Fluids and Barriers of the CNS

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CSF outflow from the human spinal canal: preliminary results from an anatomical specimen-based model

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Abstract

Background Recent discoveries focused on the role of cerebrospinal fluid (CSF) in metabolite clearance have initiated intense research on CSF circulation and outflow pathways. These studies have focused on the cranial subarachnoid space, whereas spinal outflow has been relatively less investigated. Moreover, most studies have been performed on rodent models, which allows thorough anatomical investigation, whereas evidence from humans has been generated primarily from in vivo neuroimaging techniques. In this paper, we introduce an anatomical specimen-based preparation for studying spinal CSF outflow in humans and present preliminary results from our initial studies.

Methods Unfixed anatomical specimens of the thoracolumbar spinal dural sac along with the spinal nerves were obtained from cadavers. Experiments involving low-pressure infusion of contrast medium (barium sulfate) into the spinal subarachnoid space with video recording of contrast spread were performed. After fixation, contrast agent distribution of the samples was assessed via histological and radiological analyses including 3D X-ray microscopy.

Results Five human anatomical specimens of the dural sac were assessed. Filling of spaces extending to the spinal dura (arachnoid granulations, cuffs around the proximal spinal nerves) and unrestricted outflow from postganglionic spinal nerve cross-sections were both observed. Histological and radiological results confirmed the presence of contrast around the spinal nerve fascicles under the perineurium, in the arachnoid granulations and within the lumens of vessels within the dura or in the surrounding epidural adipose tissue.

Conclusions The described model makes it possible to examine CSF outflow routes from the human spinal subarachnoid space. The methodology is reproducible, feasible, and does not require specialized equipment. Preliminary results have revealed two potential CSF outflow pathways that have been previously observed in animal models: along the spinal nerves and to the epidural tissue and vessels.

Keywords Cerebrospinal fluid, CSF outflow, Dural lymphatics, Spinal nerves, Vertebral canal

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Background

In recent years there has been an intense research focus on determining the mechanisms controlling cerebrospinal fluid (CSF) circulation and elucidating the anatomical pathways responsible for the clearance of CSF and brain interstitial fluid [1–3]. The rediscovery of dural lymphatics, as well as a renewed focus on CSF outflow sites along the perineural spaces of cranial and spinal nerves, has invigorated a debate on whether the textbook understanding of CSF outflow through arachnoid villi or granulations is still valid [1, 4–6]. Most of the focus of this research has been on identifying outflow pathways from the brain through the cranial compartment, with several recent studies using tracer injections to demonstrate that connections exist between the subarachnoid space (SAS) and lymphatics in the nasal mucosa and nasopharyngeal region in mice [7–9]. In addition, routes alongside several other cranial nerves to extracranial lymphatics have also been identified [1, 6].

There has been relatively less interest in investigating potential outflow pathways from the spinal cord. The spinal clearance pathways have been estimated in studies with cats and sheep to account for less than 25% of total CSF turnover [10, 11]. However, due to hydrostatic pressure gradients, this pathway may theoretically be more important in bipedal species such as humans [12]. Furthermore, spinal pathways may account for a greater percentage of clearance when cranial outflow pathways are blocked, as shown in models of hydrocephalus or glioblastoma [13, 14]. While spinal arachnoid granulations have been identified [15], only a small percentage of these meningeal tissue extensions project into the spinal venous sinuses. Instead, classic experiments have identified potential outflow pathways at the lumbar and sacral regions of the spinal cord along the spinal nerve roots. At these anatomical locations, accumulations of injected India ink particles, which were termed "ink cuffs", were found and some particles were observed to enter nearby epidural lymphatic vessels [16, 17]. Miura et al. demonstrated CSF outflow from the cervical spine in monkeys to epidural lymphatic vessels but utilized high injection pressures [18]. A recent study investigated spinal outflow pathways by performing in vivo near infrared imaging of CSF tracers in transgenic reporter mice for lymphatic vessels, along with 9.4 T MRI after low-rate infusion into the lateral ventricle [19]. This study confirmed that pathways exist to lymphatic vessels near lumbar and sacral spinal nerves in mice, although the exact anatomical routes involved have not been elucidated. Interestingly, recent findings using histology of spinal cord sections at different time points after nanoparticles were injected into the cisterna magna of rats have shown that CSF may also have direct access to the peripheral nervous system through the spinal nerves [20], supporting previous evidence that has demonstrated this pathway [21].

Confirmation of CSF outflow to lymphatic vessels in humans is still emerging. Anecdotal evidence exists for pathways along cranial nerves from neuropathological examination of individuals who have succumbed to hemorrhagic stroke [22]. Most current evidence from humans is based on neuroimaging techniques, such as MRI, PET or SPECT/CT [23–27]. These studies have focused mostly on cranial pathways, including potential routes to dural lymphatic vessels located in the perisagittal sinus region of the skull [25, 28]. While lymphatic vessels have been confirmed in this region using immunohistochemistry [24, 29], the precise anatomical pathways from the CSF to the lymphatic vessels located either in the dura or along cranial and spinal nerves, have not been fully described.

In this paper, we introduce an anatomical specimen-based preparation for studying spinal CSF outflow in humans and present our preliminary results from the initial studies. We developed a methodology for obtaining unfixed specimens of the thoracolumbar dural sac with the spinal nerves and conducted a series of experiments involving infusion of barium contrast agent into the subarachnoid space. We observed contrast leakage from the spinal nerve cross-sections and filling of intradural structures (meningeal cuffs and arachnoid granulations). The spread of the contrast agent was confirmed by histology and X-ray microscopy.

Methods

Study approval

The material for the study was provided by the Department of Descriptive and Clinical Anatomy at the Medical University of Warsaw. Unfixed specimens of the dural sac of the spinal canal, along with proximal sections of the postganglionic spinal nerves were obtained from five cadavers (1 female, 4 males, aged 30–63 years). In each case a central nervous system-related cause of death was ruled out and abnormalities or diseases of the vertebral column, meninges and spinal cord were not present. The study protocol was approved by the Ethics Committee of the Medical University of Warsaw, Poland (number 260/2023).

Sample preparation

The anatomical specimens were prepared with the use of standard neurosurgical technique. A skin incision was made in the posterior median sagittal line over the spinous processes of the thoracic and lumbar vertebrae and the median sacral crest. After reaching the spinous processes, the erector spinae were detached laterally, exposing the laminae of the vertebral arches lateral to the intervertebral joints. A multilevel laminectomy

was performed, and the spinal canal was then carefully opened. The opening was extended to include the upper part of the sacral canal. A foraminotomy was performed to expose the proximal segments of the spinal nerves. This provided access to the dural sac and the postganglionic segments of the spinal nerves, since the lumbar spinal nerve ganglia lie near the intervertebral foramina (Fig. 1a) [30]. The exposed specimen was removed from the cadaver by cutting off the spinal nerves as laterally as possible and excising the dura mater both caudally and cranially. The technique used made it possible to preserve the anatomical compartment at the subarachnoid angle.

Infusion of the contrast agent

The dural sac was closed with forceps from the caudal side, whereas from the cranial side, a silicone tube connected to a reservoir of contrast mixture was inserted into the subarachnoid space (Fig. 1b). The reservoir was suspended 15–20 cm above the sample and the contrast medium contained a 1:1 mixture of normal saline and barium sulfate (Barium sulphuricum 1.0 g/ml, Medana), corresponding to a pressure of 20–25 cm H20. The drain was opened, which allowed contrast agent to freely flow into the subarachnoid space. The reservoir volume was large enough to maintain constant infusion pressure which was regulated by the suspension height of the reservoir. The internal diameter of the connecting drain was 12 mm, which provided negligible flow resistance. The course of the experiment was documented via an OPMI Pico microsurgical microscope (Carl Zeiss, Germany) allowing video or image acquisition. The observations were carried out for 15 min.

Histological and radiologic studies

After the experiment, the samples were fixed in 10% buffered formalin. To identify the contrast-filled spaces, additional histological examinations were performed. Cross-sections were made along the long and short axes of the spinal nerve, and hematoxylin and eosin staining was performed. The slides were analyzed with an Olympus BX53 microscope, and photographs were taken using an Olympus UC90 camera and CellSens Dimension software without additional processing (Olympus Corporation, Japan). In addition, one of the spinal nerves was scanned via a Zeiss Varia 510 X-ray microscope (Carl Zeiss, Germany; voxel size, 9 µm). Owing to the limited sample size, the nerve was excised with a fragment of the meninges from the dural sac specimen, preserving the anatomical compartment at the subarachnoid angle. The radiological results were analyzed via Dragonfly software, version 2022.2 (Comet Technologies Canada Inc., Montreal, Canada).

Results

Five specimens of the thoracolumbar dural sac were subjected to the experimental protocol and a total of 48 spinal nerves were evaluated. The experiments provided information on the distribution of the contrast mixture, which appeared as an opaque, white liquid within the specimen. Immediately after the drain was opened, the dural sac was filled with contrast agent. The irregular spaces within the dura covering the proximal segments of the spinal nerves were filled with contrast agent within seconds (Video 1). The spaces were apparent on each spinal nerve and took diverse forms, from shapes

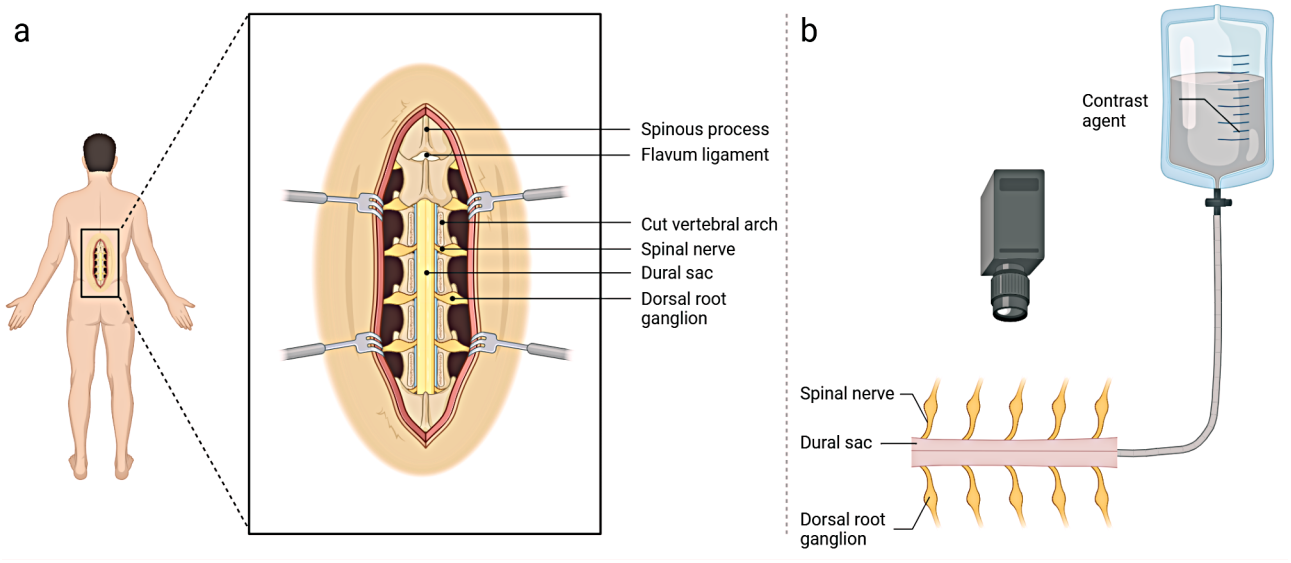

Fig. 1 Schemes of the anatomical specimen preparation (**a**) and the experimental setup (**b**). The reservoir of the contrast agent is connected to the subarachnoid space of anatomical specimen of the dural sac with spinal nerves. Infusion pressure can be adjusted by changing the height of the reservoir suspension. The course of the experiment is recorded by a surgical microscope

resembling arachnoid granulations (Fig. 2c) to cuffs surrounding the nerves (Fig. 2b and d). An incision made along the cuff revealed that the contrast filled the space between the dural layers (Fig. 2e and f). Within a single spinal nerve, a spectrum of contrast-filled structures could be seen: both arachnoid granulations (single larger structures or multiple smaller structures, possibly representing arachnoid villi) and cuffs. Close inspection with a surgical microscope of the dural surface in close proximity to the granulations revealed the presence of thin walled, collapsed vessels not filled with blood connected to the granulations in each case (Fig. 2c).

A few seconds after contrast inflow began, free leakage of the contrast agent appeared on the postganglionic spinal nerve cross-sections (Fig. 2d and Video 2). A thin layer of contrast separated the nerve fibers from the perineurium and the individual bundles of nerve branches from one another. Notably, this leakage did not occur from every branch of the spinal nerve, but instead exhibited a tendency toward more abundant outflow from the lower lumbar nerves. Owing to the short postganglionic segment of the spinal nerve, it was impossible to determine from which spinal nerve branch the outflow occurred. Moving microsurgical forceps along the nerve laterally squeezed out the contrast agent. Contrast was

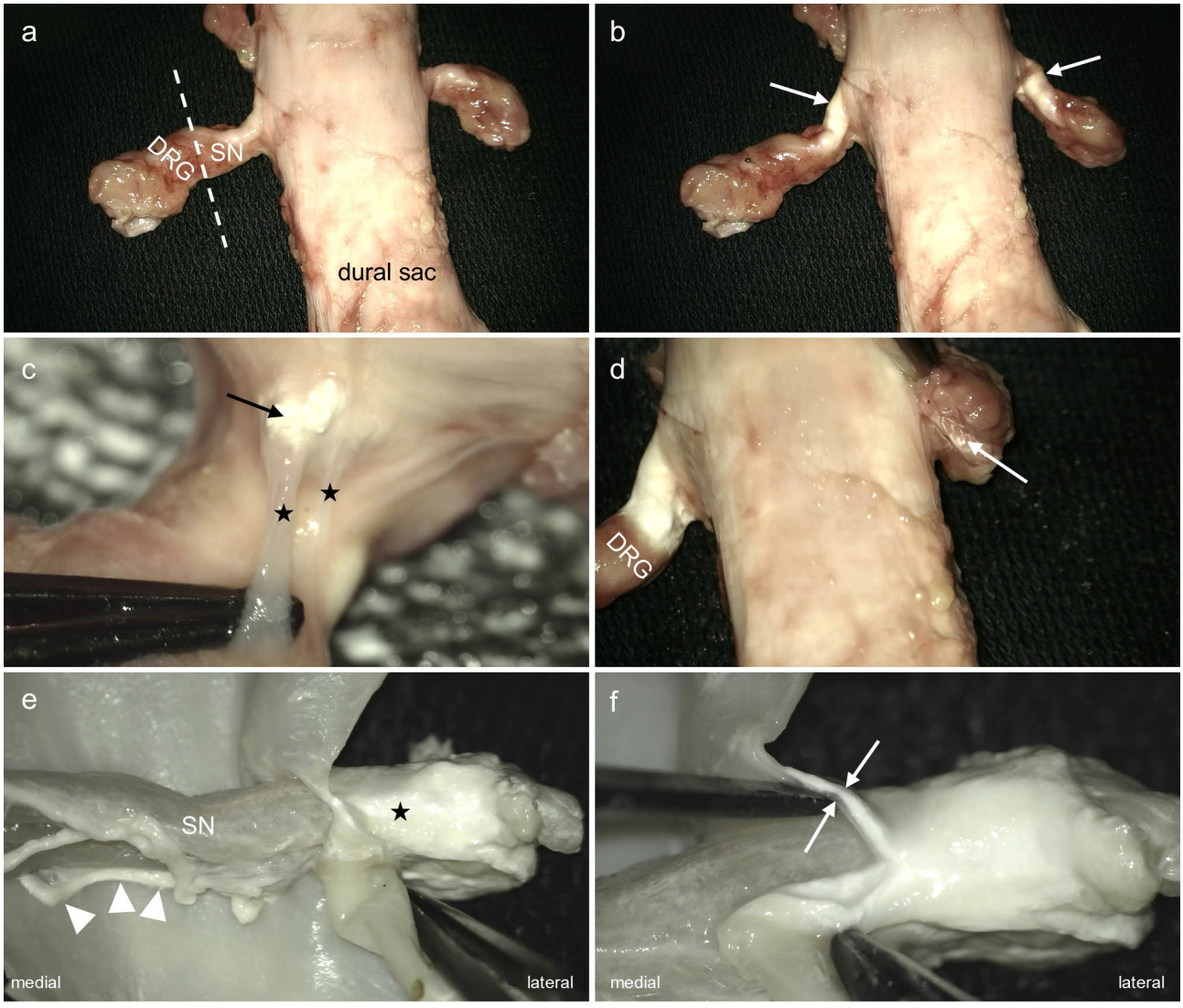

Fig. 2 Observations of contrast spread. **a** General view of the specimen with the spinal nerves (SN) and dorsal root ganglion (DRG) marked; the dashed line represents the intervertebral foramen. **b** Contrast-filled cuffs around the spinal nerves (arrows). **c** Apparent arachnoid granulation (arrow) with thin-walled vessels (asterisks). **d** Contrast visible on the postganglionic spinal nerve cross-section (arrow). **e** and **f** Inspection of a contrast-filled cuff (after specimen fixation). The dura and arachnoid were carefully cut to show the area where the nerve passes through the meninges. The connection between the dura and the arachnoid is loose, therefore the arachnoid lies on the spinal nerve roots (SN) and the tip of the forceps is located in the subdural space (f). The contrast medium is visible between dural layers (arrows). Similar observations were made in all five specimens

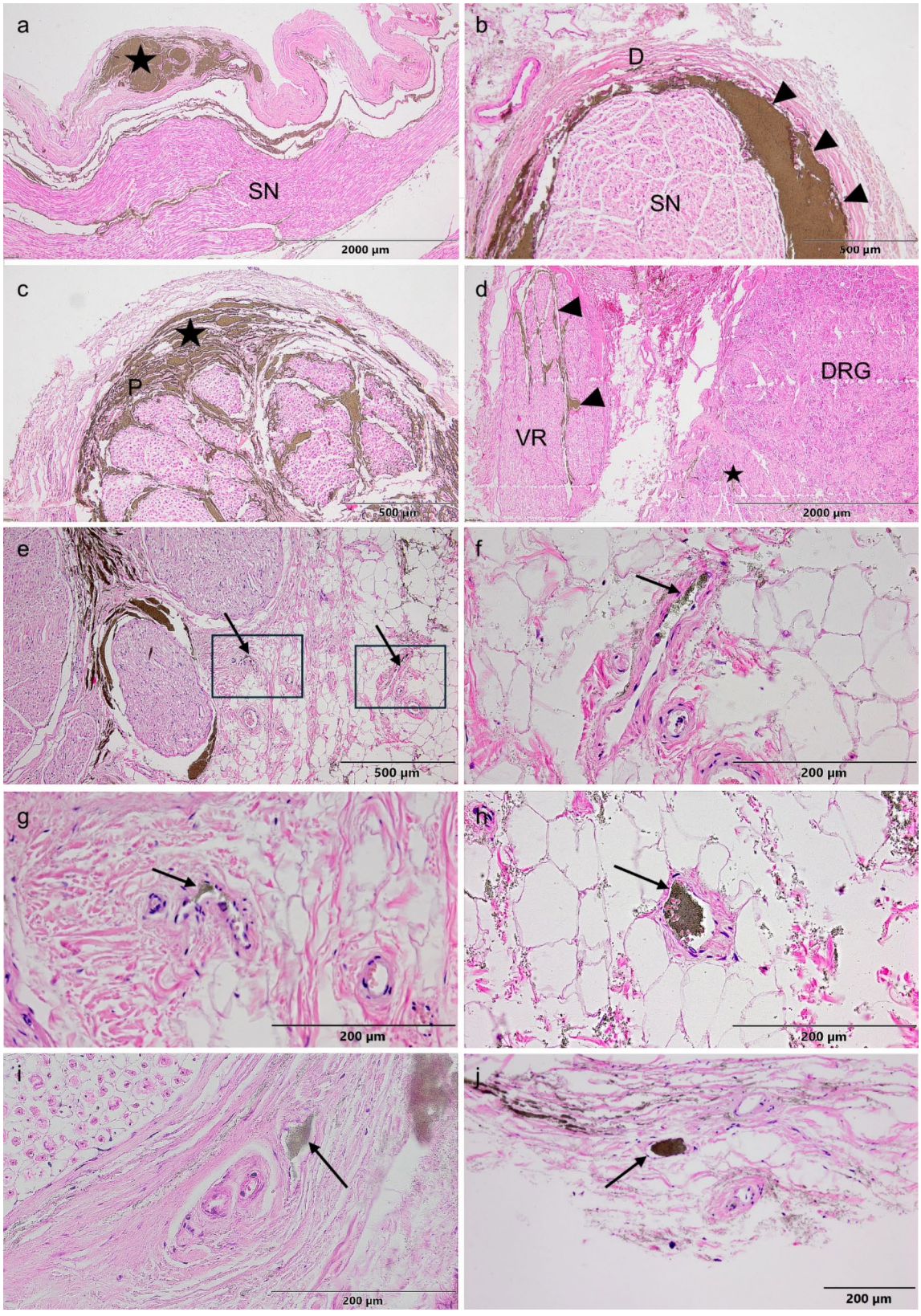

Fig. 3 (See legend on next page.)

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Fig. 3 Representative resuls of the histological examinations (hematoxylin and eosin staining). **a** Longitudinal section through the preganglionic spinal nerve (SN), arachnoid granulation in the dura (asterisk). **b** Cross-section through the proximal spinal nerve (preganglionic), contrast (arrowheads) between the dura (D) and the spinal nerve (SN, both roots). **c** Cross-section through the spinal nerve at the level of subdivision into rami of spinal nerve (in the vicinity of the dorsal root ganglion), extensive contrast infiltration (asterisk) between and around the nerve fascicles and between the perineurial layers (P). **d** Cross-section through lateral part of the dorsal root ganglion (DRG); contrast is present between the ventral root fascicles (arrowheads, ventral root VR), while in the ganglion the infiltration is discrete (asterisk). **e** Cross-section through the trunk of spinal nerve, contrast around the nerve fascicles and in the vessels of the perineurium and in the adipose tissue (arrows). **f** and **g** Zoom of the areas showed in e; barium contrast in the vessels (arrows) in the epidural adipose tissue (**f**) and in the perineurium (**g**). **h** Barium-filled vessel (arrow) in the adipose tissue (note the erythrocytes present in the vessel). **i** and **j** Thin-walled vessels filled with contrast agent in the dura (**i**) and on its surface (**j**)

also apparent in the epidural connective tissue without an identifiable source of leakage.

Histological examinations revealed several apparent contrast-filled arachnoid granulations (Fig. 3a) and confirmed the presence of contrast agent within the meninges and between the spinal nerve fascicles (Fig. 3a-e). Proximal to the dorsal root ganglion contrast surrounded both roots under the dura and arachnoid (Fig. 3ab). Distal to the dorsal root ganglion, where roots of the spinal nerve exchange the fascicles and form rami of the spinal nerve, contrast was present under the perineurium, in selected fascicles (Fig. 3c). Moreover, barium was also present between the perineurium layers which was especially noticeable laterally, in the vicinity of the dorsal root ganglion (Fig. 3c). Examination of both spinal nerve roots revealed contrast present in the ventral root under the perineurium, while inside the dorsal root ganglion the spread seemed to be blocked (Fig. 3d). In addition, barium-filled vessels were identified within the dura and surrounding adipose tissue (Fig. 3e). Some of these vessels contained red blood cells in addition to the contrast (Fig. 3f-h) and some were thin-walled with no apparent red blood cells, suggestive of lymphatics (Fig. 3i and j).

Radiological studies via 3D X-ray microscopy visualized the anatomical structures (the dura mater, the spinal nerves, etc.) in relation to the barium contrast. On a cross-sectional rendering of the spinal nerve, barium contrast was clearly visualized around the fascicles of the nerve (Fig. 4a). Contrast-filled arachnoid granulations were visualized and clearly connected to the arachnoid of the dural sac (Fig. 4a). Barium was present around the spinal nerve bundles but did not access the dorsal root ganglion itself (Fig. 4b). However, the contrast was visible around the ventral root distal to the dorsal root ganglion. Histological assessment of the same spinal nerve confirmed the presence of barium around the nerve roots up to the dorsal root ganglion (Fig. 4c).

Discussion

The method described in this study, which is based on human anatomical specimens, allows for the study of cerebrospinal fluid outflow pathways from the human spinal subarachnoid space. This preliminary study has shown that the method is feasible, reproducible, and does not require expensive reagents. Barium contrast is clearly visible both macroscopically and microscopically. Importantly, the specimens can also be analyzed radiologically via microtomography or 3D X-ray microscopy, which we show may provide valuable observations without the need for tissue sectioning of the specimen. The authors' experience with microtomography and barium contrast indicates its utility for analyzing the geometry of small vessels [31–35], as it quickly provides high resolution 3D data and allows better planning of subsequent histological and immunohistochemical studies, the extent of which depends on the needs of the researchers. Modifications to the methodology aimed at simulating specific conditions are also possible.

Although the series of experiments presented is a pilot study, the results indicate the presence of two pathways (Fig. 5) of passive CSF outflow from the human spine: a route along the spinal nerves and within tissue spaces in the dura (meningeal route), both of which have been previously observed in animal studies. The spread of contrast beneath the perineurium between the nerve fascicles (confirmed both histologically and radiologically) suggests continuity of CSF flow from the central nervous system to the peripheral nervous system, at least in the ventral root of the spinal nerve. These observations are in line with an early report from Pettersson et al. [21] and the more recent study of Ligocki et al. [20] that small nanoparticles infused into the lateral ventricle in rats can be visualized in the sciatic nerve. These findings also support the classic work of McCabe and Low, who reported that the cells covering the spinal nerve roots in the subarachnoid space (termed the root sheath by these authors) do not form a barrier between the SAS and the endoneurial fluid [36].

This finding raises an analogy with clinical experience in performing spinal anesthesia [37]. Patients often report the onset of local anesthetic drug action (change in skin temperature or tingling in the lower extremities) while the drug is still being injected into the subarachnoid space, which lasts only a few seconds. This phenomenon is observed only with intrathecal spinal anesthesia; ultrasound-guided peripheral nerve blocks with a concentrated local anesthetic drug injected directly around the nerves do not produce immediate effects (the onset is delayed by at least a few minutes). Thus, it seems that in the case of spinal anesthesia, the drug does not have

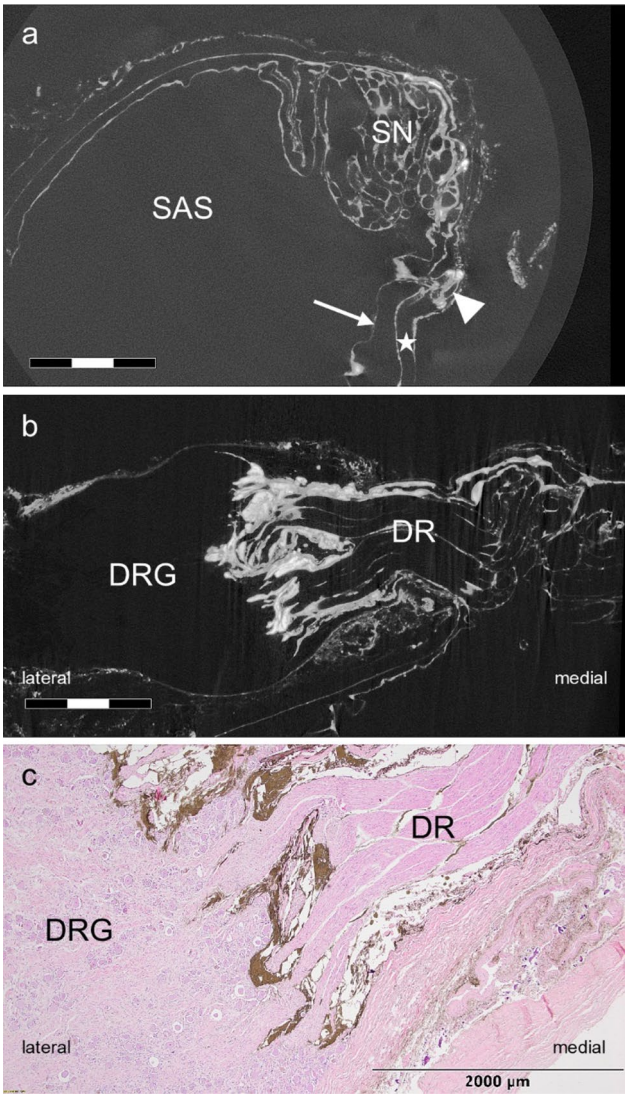

Fig. 4 Representative results of X-ray microscopy on one specimen of the spinal nerve. **a** Cross-section through the roots of spinal nerve (SN) in the subarachnoid space (SAS); the dura mater (asterisk), the arachnoid (arrow), the arachnoid granulation (arrowhead). **b** Longitudinal section through the distal dorsal root (DR). The contrast medium is visible between the nerve fascicles with accumulation in the dorsal root ganglion (DRG). Length of the scale bars is 3 mm. **c** Corresponding histological picture (hematoxylin and eosin staining)

to slowly diffuse either through the nerve root sheaths or gradually through the fascicles, but, like barium in the experiment, may immediately access the endoneurial space (Fig. 3c).

Although a consensus is slowly being reached that CSF outflow from the spine occurs to the lymphatics, the specific anatomical routes remain unknown [1]. The second CSF outflow pathway shown by the method involved the meninges. A spectrum of intradural spaces was apparent on the spinal nerves a few seconds after the experiment began: arachnoid granulations of various sizes projecting into the dura and intradural cuffs located around the proximal segments of the spinal nerves. In some cases, contrast was apparent in the epineurium distal to the dorsal root ganglion suggesting a potential passage from the meningeal coverings of the nerve roots to the perineurium of the spinal nerve. Moreover, histological studies revealed several contrast-filled, thin-walled vessels (venous or lymphatic) in the dura and epidural adipose tissue. Some of these vessels also contained red blood cells, suggesting that venous outflow pathways may be active, at least under the post-mortem conditions of our study.

The presence of the arachnoid granulations in the spinal canal is not widely known, despite their careful description by Kido et al. [38], who suggested their direct connection to the extradural veins. In our study, the presence of contrast in the vessels directly arising from the arachnoid granulations was not evident. Considering low infusion pressure during the experiments, this observation supports the hypothesis that the arachnoid granulations serve as a pathway for CSF outflow only under conditions of elevated pressure and that the connection with the venous system is indirect [39, 40].

Future studies should employ further use of X-ray microscopy because this method allows for the visualization of small arachnoid granulations that do not extend through the dura without the need to section the specimen, which is necessary for histological studies. Although studies on monkeys and humans suggest that the CSF drains into the lymph nodes [12, 18], several questions about the interconnections of the subarachnoid space, the endoneurial space, arachnoid granulations, intradural cuffs, and lymphatics in humans should be explored further.

The described method and its results are subject to several limitations. First, the experiments were performed ex vivo. However, there is currently no in vivo method to study CSF outflow in humans with microscopic resolution. Recent studies have shown different outflow pathways for particles of different sizes, so it is necessary to accurately control the barium particle size [20]. After performing these series of experiments, the authors noted areas for potential improvement before further research is undertaken. Filling the dural sac with contrast can be performed in situ, i.e., without excising from the cadaver, which will allow even more faithful reproduction of anatomical conditions. It would also be worthwhile to conduct studies at different infusion pressures in the subarachnoid space and to continue observation for different time periods. Additional immunohistochemical staining will also be necessary to identify vessel types.

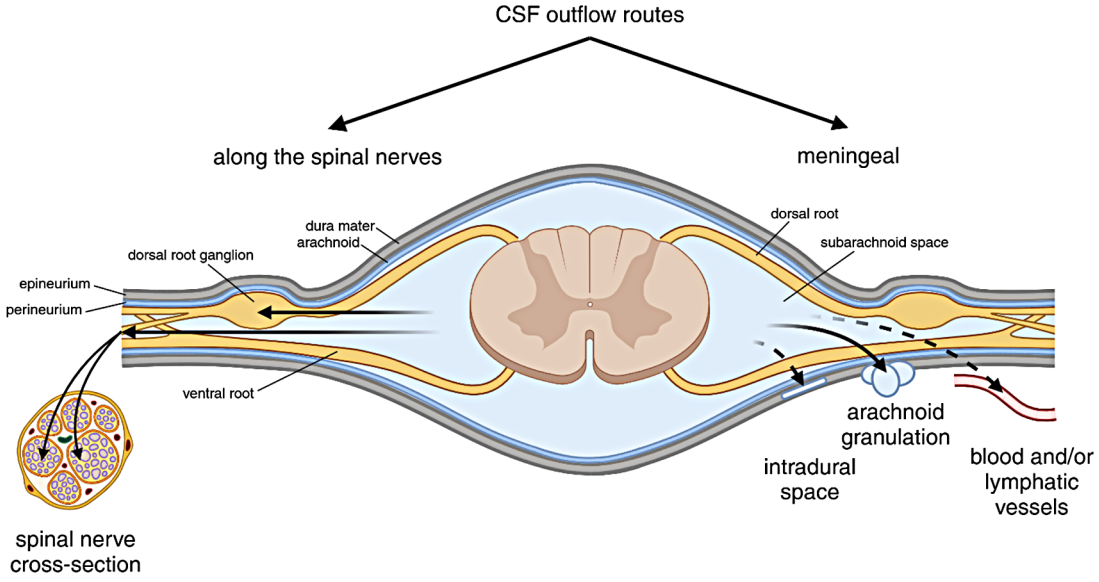

Fig. 5 Schematic representation of observed CSF outflow pathways: along the spinal nerves (left) and meningeal (right). The spread of the contrast along the spinal nerves was observed macroscopically during the experiments (Fig. 2d, Supplementary Video 2) and confirmed by histological (Fig. 3bce) and radiological studies (Fig. 4bc). The upper arrow on the left side represents the observation that the contrast reached the dorsal root ganglion but further spread within the *dorsal* root seemed to be blocked, unlike along the *ventral* root (Figs. 3d and 4bc). However, a potential perineurial route around the dorsal root ganglion may be present (Fig. 4bc). The meningeal route involves arachnoid granulations (Figs. 2c, 3a and 4a) as well as passage to the intradural spaces (Fig. 2bef) and vessels (Fig. 3f-j), to the latter two by still to be elucidated routes marked with dashed arrows.

Conclusions

In this study, we have shown that cerebrospinal fluid outflow from the human spinal canal can be studied using anatomical specimens from cadavers. The methodology presented is repeatable and feasible. Preliminary results indicate the presence of CSF outflow pathways similar to those observed in animal models, including along the spinal nerves to the peripheral nervous system and meningeal to lymphatic or blood vessels. Further research on human tissues is needed to translate findings from animal models and to describe specific anatomical relationships between the subarachnoid space and suggested pathways of CSF clearance.

Abbreviations

CSF Cerebrospinal fluid
DRG Dorsal root ganglion
SAS Subarachnoid space

Supplementary Information

The online version contains supplementary material available at https://doi.org/10.1186/s12987-025-00645-w.

Supplementary Material 1: Video 1 Infusion of the contrast medium to the dural sac. Please note the immediate filling of the irregular spaces within the dura covering the proximal segments with contrast agent.

Supplementary Material 2: Video 2 Unrestricted outflow of the contrast from the postganglionic lumbar spinal nerve cross-section. The outflow is clearly visible from the right upper fascicle.

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Author contributions

R.R. and S.T.P. designed the study and directed the project. R.R., S.T. and T.S. performed the experiments and collected the histological and imaging data. All authors performed data analysis. R.R. and S.T.P. drafted the manuscript. All authors provided important intellectual content in manuscript drafting or manuscript revision. All authors have approved the final version of the manuscript and have agreed to be accountable for all aspects of the work.

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Data availability

Data supporting the conclusions are presented in the article.

Declarations

Ethics approval and consent to participate

All procedures performed in the study were in accordance with the ethical standards of the institutional research committee and with the 1964 Helsinki Declaration and its later amendments. The study protocol was approved by the Ethics Committee of the Medical University of Warsaw, Poland (number 260/2023). The material for the study was provided by the Department of Descriptive and Clinical Anatomy at the Medical University of Warsaw. The authors sincerely thank those who donated their bodies to science so that anatomic research could be performed. The results from such research can potentially increase overall knowledge that can improve patient care. Therefore, these donors and their families deserve our highest gratitude.

Consent for publication
Not applicable.

Competing interests
S.T.P. is an Editorial Board Member but was not involved in the peer review process. The authors declare no other conflicts of interest.

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