High Levels of Gadolinium Deposition in the Skin of a Patient With Normal Renal Function

Donna R. Roberts, MD, Scott M. Lindhorst, MD,† Cynthia T. Welsh, MD,‡ Kenneth R. Maravilla, MD,§ Mary N. Herring, MD,‖ K. Adam Braun, MD,¶ Bruce H. Thiers, MD,¶ and W. Clay Davis, PhD#*

Objective: The aim of this study was to assess gadolinium deposition in the skin of a patient with normal renal function, based on estimated glomerular filtration rate values greater than 59 mL/min/1.73 m^2 after exposure to large cumulative doses of gadolinium-based contrast agents (GBCAs).

Materials and Methods: The patient underwent 61 contrasted brain MRI scans over the course of 11 years. Skin biopsies from the forearm and lower extremity were analyzed with inductively coupled plasma mass spectrometry (ICP-MS), laser ablation ICP-MS, and hydrophilic interaction liquid chromatography ICP-MS.

Results: The ICP-MS demonstrated high levels of gadolinium deposition (14.5 ± 0.4 μg/g), similar to previously reported gadolinium levels within the skin of patients with nephrogenic systemic fibrosis. The laser ablation ICP-MS demonstrated deposition of gadolinium within the deep layers of skin. Speciation analysis using hydrophilic interaction liquid chromatography ICP-MS demonstrated the presence of intact gadolinium-chelate species, although most of the gadolinium present could not be further characterized. Light microscopy demonstrated increased CD34 immunoreactivity in the connective tissue septations of the subcutaneous adipose tissue. The patient had no history of skin disorders and did not have a history of nephrogenic systemic fibrosis but did have severe joint contractures of unknown etiology.

Conclusions: Our results, in contradiction to published literature, suggest that in patients with normal renal function, exposure to GBCAs in extremely high cumulative doses can lead to significant gadolinium deposition in the skin. This finding is in line with more recent reports of gadolinium deposition in the brain of patients with normal renal function. Future studies are required to address possible clinical consequences of gadolinium deposition in the skin, brain, and potentially other organs in patients with normal renal function. We recommend, in addition to following current US Food and Drug Administration and American College of Radiology guidelines based on estimated glomerular filtration rate values, that caution be used when administering large cumulative doses of GBCAs and that total cumulative dose of each agent administered is recorded in the patient's medical record.

Key Words: gadolinium retention, skin, normal renal function

(*Invest Radiol* 2016;51: 280–289)

Gadolinium is a rare Earth element that does not occur naturally in the human body. Free gadolinium, Gd^{3+}, the basis of gadolinium-based contrast agents (GBCAs) used in magnetic resonance imaging (MRI), is extremely toxic.[1] Despite this toxicity, GBCAs are safely used in patients as the contrast agents are composed of gadolinium in a chelated form.[2] Gadolinium is bound to various ligands to prevent the release of free gadolinium while the contrast agent is in the body before predominantly renal excretion.[2] Because the chelate-gadolinium complex is subject to thermodynamic processes, prolonged duration in the body, such as occurs in patients with renal failure, may allow time for ligand-gadolinium dissociation.[3] This is the leading hypothesis for the development of nephrogenic systemic fibrosis (NSF).[1]

It is a widely held belief that gadolinium does not significantly accumulate in the skin of patients exposed to GBCAs in the absence of renal disease, as has been suggested by several previous studies.[4–9] However, these studies did not include patients who had received large cumulative doses of GBCAs. For example, Boyd et al,[4] using scanning electron microscopy with energy-dispersive x-ray spectroscopy, reported finding no detectable gadolinium deposits in 20 skin biopsy specimens from 10 patients who had undergone contrast-enhanced MRI with either Omniscan (gadodiamide) or Magnevist (gadopentetate dimeglumine) GBCAs. The patients had received an average dose of 1.2 to 1.8 g of gadolinium. This would equate to approximately 1 to 2 doses of contrast agent depending on the patient's weight, if the recommended weight-adjusted dosing scheme was used for GBCA administration.

High et al,[6,7] in a study of gadolinium deposition in NSF patients, included a skin sample from a patient without NSF. No further information was given concerning this patient, such as whether the patient had been exposed to gadolinium administration and, if so, at what dose. Using inductively coupled plasma mass spectrometry (ICP-MS), a technique that allows the quantification of total gadolinium present, the gadolinium concentrations in the skin samples from the NSF patients ranged from 4.8 to 106 μg/g. No gadolinium deposition was detected in the skin sample from the control patient. The authors did not provide the limit of detection for their analysis.

Khurana et al,[9] in a study of gadolinium deposition in NSF patients, included 2 control patients with multiple sclerosis who had been exposed to 3 to 5 doses of Omniscan over a period of 1.5 to 2.5 years. Using ICP-MS, they found gadolinium concentrations in the skin tissue specimens of these control patients equal to 0.1 μg/g. This level was much lower than the concentration of gadolinium found in the skin specimens of patients with NSF, which ranged from 57.2 to 717.8 μg/g.

Christensen et al[5] examined the affected and unaffected skin of 13 patients with NSF. A control group of 13 patients without NSF were included in the study. Total gadolinium levels were determined using ICP-MS. In the patients with NSF, the gadolinium levels ranged from 6.3 to 348.7 μg/g in affected skin and from 0.6 to 68.2 μg/g in unaffected skin. In 2 of the control patients without NSF, the gadolinium levels in the skin were 0.1 μg/g, and in the remaining 11 patients, the gadolinium levels were below the detectable range. The authors reported that the 2 control patients who had very low levels of gadolinium deposition in the skin had previous GBCA exposure, 1 patient 8 months and the other patient 16 months before biopsy. The authors did not report the number of doses of GBCA that these 2 patients were exposed to nor whether the 11 patients without detectable gadolinium levels had been exposed to GBCA administration.

Received for publication December 11, 2015; and accepted for publication, after revision, January 20, 2016.

From the Departments of *Radiology and Radiological Sciences; †Neurosurgery and Hematology/Oncology, and ‡Pathology, Medical University of South Carolina, Charleston, SC; §Departments of Radiology and Neurological Surgery, University of Washington, Seattle, WA; ‖Department of Physical Medicine & Rehabilitation and ¶Dermatology & Dermatologic Surgery, Medical University of South Carolina; and #National Institute of Standards and Technology, Chemical Sciences Division, Charleston, SC.

Conflicts of interest and sources of funding: none declared.

Disclaimer: Certain commercial equipment, instruments, software, or materials are identified in this document. Such identification does not imply recommendation or endorsement by the National Institute of Standards and Technology, nor does it imply that the products identified are necessarily the best available for the purpose.

Correspondence to: Donna R. Roberts, MD, 96 Jonathan Lucas Street, MSC 323, CSB Suite 210, Charleston, SC 29425-3230. E-mail: robertdr@musc.edu.

ISSN: 0020-9996/16/5105–0280
DOI: 10.1097/RLI.0000000000000266

TABLE 1. Summary of the HILIC Chromatographic Method

Column SeQuant ZIC-cHILIC (3 μm, 100 Å) 150 × 1 mm	
Mobile phases	
A. 10 mmol L^{-1} ammonium acetate in 70% (v/v) acetonitrile	
B. Acetonitrile	
Gradient program (50 μL/min)	
0–5 min	70% A
5–20 min	70% A linear gradient to 95% A
20–30 min	95% A
30–31 min	95% A linear gradient to 70% A
31–41 min	70% A

HILIC indicates hydrophilic interaction liquid chromatography.

Here, we present a case report of a patient who underwent at least 61 contrast-enhanced MRI brain scans over an eleven year period. Renal function remained normal throughout this time with all measured estimated glomerular filtration rate (eGFR) values greater than 59 mL/min/1.73 m^2. We performed skin biopsies in this patient, which demonstrated high levels of gadolinium deposition, which we further characterized by speciation analysis. To our knowledge, this is the first reported case of a patient with normal renal function demonstrating high levels of gadolinium deposition in the skin after exposure to a large cumulative dose of GBCAs.

MATERIALS AND METHODS

The patient presented to the neurology clinic at 19 years old with symptoms of fatigue. An MRI of the brain with contrast was obtained, which revealed a cerebral mass consistent with an aggressive tumor involving the left temporal lobe. The patient underwent resection and histology revealed glioblastoma with oligodendroglial components. Palisading necrosis, mitoses, and complex microvasculature were prominent. Over the next 2 years, the patient was treated with chemotherapy and received radiation treatment to the presurgery tumor

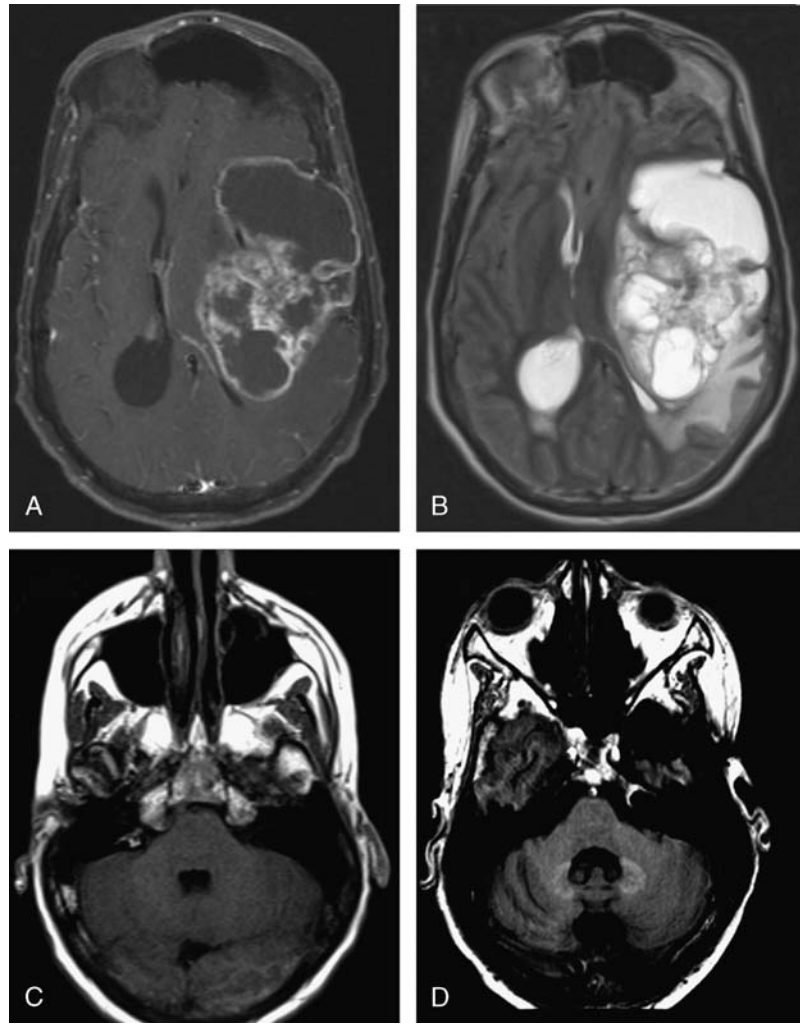

FIGURE 1. Axial postcontrast, fat-saturated, T1-weighted image (A) and axial T2-weighted image (B) through the brain demonstrated an aggressive appearing tumor involving the left temporal lobe with areas of necrosis and surrounding infiltrative edema. There was mass effect on the ventricular system and resultant hydrocephalus. Axial nonenhanced T1-weighted images through the cerebellum from the patient's initial magnetic resonance imaging (MRI) examination (C) and from the patient's 61st MRI examination (D) demonstrating the development of hyperintensity within the dentate nucleus are also shown.

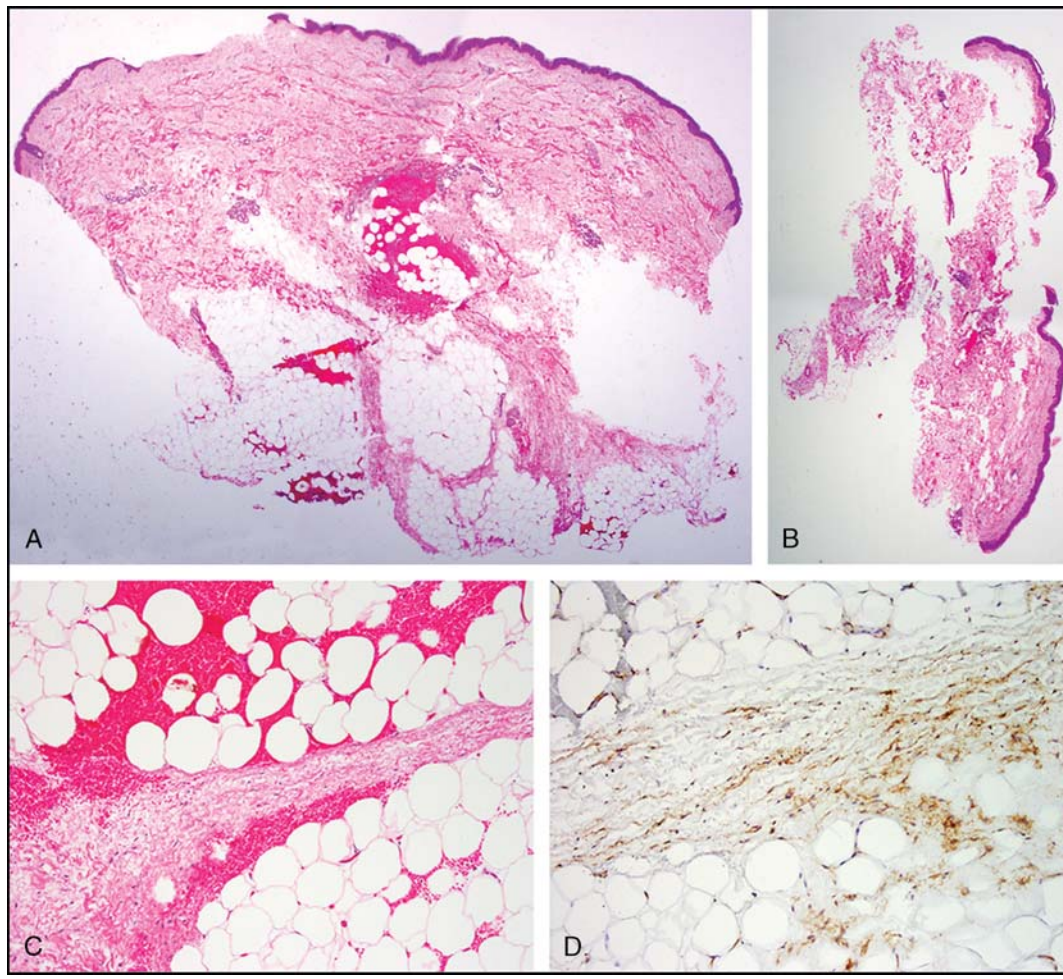

FIGURE 2. Hematoxylin and eosin staining of the arm (A) and the leg (B) skin biopsy sections showing no evidence of increased dermal cellularity, fibrosis, abnormal collagen bundles, osseous metaplasia, or abnormal numbers of fibroblast-like cells or macrophages. Skin biopsy section of the arm stained with hematoxylin and eosin (C) and antibodies against CD34 (D) demonstrating increased CD34 immunoreactivity in the connective tissue septations of the subcutaneous adipose tissue.

volume with a 2-cm margin for a total of dose of 60 Gy. Follow-up imaging and clinical examinations failed to demonstrate evidence of disease progression. Because of the lack of disease progression, the decision was made to discontinue treatment protocols, but because of the initial aggressive pathology, the patient continued to be followed closely with serial imaging. Therefore, the patient underwent brain MRI scans with contrast, on average, every 2 to 3 months for the next 11 years. In total, the patient underwent 61 contrasted examinations between the ages of 19 and 30 years. The patient was potentially exposed to multiple contrast agents, including Multihance (gadobenate dimeglumine), Magnevist, Omniscan, and ProHance (gadoteridol), as these were the agents used at our institution during the time period this patient underwent MRI. Likely the highest level of exposure was to Multihance, which is the agent used for adult brain tumor imaging at our institution. The last MRI was performed 8 months before biopsy.

The patient's medical history included glioblastoma of the left temporal lobe, seizure disorder, and hypothyroidism. The patient's surgical history included glioblastoma resection, laparoscopic cholecystectomy, vagal nerve stimulator implantation, and percutaneous endoscopic gastrostomy tube placement. The patient had significant cognitive and developmental delays with visual impairment bilaterally. The patient had no history of renal disorders or acute renal failure.

Clinic records, from approximately 4 years after initial brain tumor diagnosis, noted increased tone of the right greater than left upper and lower extremities on physical examination; however, the patient remained ambulatory. Approximately 3 years before biopsy, at age 27 years, the patient presented with choledocholithiasis with acute cholecystitis and underwent a laparoscopic cholecystectomy. Although discharged the next day without complication, following surgery, the patient experienced increasing problems with joint contractures, involving the neck and all 4 extremities. Because of the severe joint contractures, the patient became nonambulatory. The severity of the joint contractures also prohibited safely placing the patient in the bore of the magnetic resonance magnet. Therefore, the patient's last MRI scan was performed 8 months ago, and since that time, the patient has been followed with serial computed tomography imaging.

The patient had no skin issues, with no complaints of rashes, pruritus, sclerosis of the skin, discoloration, or other changes. An examination performed by a dermatologist revealed no skin rash, superficial plaque, woody induration, peau d'orange, hyperpigmentation, or violaceous coloration involving any skin surfaces.

After informed written consent was obtained, 2 skin biopsies were performed: one involving the right forearm and the other the

right shin. Each site was marked as an ellipse measuring approximately 1.3 cm. The outlined area was then excised to the subcutis.

Chemicals and Consumables

The GBCAs were obtained from the pharmaceutical companies: Magnevist (Gd-DTPA) by Bayer Schering, Omniscan (Gd-DTPA-BMA) by GE-Healthcare, and Multihance (BOPTA) and ProHance (Gd-HP-DO3A) by Bracco Imaging. Ultra-high-purity nitric acid was obtained from Thermo Fisher Scientific (Suwanee, GA), and ^{155}Gd enriched spike (Trace Sciences International) was used for isotope dilution analysis. Standard reference materials (SRMs) 3118a Gadolinium Standard Solution, SRM 3167a Yttrium (Y) Standard Solution, and 1947 Lake Michigan Fish Tissue were obtained from the National Institute of Standards and Technology (Gaithersburg, MD). Acetonitrile, ethanol, and xylene were purchased from Merck KGaA (Darmstadt, Germany). TraceSELECT ammonium acetate was purchased from Sigma Aldrich (Milwaukee, WI). Milli Q (18.2 MΩ cm^{-1}) water was used for all dilutions and mobile phases. Amicon Ultra 3 kDa centrifugal filter devices were obtained from Millipore (Billerica, MA).

Sample Preparation and Analysis

Histology

The skin biopsy samples were formalin fixed and paraffin embedded. They included epidermis, dermis, and subcutaneous adipose tissue. Three 3-μm-thick formalin fixed and paraffin embedded sections from each site were stained with hematoxylin and eosin and antibodies

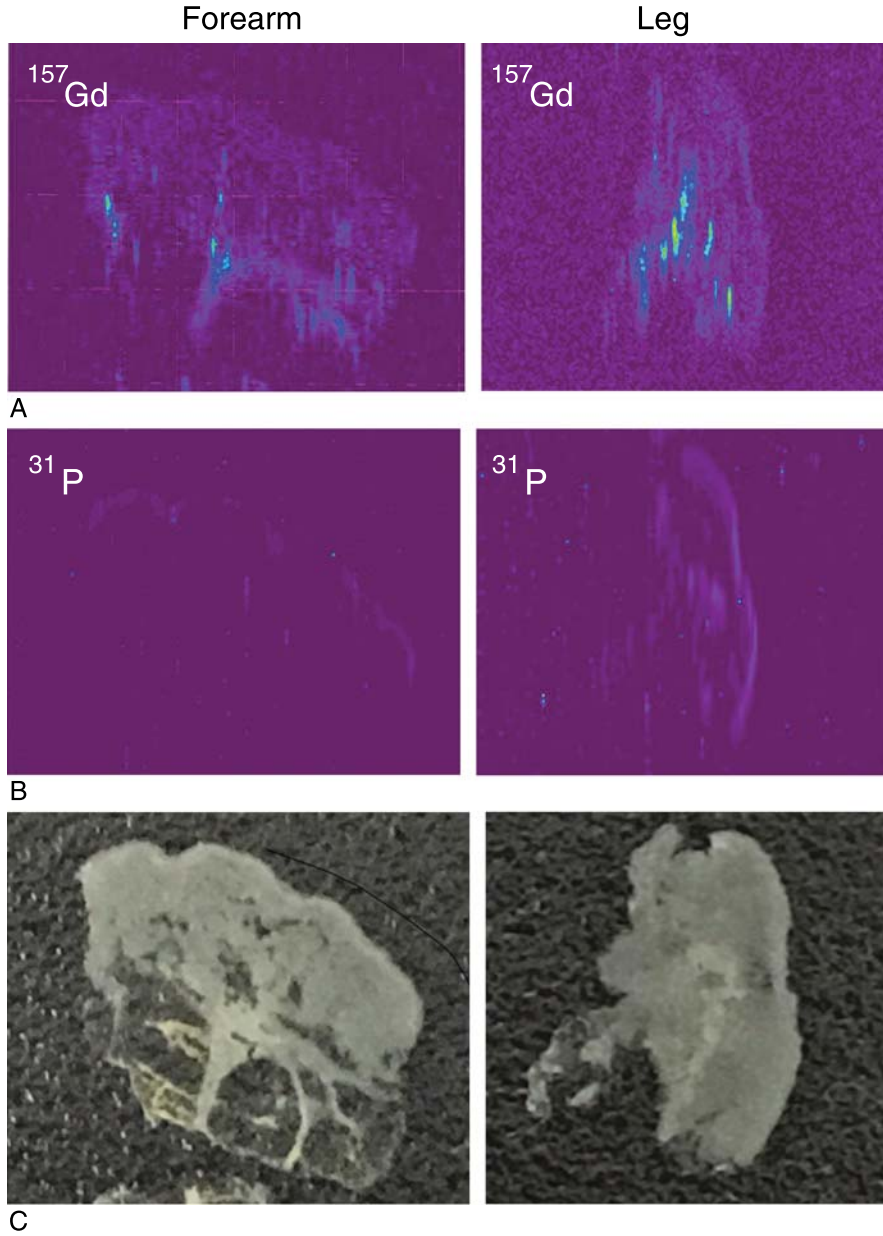

FIGURE 3. The accumulation and spatial distribution of gadolinium (A) and phosphorus in (B) in the tissue sections. Images of the deparifinized tissue sections before analysis are shown in panel C. The left column represents the biopsy sample from the forearm. The right column represents the biopsy sample from the leg. High levels of accumulated gadolinium can clearly be seen within the deep layers of the subcutis closely associated with the connective tissue septae. No significant deposition of phosphorus within the sections is noted.

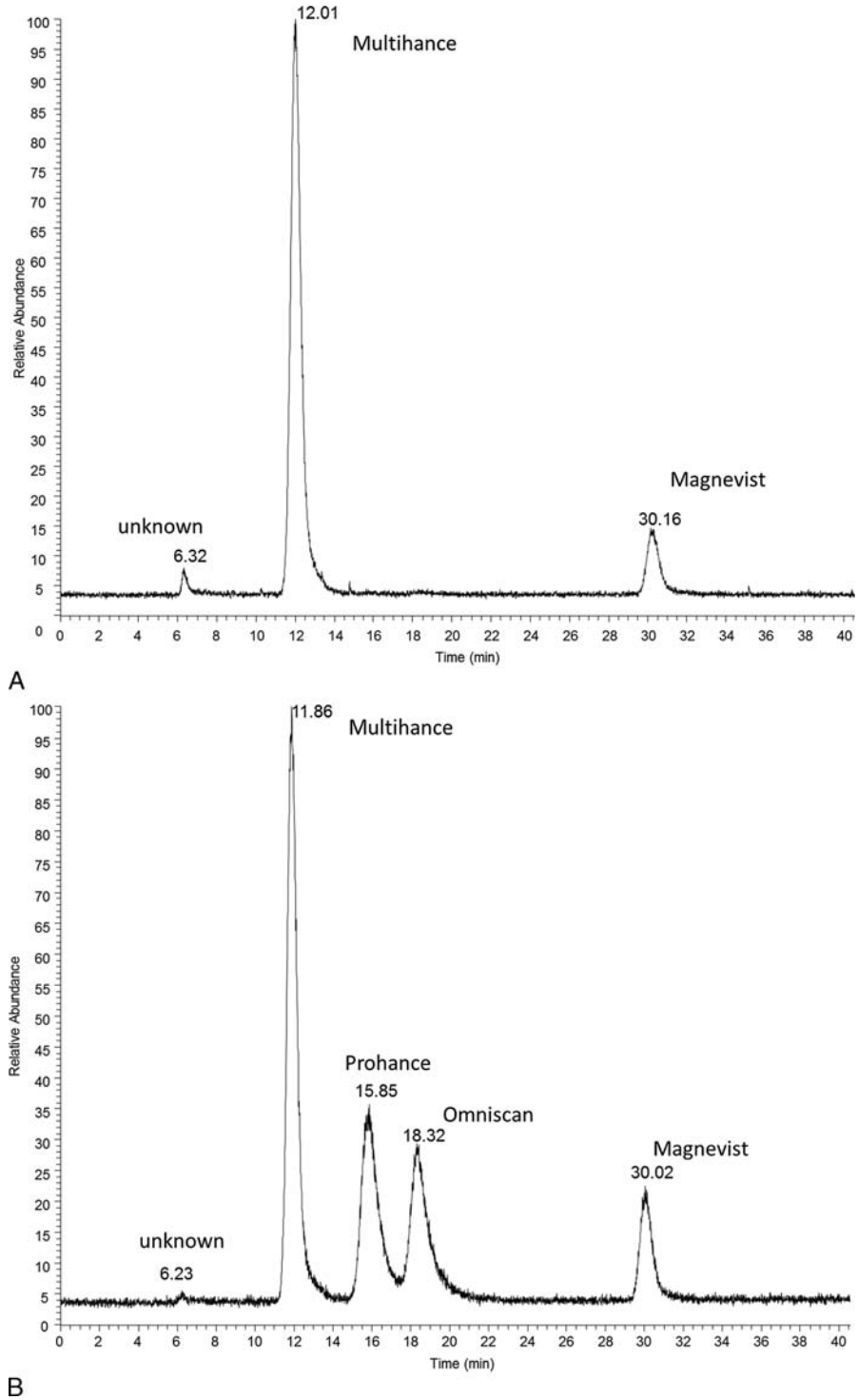

FIGURE 4. HILIC-ICP-MS chromatograms of the skin biopsy extract (A) and the biopsy extract spiked with all 4 GBCAs: Magnevist, Multihance, Omniscan, and Prohance (B).

against CD34. Eight-micron-thick unstained sections were sent for gadolinium analysis.

Inductively Coupled Plasma Mass Spectrometry

The total gadolinium of 3 subsections of skin biopsy material taken from the right lower extremity (≈100 mg each) was quantified by isotope dilution by adding a known amount of ^{155}Gd-enriched spike and subsequently dissolving the samples by microwave digestion (Multiwave, Anton Paar, Ashland, VA) after addition of 5 mL of HNO_3. The dissolved samples were subsequently diluted to a volume of 50 mL and the resulting isotope ratio ^{155}Gd/^{157}Gd was measured on a Thermo Element XR sector field ICP-MS in medium resolution

using an Aridus II (CETAC, Omaha) sample introduction system. Spike calibration was carried out by reverse isotope dilution using SRM 3118a as the calibrant. Because of a lack of reference materials certified for gadolinium content, SRM 1947 Lake Michigan Fish tissue was spiked with a known amount of gadolinium and used as a control material for total gadolinium measurements to ensure complete recovery. SRM 1947 was chosen as the reference material as it is represents an accepted, well-characterized standard reference material available from the National Institute of Standards and Technology. Although not a perfect match for human skin tissue, SRM 1947 provides a much more suitable "complex" matrix for spike recovery determinations of the GBCAs than spiked blanks do.

Laser Ablation ICP-MS

Tissue sections were embedded in paraffin and sectioned to a thickness of approximately 8 μm and fixed to glass microscope slides. Before analysis, the tissue sections were deparaffinized by washing with xylene, ethanol, and water.

Laser ablation ICP-MS experiments were carried out using a commercial New Wave Research UP213 (Fremont, CA) laser ablation system coupled to an Element XR ICP-MS and data collected for the individual gadolinium isotopes in medium resolution. Ablation of the tissue sections was performed in a line-by-line scan with a laser spot size of 55 μm, a scan rate of 55 μm/s, and 60 μm distances between each line. The laser energy was set to 85% power, with the laser operating at 15 Hz repetition frequency and the resulting aerosol was transported by helium carrier gas to the ICP-MS. A dual-sample introduction system was used, which allows for the aerosol, which passes through the ablation cell, and an internal standard solution of yttrium (1 ng/g) to be introduced simultaneously to monitor the sensitivity and drift of the instrumental setup. The individual line scans were exported as text files and images were generated with Origin 9.0 (OriginLab, Northhampton, MA).

Hydrophilic Interaction Liquid Chromatography ICP-MS

Approximately 200 mg of the skin biopsy material taken from the right forearm was cut to smaller fragments for speciation analysis using hydrophilic interaction liquid chromatography ICP-MS (HILIC-ICP-MS). A volume of 4 mL of Milli-Q water and 1 mL of acetonitrile were added to the sample and placed on a Roto-Shake Genie (Scientific Industries, Bohemia, NY) and rotated for 72 hours. In addition, aliquots of SRM 1947, SRM 1947 spiked with a known amount of GBCAs, sample blanks (Milli-Q water), and sample blanks spiked with a known amount of GBCAs were processed under the same conditions. For both the SRM 1947 and SRM 1947 spiked with GBCAs, approximately 200 mg of fish tissue and Milli-q water were vortex mixed and allowed to equilibrate for 30 minutes before the extraction process. After the extraction, the samples were centrifuged at 3000 rpm and then filtered with 3 kDa molecular weight cutoff filters. The material that passed through the 3 kDa molecular weight cutoff filters (MWCFs) was then taken to dryness using a Thermo Savant SpeedVac and reconstituted in 150 μL of 80% acetonitrile. No visible residue remained after the sample was reconstituted. A portion of the biopsy extract was spiked with a known amount of the GBCAs to verify the retention time used for identification. The remaining biopsy material and the above 3 kDa fraction of extracted material were saved for further processing.

The chromatographic separation was accomplished with a Dionex ICS-3000 Dual pump chromatography system (Sunnydale, CA) and a SeQuant ZIC-cHILIC column (3 μm, 100 Å; 150 × 1 mm, Millipore). Mobile phase A consisted of 10 mM ammonium acetate in 70% (v/v) acetonitrile in water and mobile phase B consisted of acetonitrile. The separation was accomplished with a linear gradient (Table 1) with an injection volume of 10 μL with a 50 μL/min flow rate. The liquid chromatography effluent was transferred to the Element XR ICP-MS using an Aridus II sample introduction system, and gadolinium was measured in low resolution. The individual chromatographic files were converted to .RAW files and processed in Xcalibur (Thermo Fisher Scientific).

Quantification of the gadolinium species was accomplished by online isotope dilution in which a post-column solution of ^{155}Gd was added at 100 μL/min to the liquid chromatography output via a T-connector using a Micro peripump MP-2 (Elemental Scientific, Inc, Omaha, NE). The individual chromatographic files from the online isotope dilution runs were exported as text files and processed as mass flow chromatograms using Origin 9.0.

Size Exclusion Chromatography ICP-MS

A portion of the extract that remained in the 3 kDa filter was injected into a BioSep SEC 2000 size exclusion column (4.6 mm × 300 mm; Phenominex, Torrance, CA). The separation was accomplished with a 50 mM ammonium acetate mobile phase with an injection volume of 50 μL with a 750 μL/min flow rate. The liquid chromatography effluent was transferred to the Element XR ICP-MS using an Aridus II sample introduction system and gadolinium was measured in low resolution.

RESULTS

During the 11-year period in which the patient underwent 61 contrast-enhanced brain MRI examinations, 167 renal function tests were performed. Creatinine values remained normal at all times that they were measured (mean [SD], 0.5 [0.19] mg/dL; min, 0.2 mg/dL; max, 1.3 mg/dL). At all times, the eGFR was reported as greater than 59 mL/min/1.73 m^2. The initial MRI examination demonstrated a heterogeneous mass centered in the left temporal lobe with rim enhancement, large areas of necrosis, and surrounding infiltrative edema (Fig. 1, A and B). Subsequent magnetic resonance examinations showed posttreatment related changes with no evidence of disease progression. Progressive hyperintensity on unenhanced T1-weighted images was noted in the dentate nucleus and globus pallidus (Fig. 1, C and D).

Review of histological sections of the skin biopsy by a pathologist with light microscopy revealed normal skin and subcutis. There was no evidence of increased dermal cellularity, fibrosis, abnormal collagen bundles, osseous metaplasia, or abnormal numbers of fibroblast-like cells or macrophages (Fig. 2, A and B). However, there was increased CD34 immunoreactivity in the connective tissue septations of the subcutaneous adipose tissue, indicating inflammation and/or tissue injury (Fig. 2, C and D).

Inductively Coupled Plasma Mass Spectrometry

Analysis of the gadolinium-spiked SRM 1947 samples resulted in a recovery of 99.9% (n = 3) with an overall detection limit of 0.57 ng/g. The ICP-MS analysis of the patient's skin sample demonstrated high levels of total gadolinium deposition with a total mass fraction of gadolinium of 14.5 ± 0.4 μg/g. For example, this was approximately 4 to 5 times the levels of total gadolinium previously found in a patient with renal failure and NSF (3.02–4.58 μg/g).[10]

Laser Ablation ICP-MS

The accumulation and spatial distribution of gadolinium in the tissue sections are shown in Figure 3A and of phosphorus in Figure 3B. Images of the depariffinized tissue sections before analysis are shown in Figure 3C and correspond to tissue sections obtained within 8 μm of the corresponding histological sections shown in Figure 2, A and B. The left column in Figure 3 represents the biopsy sample from the forearm. The right column in Figure 3 represents the biopsy sample from the leg. High levels of accumulated gadolinium can clearly be seen within the deep layers of the subcutis closely associated with the connective tissue septae. Unlike the skin samples from the patient with NSF described by Birka et al,[10] there was no significant colocalization of phosphorus with the observed high levels of gadolinium deposition.

Hydrophilic Interaction Liquid Chromatography ICP-MS

Speciation analysis was carried out using HILIC-ICP-MS to separate and detect the 4 GBCAs that the patient had potentially been administered. Figure 4A shows the chromatogram of the skin biopsy extract, whereas Figure 4B shows a chromatogram of an aliquot of the skin biopsy extract spiked with approximately 8 ng/g (as gadolinium) of Magnevist and Multihance and approximately 5 ng/g (as gadolinium) of ProHance and Omniscan. Separate peaks corresponding to all 4 GBCAs are clearly identified in the biopsy sample spiked with the GBCAs. In the unspiked skin biopsy sample, 3 peaks were seen, 2 of which corresponded to the retention times of Multihance (12 minutes) and Magnevist (30 minutes). An unidentified peak was also present, eluting at approximately 6 minutes. Quantitative determinations of each of the species in the biopsy sample by online isotope dilution resulted in mass fraction determinations of 34.5 ng/g of Multihance (as gadolinium) and 6.1 ng/g of Magnevist (as gadolinium). After extraction, the remaining portion of the biopsy sample was digested in nitric acid to determine the total amount of any gadolinium remaining in the biopsy sample that had not been removed by the extraction process. The total amount of this gadolinium remaining in the sample, which could not be further characterized using our methodology, was measured to be 2.1 μg/g (Fig. 5A). The low extraction efficiency was a result of the mildness of the extraction technique that we chose to use to keep any potential ligand-GBCA complex within the sample intact.

To determine percentage recovery, a sample of SRM 1947 spiked with known amounts of the 4 GBCAs was carried through the extraction method, which resulted in recoveries of only approximately 1% for each of the contrast agents (Fig. 5B). Most of the gadolinium that was spiked into the sample had been removed by the 3 kDa MWCF. Analysis of the blank sample spiked with the 4 GBCAs resulted in recoveries of approximately 60% for each of the contrast agents. This 40% loss from the spiked blanks would suggest that a large portion of the GBCAs could be absorbed onto the cellulose membrane or tube.

Using size exclusion chromatography coupled to the ICP-MS, a portion of the greater than 3 kDa fraction was analyzed for residual gadolinium-containing species in the skin biopsy and spiked 1947 samples that had been excluded in the original analysis. Both the biopsy sample and the spiked 1947 sample demonstrated a large

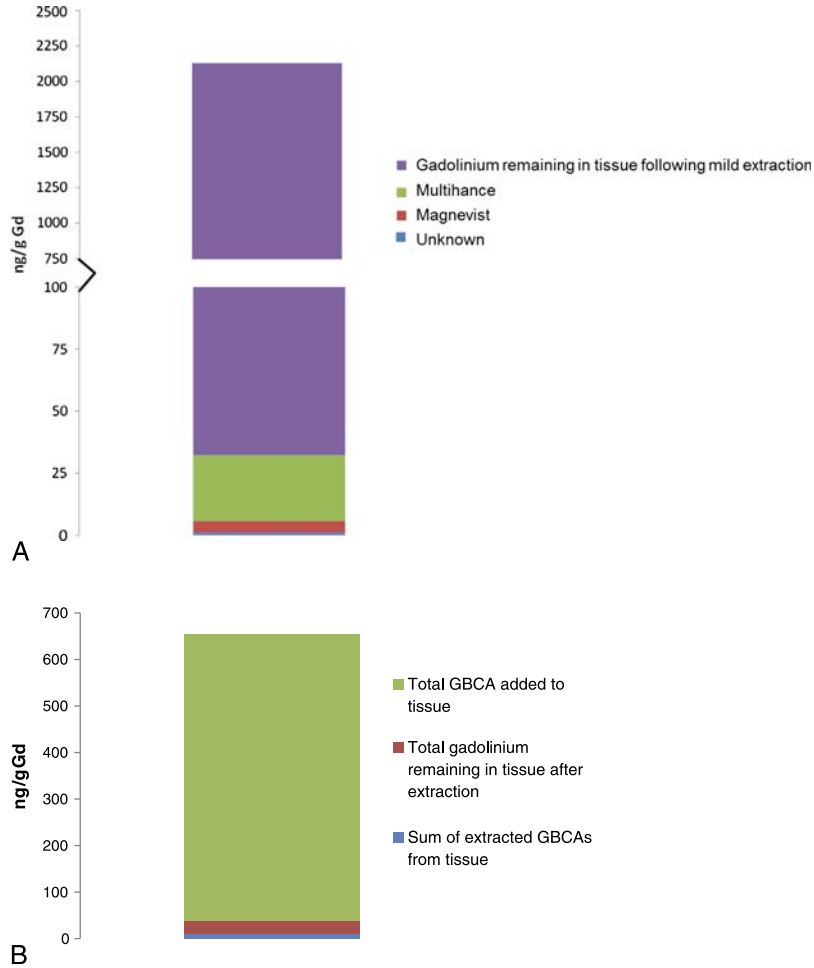

FIGURE 5. A, Quantitative determinations of each of the species in the skin biopsy sample: green, 34.5 ng/g of Multihance (as gadolinium); red, 6.1 ng/g of Magnevist (as gadolinium); and blue, a small fraction of an unknown gadolinium species. The total amount of gadolinium remaining in the biopsy sample after extraction, which could not be further characterized, was measured to be 2.1 μg/g, shown in purple. Note: This figure does not include the fraction of gadolinium within the skin biopsy sample that had been excluded by the 3 kDa filter. B, Quantitative determinations of each of the species in a sample of SRM 1947 spiked with known amounts of the 4 GBCAs demonstrated: green, total amount of gadolinium added (176 ng/g Magnevist, 145 ng/g ProHance, 142 ng/g Multihance, and 151 ng/g Omniscan); red, total gadolinium remaining in the tissue after undergoing the mild extraction technique; and blue, gadolinium in chelated form corresponding to the 4 GBCAs with approximately 1% recovery of each of the contrast agents. Figure 5 can be viewed online in color at www.investigativeradiology.com.

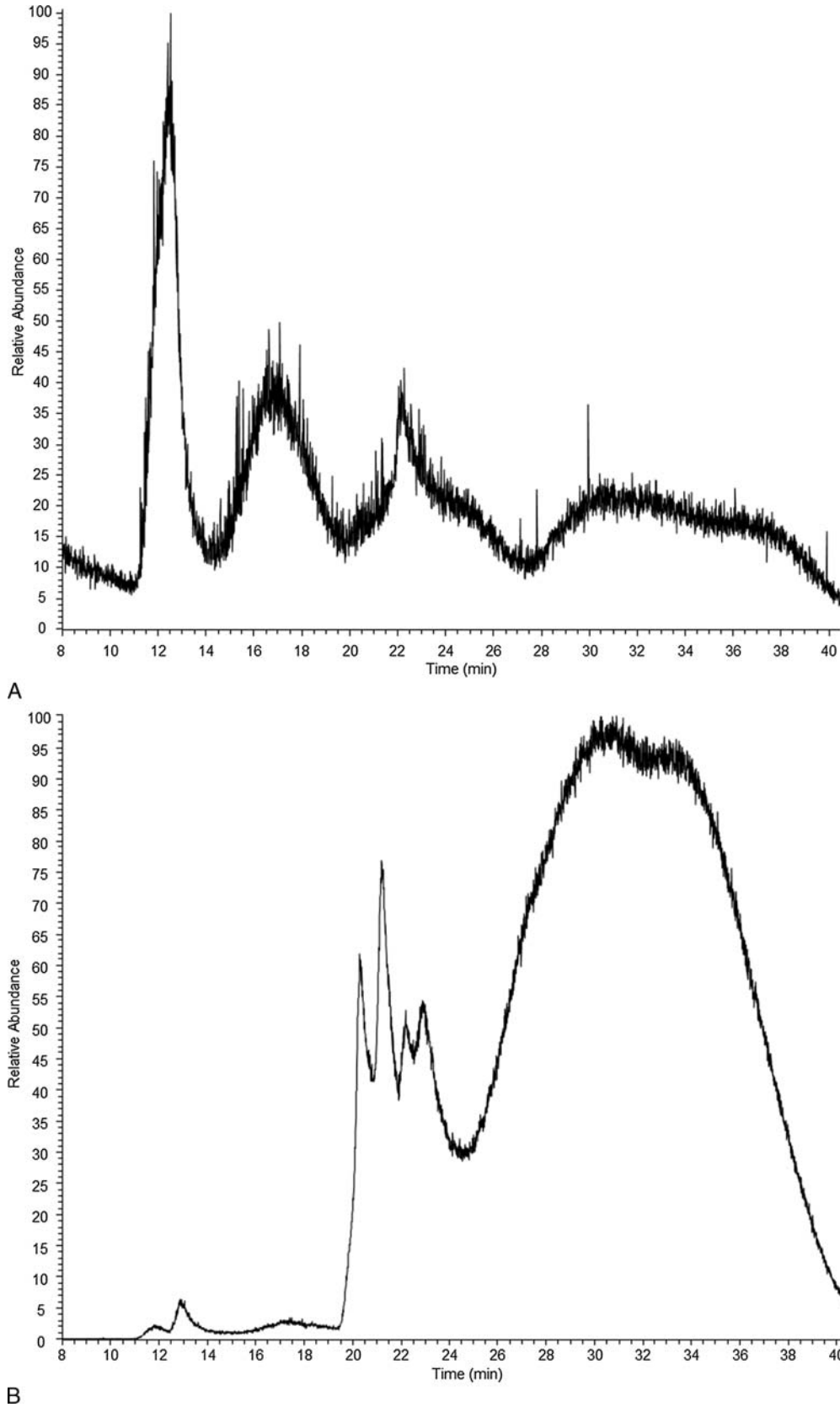

FIGURE 6. A, The >3 kDa size exclusion chromatography ICP-MS chromatogram of the skin biopsy sample. B, The >3 kDa SEC-ICP-MS chromatogram of the SRM 1947 sample spiked with the GBCAs.

gadolinium signal, although the gadolinium content was not quantified (Fig. 6, A and B). This additional portion of the total gadolinium that did not pass through the filter presumably represented protein- or particulate-bound gadolinium species, although there was also a small amount of gadolinium signal detected from the spiked blanks.

DISCUSSION

It is thought that, in the presence of normal renal function, significant levels of gadolinium are not deposited in the skin.[4–9] More recently, however, it has been shown that gadolinium deposition occurs in deep brain nuclei (globus pallidus and dentate nucleus) in patients with normal renal function who receive GBCAs in measurable cumulative doses.[11–20] However, the form of gadolinium retained in the body remains unclear. Furthermore, whether renal function status may influence which specific gadolinium species are deposited is also unknown. Here, we demonstrate high levels of gadolinium retention within the skin of a patient with normal renal function, similar to those reported in patients with NSF, and the presence of intact GBCAs.

Most importantly, it is unclear whether patients with normal renal function may exhibit subclinical signs and symptoms of gadolinium toxicity. Our patient had no history of skin disorders or any skin changes on physical examination, and review of histological sections of the skin biopsy revealed normal skin and subcutis. However, there was increased CD34 immunoreactivity in the connective tissue septations of the subcutaneous adipose tissue, a finding indicating inflammation and/or tissue injury. The patient also had a history of joint contractures of unknown etiology, but possibly multifactorial in nature. Without joint biopsy, a definite association of the joint contractures with the high levels of gadolinium could not be confirmed or excluded.

Given the clinical and pathological findings, the patient did not meet criteria for diagnosis of NSF.[21] The exact mechanism of NSF causation is unknown. The most widely held hypothesis is that gadolinium ions dissociate from the chelates in GBCAs in patients with poor renal function owing to the prolonged clearance times of the GBCAs, as well as to other metabolic factors associated with this level of renal disease.[1] The free gadolinium then binds with an anion such as phosphate, and the resulting insoluble precipitate is deposited in various tissues, inciting the fibrotic reaction seen in NSF patients.[1] It is interesting that we did not find colocalization of significant levels of phosphorus with gadolinium in our tissue samples.

As a limitation to our study, it should be noted that speciation analysis was able to characterize only a small fraction of the total gadolinium present in the skin samples (Fig. 5, A and B). This can be accounted for by 2 reasons. First, a mild extraction method was chosen for speciation analysis to keep intact any possible GBCA species present. This technique, therefore, was not efficient to yield quantitative extraction of all possible species present in the sample. If we had chosen to pursue a more complete extraction using a more vigorous extraction technique, the gadolinium-chelate molecule would not have remained intact. Second, we used a 3 kDa MWCF to separate the high-molecular-weight proteins and remaining particulates before the HILIC speciation analysis. This was necessary as the large-molecular-weight species (most likely keratin based proteins) would interfere with the chromatographic separation of the small-molecular-weight GBCAs by competing for interaction sites of the column's stationary phase, thus reducing the chromatographic peak resolution. Furthermore, these large proteins can also plug the column unless the sample has been significantly diluted and/or the chromatographic method optimized for protein-based separation (Fig. 6, A and B). Future analysis would be useful to further characterize this additional portion of the total gadolinium within the tissue sample, which presumably represents protein- or particulate-bound gadolinium species. While intact Multihance and Magnevist were present, it is unknown whether these intact GBCAs represent most of the gadolinium species deposited in this patient's skin.

Authorities have established guidelines that require assessment of renal function in vulnerable patients before GBCA administration[22–25]; however, our patient had no history of renal disease and eGFR values were well within the normal range at all times measured, including at the time of GBCA administration.

This case report, in contradiction to published literature,[4–9] suggests that in patients with normal renal function (eGFR >59 mL/min/1.73 m^2), exposure to GBCAs, particularly in extremely high cumulative doses, can lead to significant gadolinium deposition in the skin. Any long-term consequences of gadolinium accumulation in patients without a history of NSF are unknown. Future studies are required to elucidate the mechanisms and address possible clinical consequences of gadolinium deposition in the skin, brain, and potentially other organs in patients with normal renal function. In the meantime, we recommend that in addition to following current US Food and Drug Administration and American College of Radiology guidelines based on eGFR levels, caution be used in patients receiving large cumulative doses of GBCAs and that the agent administered along with total cumulative gadolinium dose be recorded in the patient's medical record.

ACKNOWLEDGMENTS

The authors would like to acknowledge the contribution of Ms. Donnie Beason, R.T. (R) (MR).

REFERENCES

1. Idee JM, Fretellier N, Robic C, et al. The role of gadolinium chelates in the mechanism of nephrogenic systemic fibrosis: a critical update. *Crit Rev Toxicol*. 2014; 44:895–913.
2. Runge VM, Ai T, Hao D, et al. The developmental history of the gadolinium chelates as intravenous contrast media for magnetic resonance. *Invest Radiol*. 2011; 46:807–816.
3. Port M, Idée JM, Medina C, et al. Efficiency, thermodynamic and kinetic stability of marketed gadolinium chelates and their possible clinical consequences: a critical review. *Biometals*. 2008;21:469–490.
4. Boyd AS, Sanyal S, Abraham JL. Gadolinium is not deposited in the skin of patients with normal renal function after exposure to gadolinium-based contrast agents. *J Am Acad Dermatol*. 2008;59:356–358.
5. Christensen KN, Lee CU, Hanley MM, et al. Quantification of gadolinium in fresh skin and serum samples from patients with nephrogenic systemic fibrosis. *J Am Acad Dermatol*. 2011;64:91–96.
6. High WA, Ayers RA, Chandler J, et al. Gadolinium is detectable within the tissue of patients with nephrogenic systemic fibrosis. *J Am Acad Dermatol*. 2007;56: 21–26.
7. High WA, Ayers RA, Cowper SE. Gadolinium is quantifiable within the tissue of patients with nephrogenic systemic fibrosis. *J Am Acad Dermatol*. 2007;56: 710–712.
8. High WA, Ranville JF, Brown M, et al. Gadolinium deposition in nephrogenic systemic fibrosis: an examination of tissue using synchrotron x-ray fluorescence spectroscopy. *J Am Acad Dermatol*. 2010;62:38–44.
9. Khurana A, Greene JF Jr, High WA. Quantification of gadolinium in nephrogenic systemic fibrosis: re-examination of a reported cohort with analysis of clinical factors. *J Am Acad Dermatol*. 2008;59:218–224.
10. Birka M, Wentker KS, Lusmoller E, et al. Diagnosis of nephrogenic systemic fibrosis by means of elemental bioimaging and speciation analysis. *Anal Chem*. 2015;87:3321–3328.
11. Errante Y, Cirimele V, Mallio CA, et al. Progressive increase of T1 signal intensity of the dentate nucleus on unenhanced magnetic resonance images is associated with cumulative doses of intravenously administered gadodiamide in patients with normal renal function, suggesting dechelation. *Invest Radiol*. 2014;49:685–690.
12. Kanda T, Fukusato T, Matsuda M, et al. Gadolinium-based contrast agent accumulates in the brain even in subjects without severe renal dysfunction: evaluation of autopsy brain specimens with inductively coupled plasma mass spectroscopy. *Radiology*. 2015;276:228–232.
13. Kanda T, Ishii K, Kawaguchi H, et al. High signal intensity in the dentate nucleus and globus pallidus on unenhanced T1-weighted MR images: relationship with increasing cumulative dose of a gadolinium-based contrast material. *Radiology*. 2014;270:834–841.
14. Kanda T, Osawa M, Oba H, et al. High signal intensity in dentate nucleus on unenhanced T1-weighted MR images: association with linear versus macrocyclic gadolinium chelate administration. *Radiology*. 2015;275:803–809.

15. McDonald RJ, McDonald JS, Kallmes DF, et al. Intracranial gadolinium deposition after contrast-enhanced MR imaging. *Radiology*. 2015;275:772–782.
16. Quattrocchi CC, Mallio CA, Errante Y, et al. Gadodiamide and dentate nucleus T1 hyperintensity in patients with meningioma evaluated by multiple follow-up contrast-enhanced magnetic resonance examinations with no systemic interval therapy. *Invest Radiol*. 2015;50:470–472.
17. Radbruch A, Weberling LD, Kieslich PJ, et al. Gadolinium retention in the dentate nucleus and globus pallidus is dependent on the class of contrast agent. *Radiology*. 2015;275:783–791.
18. Radbruch A, Weberling LD, Kieslich PJ, et al. High-signal intensity in the dentate nucleus and globus pallidus on unenhanced T1-weighted images: evaluation of the macrocyclic gadolinium-based contrast agent gadobutrol. *Invest Radiol*. 2015;50:805–810.
19. Runge VM. Macrocyclic versus linear gadolinium chelates. *Invest Radiol*. 2015;50:811.
20. Weberling LD, Kieslich PJ, Kickingereder P, et al. Increased signal intensity in the dentate nucleus on unenhanced T1-weighted images after gadobenate dimeglumine administration. *Invest Radiol*. 2015;50:743–748.
21. Girardi M, Kay J, Elston DM, et al. Nephrogenic systemic fibrosis: clinicopathological definition and workup recommendations. *J Am Acad Dermatol*. 2011;65:1095.e7–1106. e7.
22. EMEA. European Medicines Agency makes recommendations to minimise risk of nephrogenic systemic fibrosis with gadolinium-containing contrast agents. 2009. Doc. ref. EMEA/CHMP/739818/2009. Available at: http://www.ema.europa.eu/ema/index.jsp?curl=pages/news_and_events/news/2009/11/news_detail_000408.jsp&mid=WC0b01ac058004d5c1. Accessed October 12, 2015.
23. FDA. FDA drug safety communication: new warnings for using gadolinium-based contrast agents in patients with kidney dysfunction. Safety Announcement, August 9, 2010. http://www.fda.gov/Drugs/DrugSafety/ucm223966.htm. Accessed October 12, 2015.
24. Contrast Media Safety Committee of the European Society of Urogenital Radiology. *ESUR Contrast Media Guidelines Version 8.1*. 2012. http://www.esur.org/guidelines/.
25. ACR Committee on Drugs and Contrast Media. *ACR Manual on Contrast Media Version 10.1*. 2015. http://www.acr.org/quality-safety/resources/contrast-manual.