	As-cast	0.7	0.9
		12			0.9	1.1
		24			1.3	1.3
	Garbuz et al [3]	12	Durom	Wrought	5.1	2.1
	Lavigne et al [20]	3	Durom	Wrought	1.2	1.0
		12			2.3	1.0
		24			2.7	1.3
	Present study	3	Cormet	Cast, heat	1.4	1.4
		12			2.3	2.1
		24			2.3	1.6

Heat indicates hot-isostatic pressing and solution annealing (double-heat treatment). BHR, or Birmingham hip resurfacing, is a product of Midland Medical Technologies (Birmingham, UK) or Smith and Nephew Orthopaedics (Warwick, UK); Cormet, Corin (Cirencester, UK); Conserve Plus, Wright Medical Technology, Inc (Arlington, Tenn); Durom, Metasul, Zimmer (Warsaw, Ind); Ultamet, DePuy (Warsaw, Ind); M2a-Magnum, Biomet (Warsaw, Ind); ASR XL, DePuy.

* Mean.

levels. On the other hand, some authors have reported significantly higher levels of metal ions in patients with steeply inclined components [29,33]. Langton et al [33] showed that higher acetabular anteversion angles were associated with revision.

A metal-on-metal hip simulator study demonstrated an increased wear particle surface area due to high patient activity [34]. This is in agreement with a study showing an increase in levels of cobalt but not chromium after exercise [35]. However, another study showed that metal ion levels were not acutely affected by patient activity [20,29,36].

Patients with pseudotumor were shown to have significantly higher serum cobalt and chromium levels compared with patients without pseudotumor [37]. Metal ion measurement is a valuable additional tool for diagnosis and patient follow-up. De Smet et al [38] described that patients presenting with high serum metal ion levels should be closely monitored. In our cases, the patient in case 1 showed higher metal levels, but the patient in case 2 showed no elevated metal levels. It is now our practice to monitor metal ion levels in all large-diameter metal-on-metal THA recipients. In asymptomatic patients with well-functioning metal-on-metal implants, levels of these ions are low, typically around 2 μg/L. In the United Kingdom, the Medicines and Healthcare Products Regulatory Agency has suggested that patients with levels of cobalt or chromium ions above 7 μg/L should be further investigated and ion measurements be repeated as part of closer follow-up.

However, measurements may also need to be repeated in asymptomatic patients with levels between 3 and 7 μg/L, particularly in those with large-diameter metal-on-metal total hip arthroplasties [39,40].

Our retrieved findings indicated that increased wear at the bearing surfaces as well as the head and stem taper interface were the 2 main sources of metal ion debris. Bolland et al [41] and Langton et al [42] also indicated increased metal wear at the head and stem taper interface. Corrosion between 2 metals at tapered junctions is a known source of metal ions [13,43]. Analyses of metal particulate matter from tissues of failed metal-on-metal articulations at other centers showed that these particles were composed almost entirely of chromium, mostly in the form of a product of corrosion, chromium orthophosphate [33,41,44]. Fretting corrosion might contribute to the production of metal ions in our failed cases.

Limitations of this study included a small sample size, short follow-up periods, and using 2 different methods to measure cobalt and chromium levels. One of the strengths is that the present study is the first to evaluate metal ion levels in patients with THA using the Cormet cup with a large-diameter head, to our knowledge.

In conclusion, our results showed that patients with a large-diameter metal-on-metal THA had higher circulating levels of metal ions at 3 months and 1 year than before arthroplasty, with no additional significant increases at 2 years after surgery. Future, follow-up studies will investigate the long-term concentration of metal ions.

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Regular Article

The influence of fondaparinux on the diagnosis of postoperative deep vein thrombosis by soluble fibrin and D-dimer

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ABSTRACT

Introduction: There are many reports concerning the fondaparinux prophylaxis of deep vein thrombosis (DVT) after surgery, but little is known about the usefulness of diagnosing DVT by the thrombotic markers such as soluble fibrin (SF) and D-dimer in patients treated with fondaparinux. The main purpose of this study was to evaluate the accuracy of SF and D-dimer tests for DVT screening in patients undergoing total hip arthroplasty (THA) and total knee arthroplasty (TKA) treated with fondaparinux.

Materials and methods: A total of 519 patients who underwent THA or TKA were evaluated. SF and D-dimer levels were evaluated on postoperative days 1, 4, 7, 14 and 21. DVT was confirmed by ultrasonography 4 days after surgery.

Results: The incidence of DVT in patients treated with fondaparinux was significantly lower than in patients without fondaparinux. The SF test on postoperative day 1, and the D-dimer test on postoperative days 1, 4, and 7 were useful in untreated patients. However, in the patients treated with fondaparinux, the D-dimer test on postoperative day 7 only was useful for DVT screening.

Conclusion: The accuracy of SF and D-dimer test for the diagnosis of DVT was decreased by administration of fondaparinux. A new strategy for diagnosing DVT might be required for patients receiving fondaparinux.

Introduction

Deep vein thrombosis (DVT) and pulmonary thromboembolism (PTE) represent two expressions of a similar clinical pathological process referred to as venous thromboembolism (VTE). VTE is a common disorder that is associated with significant morbility and mortality after orthopadic surgery, especially total hip arthroplasty (THA) and total knee arthroplasty (TKA) [1-4]. Several population-based studies have indicated that VTE is a leading healthcare problem worldwide, thus highlighting the need for early and reliable diagnosis to enable appropriate triage of affected patients and to optimize their outcomes [5-8]. The clinical signs and symptoms of DVT are unreliable, and both venography and ultrasonography remain the "gold standard" methods, despite their many limitations. Venography is invasive, costly, and not easily repeatable, while ultrasonography requires skill, equipment and manpower [8]. As these methods are time consuming and/or expensive, to perform a DVT check in all patients repeatedly is nearly impossible in institutions where several thousand orthopedic surgeries are performed every year. As a result, plasma markers for the diagnosis of DVT have been developed, and are the prevailing method for diagnosing DVT. There is still debate, however, on which thrombotic markers to use, as well as their most suitable position within diagnostic algorithms.

Several studies dealing with soluble fibrin (SF) and D-dimer have shown that these tests could be useful biochemical markers for diagnosis of VTE. SF reflects thrombin activation and the cleavage of fibrinogen [3,6,9-13]. D-dimer is a degradation product of plasmin-cleaved, cross-linked fibrin.

Since the first manifestations of a VTE are often silent, routine thromboprophylaxis is recommended in clinical circumstances where the risk of VTE is high. The 8th American College of Chest Physicians (ACCP) Guidelines recommends the use of fondaparinux along with a low molecular weight heparin (LMWH) and a vitamin K antagonist for the prevention of VTE [14]. Fondaparinux is a synthetic pentasaccharide that acts as a specific inhibitor of factor Xa, with no direct inhibition of thrombin. The antithrombotic activity of fondaparinux is due to its selective binding to *antithrombin*. This inhibition of factor Xa via antithrombin results in effective inhibition of thrombin generation [15-17]. Fondaparinux is used all over the world, but the influence of fondaparinux on plasma markers such as the SF and D-dimer used for VTE screening is unknown. To the best of our knowledge, no study has yet focused on the impact of fondaparinux treatment on the SF and D-dimer levels used to monitor the incidence of VTE after THA and TKA.

In this study, we evaluated the accuracy of SF and D-dimer tests for VTE screening in patients undergoing THA and TKA with fondaparinux

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prophylaxis. The efficacy and safety outcome after fondaparinux treatment was also assessed.

Materials and methods

Patients

From September 2003 to March 2010, 519 patients without preoperative DVT undergoing THA or TKA at Mie University Hospital were eligible for the present study (THA n = 361, TKA n = 158). The surgical diagnosis was degenerative osteoarthritis in 403 patients, rheumatoid arthritis in 36 patients, deep infection after previous arthroplasty in 4 patients, and other causes in 76 patients (failure of a prosthesis, etc.) (Table 1). None of the patients received anticoagulant therapy. The patients' sex, height, weight, body mass index (weight in kilograms divided by the square of the height in meters), length of the operation, blood loss during surgery, and blood loss during drainage were recorded. The protocol was approved by the Ethics Committee of our institution. Written informed consent was obtained from all participants.

Study design

The study was designed as a prospective management trial. All patients underwent ultrasonography, and SF and D-dimer tests before surgery. Postoperative ultrasonography was performed 4 days after surgery in consideration of the rehabilitation schedule, as the popliteal and calf vein need to be examined with the patient in a sitting position, performing ultrasonography screening just after the surgery was difficult. All ultrasonographic examinations were performed by skilled physicians who were blinded to the SF and D-dimer results. Postoperative SF and D-dimer tests were performed on postoperative days 1, 4, 7, 14, and 21.

In this study, we excluded patients who had the following criteria: [1] <18 years of age, [2] body weight<40 kg, [3] renal failure (serum creatinine concentration >1.5 mg/dl) or liver insufficiency, or [4] a known hypersensitivity to heparin.

The patients were divided into three groups according to the time of their operation. From September 2003 to September 2007, 290 patients were assigned to receive no subcutaneous injection of fondaparinux (THA n = 199, TKA n = 91). From October 2007 until March 2010, 126 patients received once-daily subcutaneous injections of 2.5 mg of fondaparinux after surgery (THA n = 87, TKA n = 39), and 103 patients received 1.5 mg of fondaparinux (THA n = 75, TKA n = 28). In Japan, both 1.5 mg and 2.5 mg doses of fondaparinux were approved for use by the Japanese Ministry of Health, Labour and Welfare in June of 2007. Between September 2003 and November 2007, patients with DVT were treated with unfractionated heparin (UFH) or low-dose warfarin, from December 2007, fondaparinux treatment was scheduled to continue for 14 days.

The study was conducted according to the ethical principles stated in the Declaration of Helsinki and local regulations.

Table 1
Patient characteristics.

Parameter	Control n = 290	Fondaparinux n = 229	*p value
Gender			
Female	243	188	p = 0.61
Male	47	41	
Age, y ± (SD)	64.2 (12.2)	65.9 (10.2)	p = 0.19
BMI, kg/m²	24.0 (3.8)	24.6 (3.8)	p = 0.30
Diagnosis			
Osteoarthritis	213	190	
Rheumatoid arthritis	23	13	
Others	54	26	
Operation			
THA	199	162	p = 0.63
TKA	91	67	
Type of surgery			
Primary	239	205	p = 0.02
Revision	51	24	
Operation time, min ± (SD)	115 (41)	114 (46)	p = 0.34
Intraoperative bleeding, ml ± (SD)	412 (534)	272 (281)	p<0.0001
Drain bleeding, ml ± (SD)	565 (354)	407 (223)	p<0.0001
Total bleeding, ml ± (SD)	978 (740)	678 (407)	p<0.0001

SD: standard deviation, BMI: Body mass index; THA: Total hip arthroplasty; TKA: Total knee arthroplasty.
* Chi-square test, Spearman rank correlation.

Diagnosis of VTE

B-mode ultrasonography with compression and color Doppler imaging were performed for bilateral common femoral veins, the superficial veins, the popliteal veins, and the calf veins. Augmentation by calf squeezing or Valsalva's maneuver were included as needed. The criteria for the diagnosis of DVT were: loss of compressibility of the vein; presence of intraluminal echogenicity; and absence of venous flow determined using an Aplio (Toshiba Medical Systems Corp., Tokyo, Japan) sonographic scanner with a linear transducer (frequency, 6 MHz).

DVT was classified as proximal if the thrombus involved the iliac, femoral, or popliteal veins, and distal if the thrombus was limited to the calf veins. Patients with a proximal DVT proceeded to a PTE survey. PTE was confirmed by helical computer tomography (CT) scan, pulmonary angiogram, or ventilation-perfusion lung scan.

Prophylaxis

All patients received continuous epidural catheter analgesia from the day of surgery until postoperative day 1, and the catheter was removed at least 2 hours before the first injection of fondaparinux. All patients also wore intermittent pneumatic compression stockings from the day of surgery until postoperative day 2, and wore graduated compression stockings continuously. The first injection of fondaparinux was performed 24 hours postoperatively, and the second injection was performed more than 12 hours after the first. Treatment was scheduled to continue for 14 days based on the results of a previous Japanese clinical trial [18]. Walking exercise was started from 5 days after surgery.

Measurement of plasma SF and D-dimer levels

SF was measured using the latex agglutination method (IATRO SF, Mitsubishi Kagaku Iatron Inc., Tokyo, Japan). The normal range is <7.0 μg/ml. Plasma D-dimer levels were measured using the LPIA-ACE D-dimer (Mitsubishi Kagaku Iatron Inc., Tokyo, Japan). The normal range is <1.0 μg/ml.

Assessments

The efficacy outcome was assessed by the incidence of DVT. The primary safety outcome was the incidence of major bleeding. Major bleeding included fatal bleeding; bleeding that was retroperitoneal, intracranial, or intraspinal, or that involved any other critical organs; bleeding leading to reoperation, and overt bleeding with a bleeding index of 2 or more. The bleeding index was calculated as the number of units of packed red cells or whole-blood transfused (except autologous blood) plus the hemoglobin value before the bleeding episode minus the hemoglobin value after the episode. Fondaparinux was discontinued if major bleeding, transfusion of more than 2 units of whole blood transfusion, or packed red blood cells occurred during

fondaparinux prophylaxis, or if anemia (a decrease in hemoglobin of >2 g/dl from day 1) occurred.

Statistical analysis

We compared the patients' baseline characteristics using the Mann–Whitney *U* test, Fisher's exact test and the Chi-square test. A multiple linear regression analysis was performed to identify variables that were independently associated with postoperative DVT and to adjust for confounders. The type of surgery (primary or revision), intraoperative bleeding (<350 ml or ≥350 ml), drain bleeding (<450 ml or ≥450 ml), total bleeding (<800 ml or ≥800 ml), and fondaparinux injection were considered as confounders.

To determine the clinical performance of the SF and D-dimer tests, the diagnostic sensitivity, specificity, and best fit value (combination of sensitivity + specificity) were calculated. Receiver operating characteristics (ROC) curves were constructed. The ROC curves and the area under the curve (AUC) were calculated. All analyses were performed using the SAS software program, version 9.1 (SAS Institute, Inc., Cary, NC, USA); $p<0.05$ was considered to be statistically significant.

Results

The details of the patients' clinical features are listed in Table 1. Both intraoperative and drain blood loss appeared to have a clinically relevant effect on the outcome ($p<0.01$, Spearman rank correlation). A total of 133 of 290 patients without fondaparinux (45.9%) and 78 of 229 patients (34.1%) treated with fondaparinux developed a DVT, respectively (Table 2). *Thirty-two of 103 patients (31.1%), and 46 of 126 patients (36.5%) showed DVT in the groups treating 1.5 mg, and 2.5 mg fondaparinux respectively. Proximal DVT was diagnosed in 13 of 290 untreated patients (4.5%) and 5 of 229 patients (2.2%) treated with fondaparinux. In the patients treated with fondaparinux, 2 of 103 patients (1.9%) were treated with 1.5 mg foundaparinux and 2 of 126 patients (1.6%) were treated with 2.5 mg fondaparinux.* None of the patients developed signs or symptoms of PTE, and there were no deaths from PTE. The incidence of DVT in patients treated with fondaparinux was significantly lower than in untreated patients ($p<0.01$; Fisher's exact test). Compared with the incidence of DVT in untreated patients, the risk of DVT was only 74.3% in the patients treated with fondaparinux. The multiple linear regression demonstrated that fondaparinux treatment was the only statistically significant factor associated with postoperative DVT ($p<0.01$).

Fig. 1 presents the SF and D-dimer results for DVT. The SF level rapidly increased both in patients with DVT and in those without DVT on the next day after surgery, with a significant increase in the SF level in the DVT group (Fig. 1-a,b). The SF level showed a single peak at one day after surgery in both groups. The SF level declined to a low level by 4 days after surgery, and remained low until 21 days after surgery in both the untreated patients and in patients treated with fondaparinux (Fig. 1-a,b). There were no significant differences in the SF levels from 4 days after surgery between patients with and without DVT in both groups. As the blood collection on postoperative day 1 was performed before the first injection of fondaparinux, it can thus be concluded that the SF test for the diagnosis of DVT was not affected by the administration of fondaparinux.

The D-dimer level was also elevated both in patients with DVT and in those without DVT after surgery. In contrast to the SF levels, the D-dimer levels showed a bimodal peak. The D-dimer value peaked on postoperative day 1, decreased on postoperative day 4, peaked for a second time on postoperative days 7–14, and then decreased. The D-dimer levels were significantly higher in untreated patients with DVT than in patients without DVT on postoperative days 1, 4, and 7, while in patients treated with fondaparinux, the D-dimer levels were significantly higher in patients with DVT than in patients without DVT on postoperative day 7 (Fig. 1-c,d). Different from the results of the SF test, the D-dimer test for the diagnosis of DVT was affected by the administration of fondaparinux.

Interestingly, the SF levels in the fondaparinux-treated patients without DVT were significantly lower than those in the untreated patients on the postoperative days 4, 7, and 14 (all $p<0.01$). However, in the patients with DVT, the SF levels showed no major differences between fondaparinux-treated patients and untreated patients (Fig. 2-a, b). Otherwise, D-dimer levels were significantly lower in the patients treated without DVT than those not treated without DVT on postoperative days 14 and 21 (both $p<0.05$) (Fig. 2-c). However, D-dimer levels were significantly higher in the patients treated with DVT than those not treated with DVT on postoperative days 14 and 21 ($p<0.05$, $p<0.01$, respectively) (Fig. 2-d).

The ROC curves obtained for various cut-off values of SF on postoperative day 1 confirmed that 4.35 μg/ml was the most reasonable cut-off value for screening for DVT, yielding a high sensitivity (85.1%) and a fair specificity (38.2%) in the untreated patients and was 4.40 μg/ml in the patients treated with fondaparinux, yielding a sensitivity of 85.5% and a specificity of 42.8%. On the other hand, for the D-dimer test on postoperative day 1, 6.37 μg/ml was the most reasonable cut-off value for screening for DVT, yielding a sensitivity of 87.5% and a specificity of 42.5% in the untreated patients. On postoperative day 4, 4.64 μg/ml was the most reasonable cut-off value, yielding a sensitivity of 85.7% and a specificity of 47.8%, and on postoperative day 7, 6.43 μg/ml was the most reasonable cut-off value, yielding a sensitivity of 85.6% and a specificity of 34.7%. In contrast, for the patients treated with fondaparinux, on postoperative day 7, 6.04 μg/ml was the most reasonable cut-off value, yielding a sensitivity of 85.7%, and a poor specificity of 24.8%.

With regard to the AUC for the SF level on postoperative day 1, it was 0.68 in the untreated patients and 0.64 for the fondaparinux-treated group. For D-dimer, the AUC was 0.65 on postoperative day 1, 0.74 on postoperative day 4, and 0.67 on postoperative day 7 in the untreated patients, and was 0.55 on postoperative day 7 for the fondaparinux-treated patients.

The best fit values (sensitivity + specifity) were: 1.43 in patients without fondaparinux treatment (cut-off value, 12.8 μg/ml), and 1.28 in patients treated with fondaparinux (4.40 μg/ml) for SF on postoperative day 1; 1.30 (6.37 μg/ml) for D-dimer on postoperative day 1; 1.45 (5.09 μg/ml) on postoperative day 4, and 1.35 (8.81 μg/ml) for D-dimer on postoperative day 7 in patients without fondaparinux treatment, and were 1.23 for D-dimer on postoperative day 7 in the patients treated with fondaparinux (7.04 μg/ml) (Table 3).

Safety outcomes

There was no fatal bleeding, bleeding in a critical organ, or bleeding leading to reoperation. In fondaparinux-injected patients, there were 7 major bleeding events (4.4%) among the 229 fondaparinux-treated patients. The 7 patients were discontinued the fondaparinux treatment. *In case with 1.5 mg fondaparinux-injected patients, there were 5 major bleeding events (4.9%) among the 103 fondaparinux-treated patients.*

Table 2
The incidence of deep vein thrombosis.

Characteristics	Control (n = 290)	Fondaparinux (n = 229)	* p value
All DVT (%)	133 (45.9%)	78 (34.1%)	p = 0.01
Proximal DVT (%)	13 (4.5%)	5 (2.2%)	p = 0.15
Distal DVT (%)	124 (42.8%)	73 (31.9%)	p = 0.01
PTE (%)	0 (0)	0 (0)	
Fatal PTE (%)	0 (0)	0 (0)	
Major Bleeding (%)		7 (4.4%)	

DVT: Deep vein thrombosis, PTE: Pulmonary thromboembolism.

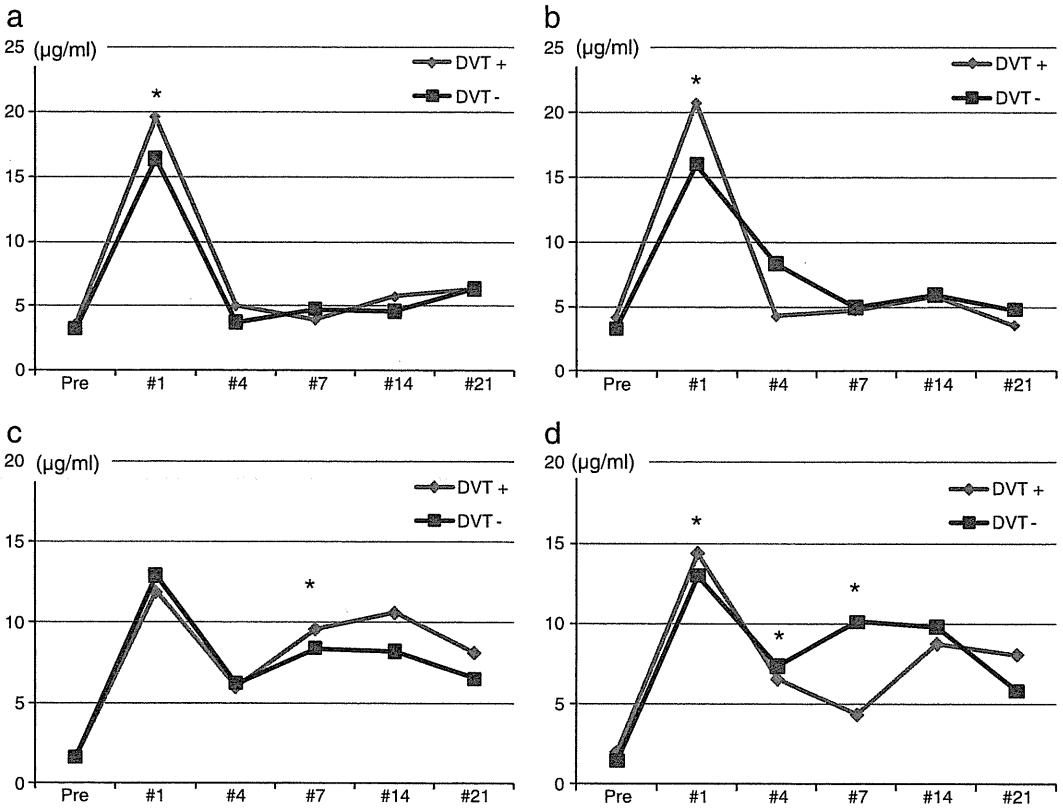

Fig. 1. a: Longitudinal changes in the soluble fibrin (SF) levels in the patients without fondaparinux treatment. The SF value peaked on postoperative day 1 and thereafter decreased consistently in both groups with a significant difference on postoperative day 1.* = $p<0.01$ (Mann–Whitney *U* test) **b: Longitudinal changes in the soluble fibrin (SF) levels in the patients with fondaparinux treatment.** The SF value peaked on postoperative day 1 and thereafter decreased consistently in both groups with a significant difference on postoperative day 1.* = $p<0.01$ (Mann–Whitney *U* test) **c: Longitudinal changes in the D-dimer levels in the patients without fondaparinux treatment.** The D-dimer values showed a bimodal peak on postoperative day 1 and postoperative days 7–14. The D-dimer levels were significantly higher in untreated patients with DVT than in patients without DVT on postoperative days 1, 4, and 7.* = $p<0.01$ (Mann–Whitney *U* test) **d: Longitudinal changes in the D-dimer levels in the patients with fondaparinux treatment.** The D-dimer levels showed a bimodal peak. The D-dimer levels were significantly higher in patients with DVT than in patients without DVT on postoperative day 7.* = $p<0.01$ (Mann–Whitney *U* test).

In case with 2.5 mg fondaparinux-injected patients, there were 2 major bleeding events (1.6%) among the 126 fondaparinux-treated patients.

Discussion

In this study, the SF test on postoperative day 1, and the D-dimer test on postoperative days 1, 4, and 7 were useful in untreated patients, otherwise in the patients treated with fondaparinux, the D-dimer test on postoperative day 7 only was useful for the diagnosis of postoperative DVT. The plasma marker tests might be affected after administration of fondaparinux, complicating the diagnosis of DVT.

VTE is a frequent, life-threatening postoperative complication of arthroplasty. The need for prevention and adequate management of VTE after THA or TKA is obvious. However, the optimal regimen to achieve this remains to be defined. There is also debate about how to achieve the most reliable diagnosis to enable appropriate triage of affected patients and to optimize outcome. The Nationwide Inpatient Sample Report supported the hypothesis that an improved and timely diagnosis, along with a more efficient therapeutic protocol, has led to a substantial decrease in hospital case fatality rates (from 12.3 to 8.2%) and the length of hospital stay (from 9.4 days to 8.6 days) [19]. Several lines of evidence attest that the introduction of laboratory testing has greatly improved the diagnosis of patients with suspected VTE.

In this study, the SF test on postoperative day 1 showed a high sensitivity (85%) and a fair specificity (38%) with an AUC of 0.68 in the untreated patients. On the other hand, the D-dimer test on postoperative day 4 showed a sensitivity of 86% and a specificity of 48%, with an AUC of 0.74 in the patients not treated with foundparinux. In previous reports, Bongard et al. [20] evaluated 173 patients undergoing hip surgery using the D-dimer test. The sensitivity and specificity for proximal DVT were 79% and 36%, respectively. Elias et al. [21] assessed the accuracy of D-dimer and SF in 231 patients with clinically suspected DVT in the diagnosis of DVT. The AUCs were 0.77 for D-dimer and 0.69 for SF. The sensitivity and specificity for DVT were 96% and 27% for the D-dimer test, and were 97% and 12% for the SF test. Strandberg et al. [22] carried out a case–control study comprising 123 patients presenting at an emergency department with clinical suspicion of DVT. The AUCs calculated to demonstrate the diagnostic performance in detecting DVT were 0.86 for D-dimer and 0.72 for SF.

Moreover, in the fondaparinux-treated patients in the present study, the D-dimer test on postoperative day 7 showed a significant difference between the patients with and without DVT, yielding a sensitivity of 86%, but a poor specificity of 25%, with an AUC of 0.55. In patients not treated with fondaparinux, the D-dimer levels were significantly higher in patients with DVT than in patients without DVT on postoperative days 1, 4, and 7, with AUCs of 0.65, 0.74 and 0.67, respectively. In the fondaparinux-treated patients, the situation was more complicated, making an accurate diagnosis more difficult.

In the DVT negative patients, the SF levels were significantly lower in the fondaparinux-treated patients than in untreated patients until 14 days after surgery. This result showed that blood coagulation appears to be suppressed effectively after surgery in patients who receive