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Supplementary Information for:

An immunohistochemical study of the lymphatic elements in the human brain

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Table S1 – Antibody specifications
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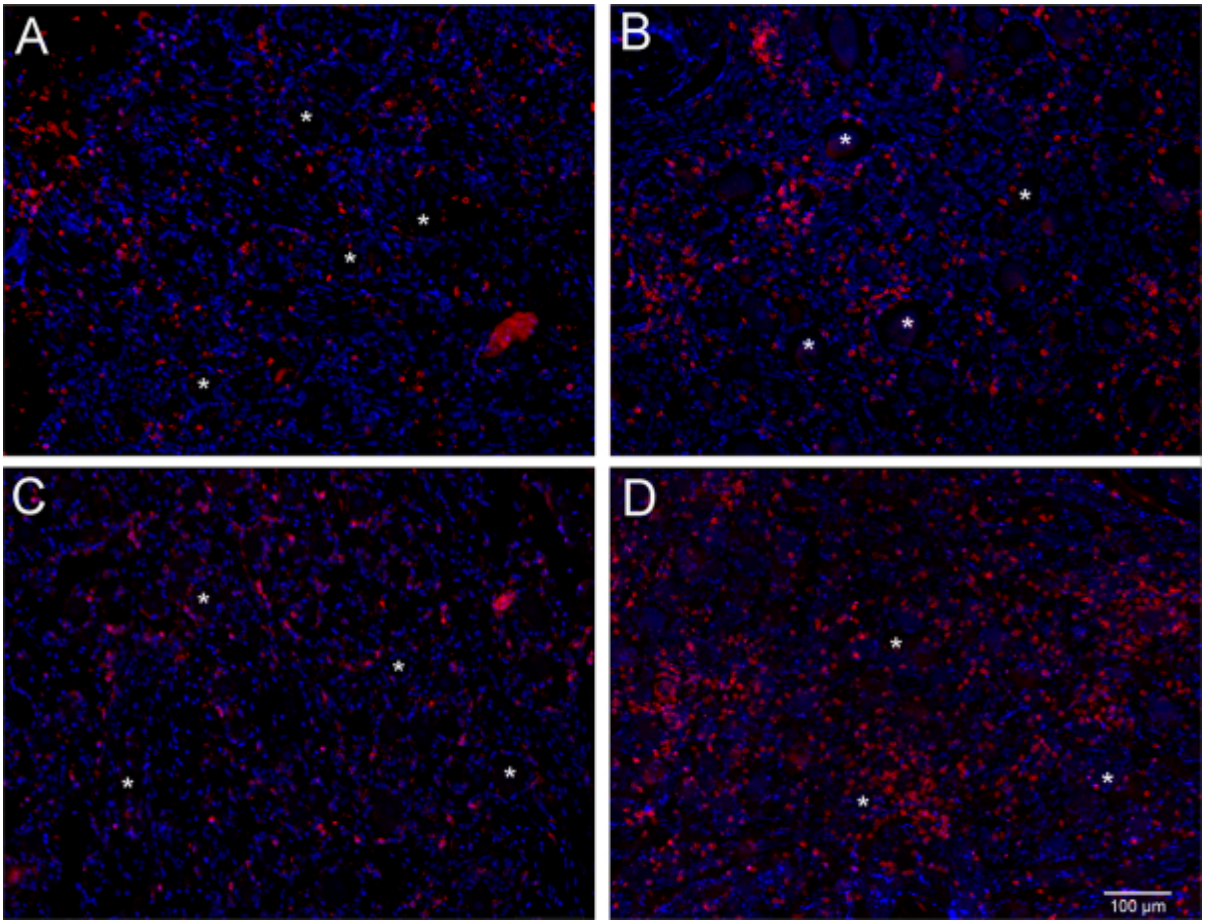
SI Fig.S1

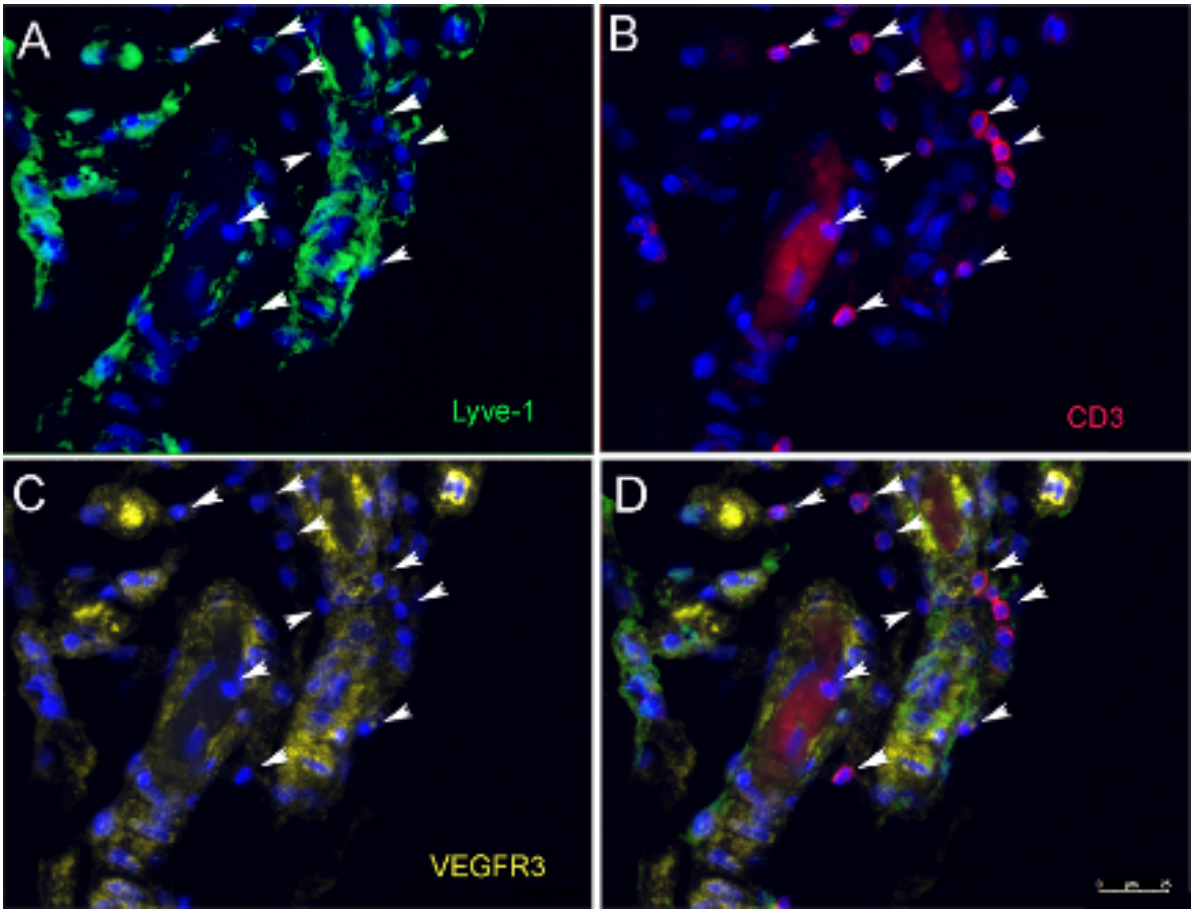


Accumulation of T cells in the trigeminal ganglion

Trigeminal ganglia dissected from people following death due to drug overdose (*A*), traffic accident (*C*) and self-inflicted strangulation (*B* and *D*) show the presence of CD3 positive (T) cells (red fluorescence) and cell nuclei in blue (DAPI). A few of the ganglion cells are labelled with stars.

Scale bar: 100 µm

SI Fig.S2

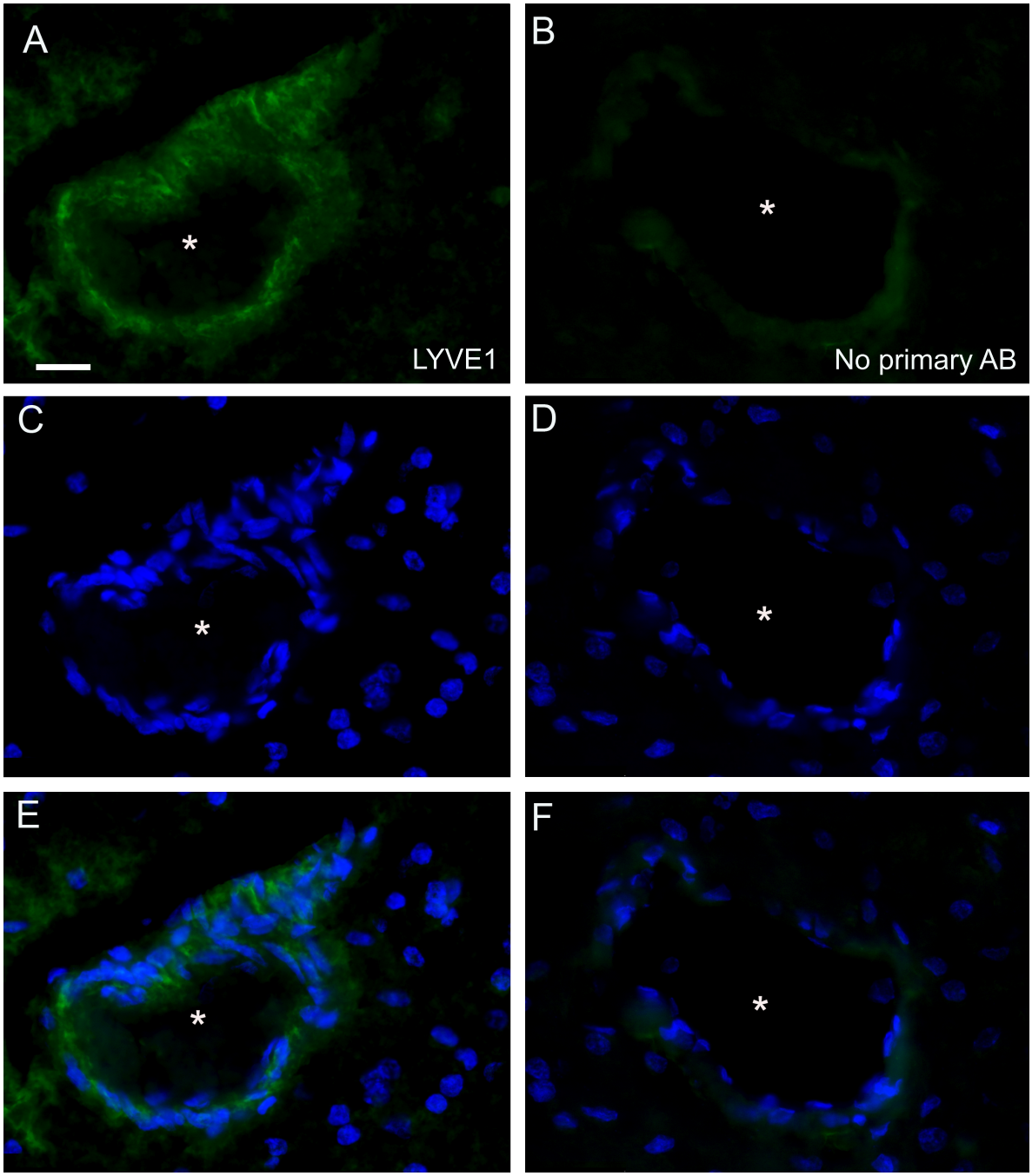


Colocalization of two lymphatic markers in the wall of a small vessel and attached T cells in the subarachnoideal space (SAS)

A - LYVE1 (green fluorescence to mark Alexa-488)) stained two vascular loops in the SAS in the same structure where VEGFR3 (C) staining (colored yellow for far-red fluorochrome, Alexa-650) is also present. CD3 positive (red fluorescence, colored for Alexa-594) T cells (B) are attached to the LMPCs as the overlay (D) demonstrates. Arrows point at T cells.

Scale bar: 25 µm

SI Fig.S3

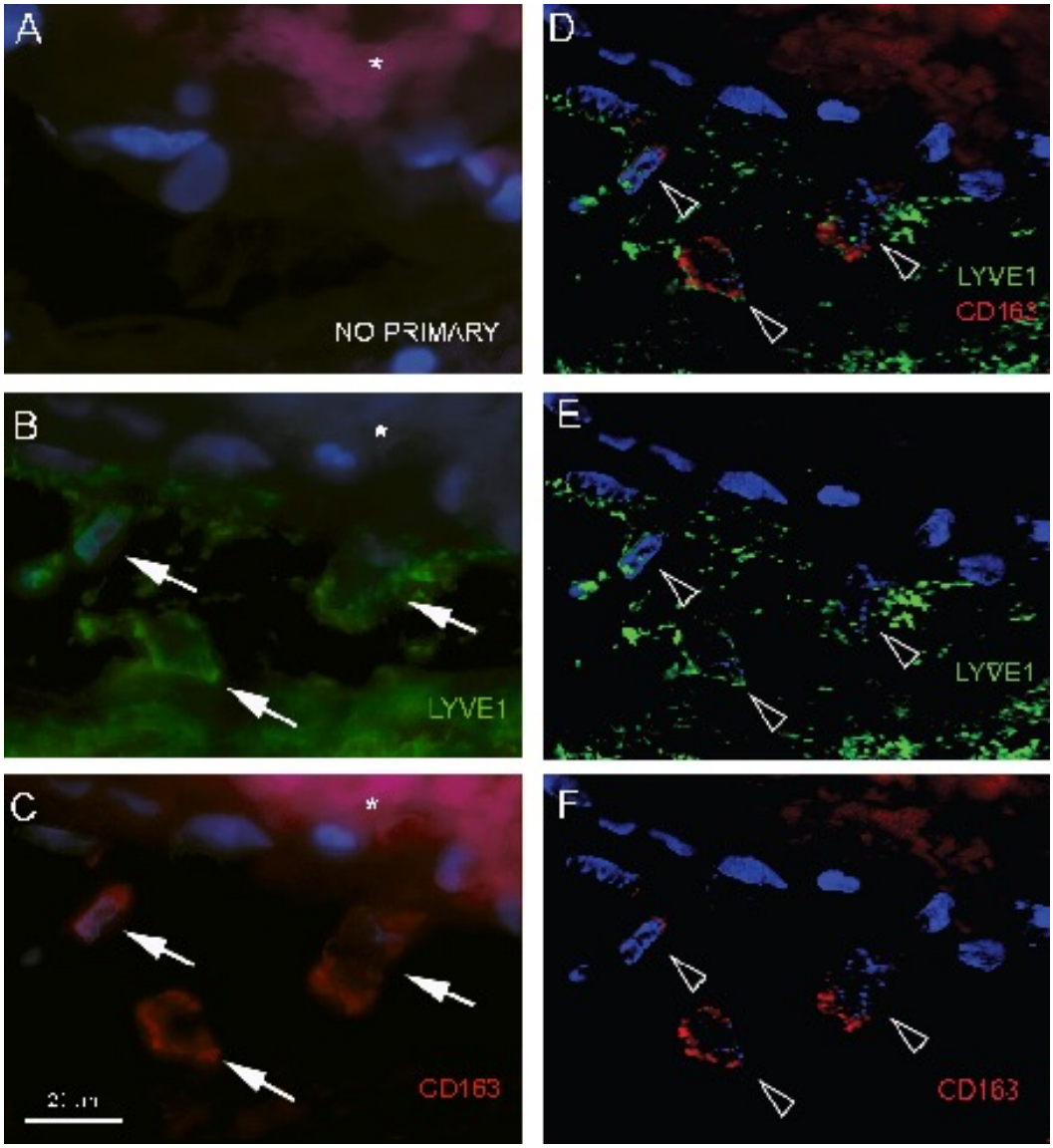


Control amplified staining for LYVE1

LYVE1 staining of vasculature in the parenchyma of a control section. A (LYVE1) and B (no primary antibody) were visualized using green fluorescence (Alexa-488). C and D are showing a nuclear staining with DAPI of sections in A and B, respectively and F are the overlay of green and blue fluorescence above. The asterisk depicts the vascular lumina.

Scale bar: 20 µm

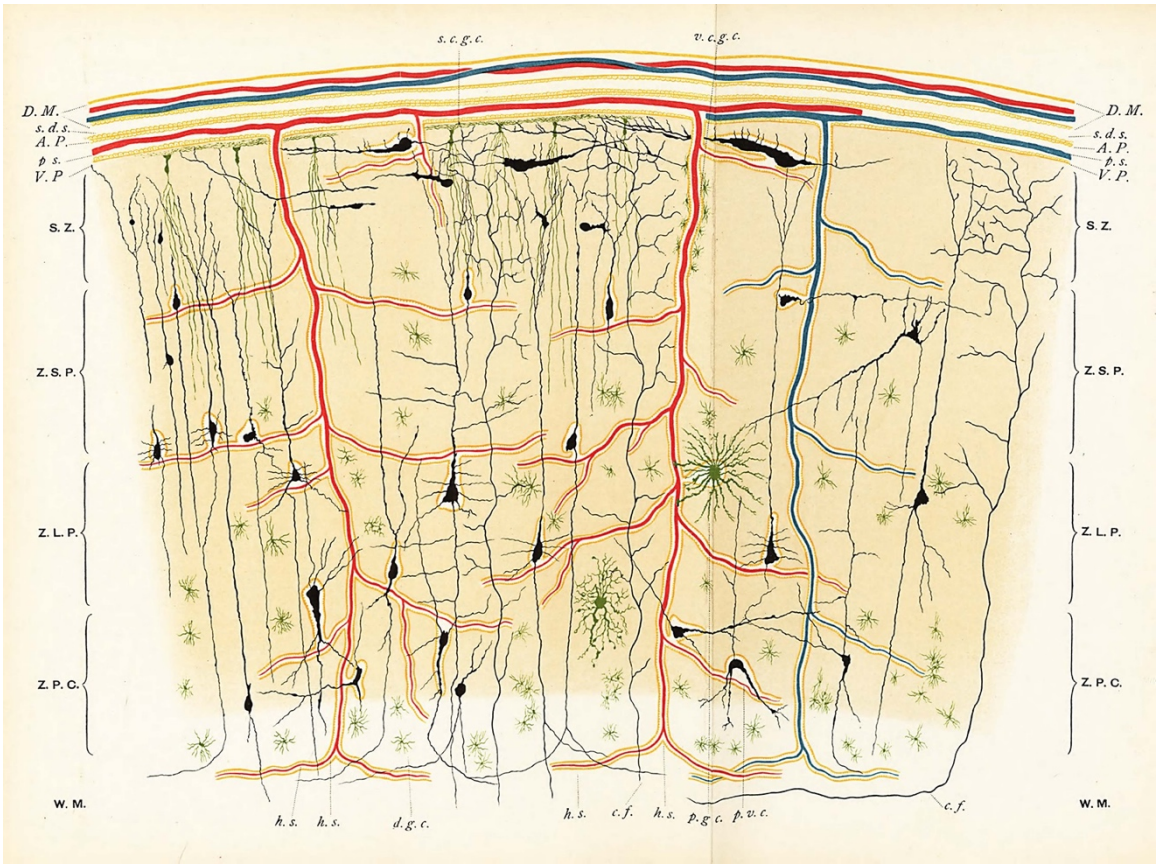
SI Fig.S4



Staining of barrier associated macrophages (BAM)s with the LYVE1 antibody

After pretreatment as described in the paper a rabbit LYVE1 and a mouse CD163 antibody were used in a control section of a human brain for double immunostaining. An overnight incubation at 4°C with the LYVE1 antibody (1:100) was followed by an anti-rabbit Alexa-488 (1:1000) secondary for 1 hour at RT. Then the mouse primary antibody to CD163 (1:100) was applied O/N and developed using an anti-mouse Alexa-594 fluorochrome. Following DAPI and Sudan black staining the section was examined using a Leica DMI6000 inverted microscope. An adjacent section was processed the same way with no primary antibodies used. Panel A demonstrates the staining with no primary antibody showing no specific signal in either filter. Panels B and C are images of the same area on an adjacent section that received primary antibody. Arrows point at three cells that seem to be double labelled. Following deconvolution D, E and F show a 0.5 µm thin optical section of the staining confirming the presence of both antigens in the same three cells (arrowheads). In the upper right corner of A and C a portion of a vessel can be seen with red blood cells causing autofluorescence in red (labelled with an asterisk). Scale bar: 25 µm

SI Fig.S5.



Dr. John Batty Tuke's drawing of perivascular pathways in the human brain from 1894

Reprinted from ref (1).

Dr. Tuke's legend to his drawing:
"DIAGRAMMATIC SCHEME OF THE CONSTITUENTS OF A CONVOLUTION. As regards the cells, fibers, and neuroglia, this diagram is founded on plates by Retzius, Cajal, and Andriezen. The arrangement of the vessels is purely diagrammatic. Endothelium is indicated by dotted yellow lines. Lymphatic spaces and channels are left white. ,' Dura mater ; s. d. s., Sub-dural space ; A. P., Arachno-pia; p. s., Pial space ; f-, Visceral pia; S. Z., Superficial zone; Z. S. P., Zone of small pyramids; Z.L.P.; Zone of large pyramids; Z. P. C., Zone of polymorphous cells; W. M., white matter; h. s., Hyaline sheath; p.g.c., Protoplasmic glia cells; s.c.g.c., superficial condensation of glia fibre cells; v. c. g. c., Vascular condensation of glia fibre cells."

List of historical references

Bold: human studies

1787

Mascagni, P. Vasorum lymphaticorum corporis humani historia et ichnographia. (Pazzini Carli: Siena, 1787). PMID: -

1816

Mascagni, P. & Bellini, G. B. Istoria completa dei vasi linfatici. Vol. 2., 195 pp. (Eusebio Pacini e Figlio: Florence, 1816). PMID: -

1869

Schwalbe, G. Der Arachnoidalraum, ein Lymphraum und sein Zusammenhang mit dem Perichorioidealraum. Zbl Med Wiss 7, 465-467 (1869). PMID: -

1875

Key, A. & Retzius, G. Studien in der Anatomie des Nervensystems und des Bindegewebes. (Norstedt & Söner: Stockholm, 1875-1876). PMID: -

1877

Lewis, B. The relationships of the nerve-cells of the cortex to the lymphatic system of the brain. Proc Roy Soc Lond 26, 326-334 (1877). PMID: -

1882

Tuke, J. B. Note on the anatomy of the pia mater. Trans Med Chir Soc Edinb 1, 118-122 (1882). PMID: 29583735

1884

Obersteiner, H. The cerebral blood-vessels in health and disease. Brain 7, 289-309, doi: 10.1093/brain/7.3.289 (1884). PMID: -

1890

Obersteiner, H. The anatomy of the central nervous organs in health and in disease, pp. 174-175 (Charles Griffin: London, 1890). PMID: -

1894

Tuke, J. B. The Morisonian Lectures, Delivered before the Royal College of Physicians of Edinburgh: Session 1894. Edinb Med J 39, 673-683 (1894). PMID: -

1910

Mott, F. W., Lond, M. D. & Lond, F. R. C. P. The Oliver-Sharpey lectures on the cerebrospinal fluid. Lancet 176, 1-8 (1910). PMID: -

1911

Bruce, A. & Dawson, J. W. On the relations of the lymphatics of the spinal cord. J Pathol 15, 169-178 (1911). PMID: -

1912

Zwillinger, H. Die Lymphbahnen des oberen Nasalschnittes und deren Beziehungen zu den perimeningealen Lymphraumen. Arch Laryngol und Rhinol 26, 66-78 (1912). PMID: -

1914

Weed, L. H. Studies on cerebro-spinal fluid. No. II: The theories of drainage of cerebro-spinal fluid with an analysis of the methods of investigation. J Med Res 31, 21-49 (1914). PMID: 19972193

1933

Mortensen, O. A. & Sullivan, W. E. The cerebrospinal fluid and the cervical lymph nodes. Anat Rec 56, 359-363, doi: 10.1002/ar.1090560405 (1933). PMID: -

1937

Faber, W. M. The nasal mucosa and the subarachnoid space. Am J Anat 62, 121-148 (1937). PMID: -

1939

Yoffey, J. M. & Drinker, C. K. Some observations on the lymphatics of the nasal mucous membrane in the cat and monkey. J Anat 74, 45-52.3. (1939). PMID: 17104799

1948

Brierley, J. B. & Field, E. J. The connexions of the spinal sub-arachnoid space with the lymphatic system. J Anat 82, 153-166 (1948). PMID: 17105055

1949

Field, E. J. & Brierley, J. B. The retro-orbital tissues as a site of outflow of cerebrospinal fluid. Proc Roy Soc Med 42, 447-450 (1949). PMID: 18153751

1957

Bowsher, D. Pathways of absorption of protein from the cerebrospinal fluid: an autoradiographic study in the cat. Anat Rec 128, 23-39 (1957). PMID: 13458825

1963

Csanda, E., Zoltán, T.Ö. & Földi, M. Elevation of cerebrospinal fluid pressure in the dog after obstruction of cervical lymphatic channels. Lancet 281, 832 (1963). PMID: 14024097

Földi, M., Csanda, E., Obál, F., Madarász, I., Szeghy, G. & Zoltán, O. T. [On effects of ligation of the lymphatic vessels and lymph nodes of the neck on the central nervous system in animal experiments. I]. Über Wirkungen der Unterbindung der Lymphgefäße und Lymphknoten des Halses auf das Zentralnervensystem im Tierversuch. Z Ges Exp Med 137, 483-510 (1963). PMID: 14097189

Szeghy, G., Csanda, E., Kozma, M., Poberai, M., Gellért, A. & Földi, M. Oedema of optic nerve produced by lymphatic stasis in dog. Lancet 281, 832 (1963). PMID: 13979893

1966

Földi, M., Gellért, A., Kozma, M., Poberai, M., Zoltán, Ö. T. & Csanda, E. New contributions to the anatomical connections of the brain and the lymphatic system. Acta Anat 64, 498-505 (1966). PMID: 5957959

1967

Földi, M., Csanda, E., Zoltán, Ö.T. & Dobranovics, I. Oedema of the optic nerve and the retina as a consequence of experimental cervical lymphatic blockage. Angiologica 4, 341-347 (1967). PMID: 6075771

1968

Csanda, E., Földi, M., Obál, F. & Zoltán, Ö. T. Cerebral oedema as a consequence of experimental cervical lymphatic blockage. Angiologica 5, 55-63 (1968). PMID: 5637028

Földi, M., Csanda, E., Simon, M., Obál, F., Schneider, I., Dobranovics, I., Zoltán, Ö. T., Kozma, M. & Poberai, M. Lymphogenic haemangiopathy. "Prelymphatic" pathways in the wall of cerebral and cervical blood vessels. Angiologica 5, 250-262 (1968). PMID: 5699286

1971

Arnold, W., Nitze, H. R. & Ritter, R. Anatomical relations between the cerebrospinal fluid (CSF) and the lymphatic system in the region of the head and neck. Arch Klin Exp Ohren Nasen Kehlkopfheilkd 198, 361 (1971). PMID: 5569463

1976

Casley-Smith, J. R., Földi-Börcsök, E. & Földi, M. The prelymphatic pathways of the brain as revealed by cervical lymphatic obstruction and the passage of particles. Br J Exp Pathol 57, 179-188 (1976). PMID: 773400

1977

Casley-Smith, J. R., Clodius, L. & Földi, M. Experimental blood vascular and lymphatic occlusion in the rabbit ear and the effect of benzopyrones. Arzneimittelforschung 27, 379-382 (1977). PMID: 577157

Csanda, E., Joó, F., Somogyi, I., Szücs, A., Saal, M., Auguszt, A. & Komoly, S. Structural, ultrastructural and functional reactions of the brain after implanting yttrium 90 rods used in stereotactic neurosurgery. Acta Neurochir (Wien) Suppl 24, 139-147 (1977). PMID: 335803

1979

Jackson, R. T., Tigges, J. & Arnold, W. Subarachnoid space of the CNS, nasal mucosa, and lymphatic system. Arch Otolaryngol 105, 180-184 (1979). PMID: 85446

Mann, J. D., Butler, A. B., Johnson, R. N. & Bass, N. H. Clearance of macromolecular and particulate substances from the cerebrospinal fluid system of the rat. J Neurosurg 50, 343-348 (1979). PMID: 422986

1980

Bradbury, M. W. & Cole, D. F. The role of the lymphatic system in drainage of cerebrospinal fluid and aqueous humour. J Physiol 299, 353-365 (1980). PMID: 6155466

1981

Bradbury, M. W., Cserr, H. F. & Westrop, R. J. Drainage of cerebral interstitial fluid into deep cervical lymph of the rabbit. Am J Physiol 240, F329-F336 (1981). PMID: 7223890

1984

Pile-Spellman, J. M., McKusick, K. A., Strauss, H. W., Cooney, J. & Taveras, J. M. Experimental in vivo imaging of the cranial perineural lymphatic pathway. AJNR Am J Neuroradiol 5, 539-545 (1984). PMID: 6435420

Szentistványi, I., Patlak, C. S., Ellis, R. A. & Cserr, H. F. Drainage of interstitial fluid from different regions of rat brain. Am J Physiol 246, F835-F844 (1984). PMID: 6742132

1985

Rennels, M. L., Gregory, T. F., Blaumanis, O. R., Fujimoto, K. & Grady, P. A. Evidence for a 'paravascular' fluid circulation in the mammalian central nervous system, provided by the rapid distribution of tracer protein throughout the brain from the subarachnoid space. Brain Res 326, 47-63 (1985). PMID: 3971148

Shen, J. Y., Kelly, D. E., Hyman, S. & McComb, J. G. Intraorbital cerebrospinal fluid outflow and the posterior uveal compartment of the hamster eye. Cell Tissue Res 240, 77-87 (1985). PMID: 3995547

1986

Erlich, S. S., McComb, J. G., Hyman, S. & Weiss, M. H. Ultrastructural morphology of the olfactory pathway for cerebrospinal fluid drainage in the rabbit. J Neurosurg 64, 466-473 (1986). PMID: 3950724

1989

Erlich, S. S., McComb, J. G., Hyman, S. & Weiss, M. H. Ultrastructure of the orbital pathway for cerebrospinal fluid drainage in rabbits. J Neurosurg 70, 926-931 (1989). PMID: 2715821

1990

Manzo, R. P., Gomez, D. G. & Potts, D. G. Cerebrospinal fluid absorption in the rabbit. Inner ear pathways. Acta Otolaryngol 109, 389-396 (1990). PMID: 2360445

1992

Cserr, H. F. & Knopf, P. M. Cervical lymphatics, the blood-brain barrier and the immunoreactivity of the brain: a new view. Immunol Today 13, 507-512 (1992). PMID: 1463583

1993

Kida, S., Pantazis, A. & Weller, R. O. CSF drains directly from the subarachnoid space into nasal lymphatics in the rat. Anatomy, histology and immunological significance. Neuropathol Appl Neurobiol 19, 480-488 (1993). PMID: 7510047

1994

Löwhagen, P., Johansson, B. B. & Nordborg, C. The nasal route of cerebrospinal fluid drainage in man. A light-microscope study. Neuropathol Appl Neurobiol 20, 543-550 (1994). PMID: 7898616

1997

Brinker, T., Lüdemann, W., Berens von Rautenfeld, D. & Samii, M. Dynamic properties of lymphatic pathways for the absorption of cerebrospinal fluid. Acta Neuropathol 94, 493-498 (1997). PMID: 9386783

1998

Boulton, M., Armstrong, D., Flessner, M., Hay, J., Szalai, J. P. & Johnston, M. Raised intracranial pressure increases CSF drainage through arachnoid villi and extracranial lymphatics. Am J Physiol 275, R889-R896 (1998). PMID: 9728088

1999

Killer, H. E., Laeng, H. & Groscurth, P. Lymphatic capillaries in the meninges of the human optic nerve. J Neuroophthalmol 19, 222-228 (1999). PMID: 10608671

2002

Podgrabinska, S., Braun, P., Velasco, P., Kloos, B., Pepper, M. S. & Skobe, M. Molecular characterization of lymphatic endothelial cells. Proc Natl Acad Sci U S A 99, 16069-16074 (2002). PMID: 12446836

2003

Zakharov, A., Papaiconomou, C., Djenic, J., Midha, R. & Johnson, M. Lymphatic cerebrospinal fluid absorption pathways in neonatal sheep revealed by subarachnoid injection of Microfil. Neuropathol Appl Neurobiol 29, 563-573 (2003). PMID: 14636163

2004

Johnston, M., Zakharov, A., Papaiconomou, C., Salmasi, G. & Armstrong D. Evidence of connections between cerebrospinal fluid and nasal lymphatic vessels in humans, non-human primates and other mammalian species. Cerebrospinal Fluid Res 1, 2 (2004). PMID: 15679948

2005

Lüdemann, W., Berens von Rautenfeld, D., Samii, M. & Brinker, T. Ultrastructure of the cerebrospinal fluid outflow along the optic nerve into the lymphatic system. Childs Nerv Syst 21, 96-103 (2005). PMID: 15338179

2006

Goldmann, J., Kwidzinski, E., Brandt, C., Mahlo, J., Richter, D. & Bechmann, I. T cells traffic from brain to cervical lymph nodes via the cribroid plate and the nasal mucosa. J Leukoc Biol 80, 797-801 (2006). PMID: 16885505

Hadaczek, P., Yamashita, Y., Mirek, H., Tamas, L., Bohn, M. C., Noble, C., Park, J. W. & Bankiewicz, K. The “perivascular pump” driven by arterial pulsation is a powerful mechanism for the distribution of therapeutic molecules within the brain. Mol Ther 14, 69-78 (2006). PMID: 16650807

Nagra, G., Koh, L., Zakharov, A., Armstrong, D. & Johnston, M. Quantification of cerebrospinal fluid transport across the cribriform plate into lymphatics in rats. Am J Physiol Regul Integr Comp Physiol 291, R1383-R1389 (2006). PMID: 16793937

Walter, B. A., Valera, V. A., Takahashi, S. & Ushiki, T. The olfactory route for cerebrospinal fluid drainage into the peripheral lymphatic system. Neuropathol Appl Neurobiol 32, 388-396 (2006). PMID: 16866984

2007

Gausas, R. E., Daly, T. & Fogt, F. D2-40 expression demonstrates lymphatic vessel characteristics in the dural portion of the optic nerve sheath. Ophthal Plast Reconstr Surg 23, 32-36 (2007). PMID: 17237687

Johnston, M., Armstrong, D. & Koh, L. Possible role of the cavernous sinus veins in cerebrospinal fluid absorption. Cerebrospinal Fluid Res 4, 3 (2007). PMID: 17437642

2008

Carare, R. O., Bernardes-Silva, M., Newman, T. A., Page, A. M., Nicoll, J. A., Perry, V. H. & Weller, R. O. Solutes, but not cells, drain from the brain parenchyma along basement membranes of capillaries and arteries: significance for cerebral amyloid angiopathy and neuroimmunology. Neuropathol Appl Neurobiol 34, 131-144 (2008). PMID: 18208483

Furukawa, M., Shimoda, H., Kajiwara, T., Kato, S. & Yanagisawa, S. Topographic study on nerve-associated lymphatic vessels in the murine craniofacial region by immunohistochemistry and electron microscopy. Biomed Res 29, 289-296 (2008). PMID: 19129672

Johanson, C. E., Duncan, J. A. 3rd, Klinge, P. M., Brinker, T., Stopa, E. G. & Silverberg, G. D. Multiplicity of cerebrospinal fluid functions: New challenges in health and disease. Cerebrospinal Fluid Res 5, 10 (2008). PMID: 18479516

2010

Birke, K., Lutjen-Drecoll, E., Kerjaschki, D. & Birke, M. T. Expression of podoplanin and other lymphatic markers in the human anterior eye segment. Invest Ophthalmol Vis Sci 51, 344-354 (2010). PMID: 19737877

Clapham, R., O'Sullivan, E., Weller, R. O. & Carare, R. O. Cervical lymph nodes are found in direct relationship with the internal carotid artery: significance for the lymphatic drainage of the brain. Clin Anat 23, 43-47 (2010). PMID: 19918869

2012

Iliff, J. J., Wang, M., Liao, Y., Plogg, B. A., Peng, W., Gundersen, G. A., Benveniste, H., Vates, G. E., Deane, R., Goldman, S. A., Nagelhus, E. A. & Nedergaard, M. A paravascular pathway facilitates CSF flow through the brain parenchyma and the clearance of interstitial solutes, including amyloid beta. Sci Transl Med 4, 147ra111 (2012). PMID: 22896675

2013

Chaitanya, G. V., Omura, S., Sato, F., Martinez, N. E., Minagar, A., Ramanathan, M., Guttman, B. W., Zivadinov, R., Tsunoda, I. & Alexander, J. S. Inflammation induces neuro-lymphatic protein expression in multiple sclerosis brain neurovasculature. J Neuroinflammation 10, 125 (2013). PMID: 24124909

Iliff, J. J., Lee, H., Yu, M., Feng, T., Logan, J., Nedergaard, M. & Benveniste, H. Brain-wide pathway for waste clearance captured by contrast-enhanced MRI. J Clin Invest 123, 1299-1309 (2013). PMID: 23434588

Iliff, J. J., Wang, M., Zeppenfeld, D. M., Venkataraman, A., Plog, B. A., Liao, Y., Deane, R. & Nedergaard, M. Cerebral arterial pulsation drives paravascular CSF-interstitial fluid exchange in the murine brain. J Neurosci 33, 18190-18199 (2013). PMID: 24227727

Mathieu, E., Gupta, N., Macdonald, R. L., Ai, J. & Yücel, Y. H. In vivo imaging of lymphatic

drainage of cerebrospinal fluid in mouse. Fluids Barriers CNS 10, 35 (2013). PMID: 24360130

Rangroo Thrane, V., Thrane, A. S., Plog, B. A., Thiyagarajan, M., Iliff, J. J., Deane, R., Nagelhus, E. A. & Nedergaard, M. Paravascular microcirculation facilitates rapid lipid transport and astrocyte signaling in the brain. Sci Rep 3, 2582 (2013). PMID: 24002448

Thrane, V. R., Thrane, A. S., Plog, B. A., Thiyagarajan, M., Iliff, J. J., Deane, R., Nagelhus, E. A. & Nedergaard, M. Paravascular microcirculation facilitates rapid lipid transport and astrocyte signaling in the brain. Sci Rep 3, 2582 (2013). PMID: 24002448

Xie, L., Kang, H., Xu, Q., Chen, M. J., Liao, Y., Thiyagarajan, M., O'Donnell, J., Christensen, D. J., Nicholson, C., Iliff, J. J., Takano, T., Deane, R. & Nedergaard, M. Sleep drives metabolite clearance from the adult brain. Science 342, 373-377 (2013). PMID: 24136970

Yang, L., Kress, B. T., Weber, H. J., Thiyagarajan, M., Wang, B., Deane, R., Benveniste, H., Iliff, J. J. & Nedergaard, M. Evaluating glymphatic pathway function utilizing clinically relevant intrathecal infusion of CSF tracer. J Transl Med 11, 107 (2013). PMID: 23635358

2014

Kress, B. T., Iliff, J. J., Xia, M., Wang, M., Wei, H. S., Zeppenfeld, D., Xie, L., Kang, H., Xu, Q., Liew, J. A., Plog, B. A., Ding, F., Deane, R. & Nedergaard, M. Impairment of paravascular clearance pathways in the aging brain. Ann Neurol 76, 845-861 (2014). PMID: 25204284

2015

Aspelund, A., Antila, S., Proulx, S. T., Karlen, T. V., Karaman, S., Detmar, M., Wiig, H. & Alitalo, K. A dural lymphatic vascular system that drains brain interstitial fluid and macromolecules. J Exp Med 212, 991-999 (2015). PMID: 26077718

Coles, J. A., Myburgh, E., Ritchie, R., Hamilton, A., Rodgers, J., Mottram, J. C., Barrett, M. P. & Brewer, J. M. Intravital imaging of a massive lymphocyte response in the cortical dura of mice after peripheral infection by trypanosomes. PLoS Negl Trop Dis 9, e0003714 (2015). PMID: 25881126

Eide, P. K. & Ringstad, G. MRI with intrathecal MRI gadolinium contrast medium administration: a possible method to assess glymphatic function in human brain. Acta Radiol Open 4, 2058460115609635 (2015). PMID: 26634147

Jessen, N. A., Munk, A. S., Lundgaard, I. & Nedergaard, M. The glymphatic system: A beginner's guide. Neurochem Res 40, 2583-2599 (2015). PMID: 25947369

Lee, H., Xie, L., Yu, M., Kang, H., Feng, T., Deane, R., Logan, J., Nedergaard, M. & Benveniste, H. The effect of body posture on brain glymphatic transport. J Neurosci 35, 11034-11044 (2015). PMID: 26245965

Louveau, A., Smirnov, I., Keyes, T. J., Eccles, J. D., Rouhani, S. J., Peske, J. D., Derecki, N. C., Castle, D., Mandell, J. W., Lee, K. S., Harris, T. H. & Kipnis, J. Structural and functional features of central nervous system lymphatic vessels. Nature 523, 337-341 (2015). PMID: 26030524

Schrödl, F., Kaser-Eichberger, A., Trost, A., Strohmaier, C., Bogner, B., Runge, C., Motloch, K., Bruckner, D., Laimer, M., Heindl, L. M. & Reitsamer, H. A. Lymphatic markers in the adult human choroid. Invest Ophthalmol Vis Sci 56, 7406-7416 (2015). PMID: 26574800

2016

Achariyar, T. M., Li, B., Peng, W., Verghese, P. B., Shi, Y., McConnell, E., Benraiss, A., Kasper, T., Song, W., Takana, T., Holtzman, D. M., Nedergaard, M. & Deane, R. Glymphatic distribution of CSF-derived apoE into brain is isoform specific and suppressed

during sleep deprivation. Mol Neurodegener 11, 74 (2016). PMID: 27931262

Asgari, M., de Zélicourt, D. & Kurtcuoglu, V. Glymphatic solute transport does not require bulk flow. Sci Rep 6, 38635 (2016). PMID: 27929105

Jin, B. J., Smith, A. J. & Verkman, A. S. Spatial model of convective solute transport in brain extracellular space does not support a "glymphatic" mechanism. J Gen Physiol 148, 489-501 (2016). PMID: 27836940

Lohrberg, M. & Wilting, J. The lymphatic vascular system of the mouse head. Cell Tissue Res 366, 667-677 (2016). PMID: 27599481

Morris, A. W., Sharp, M. M., Albargothy, N. J., Fernandes, R., Hawkes, C. A., Verma, A., Weller, R. O. & Carare, R. O. Vascular basement membranes as pathways for the passage of fluid into and out of the brain. Acta Neuropathol 131, 725-736 (2016). PMID: 26975356

2017

Absinta, M., Ha, S. K., Nair, G., Sati, P., Luciano, N. J., Palisoc, M., Louveau, A., Zaghloul, K. A., Pittaluga, S., Kipnis, J. & Reich, D. S. Human and nonhuman primate meninges harbor lymphatic vessels that can be visualized noninvasively by MRI. eLlife 6, pii: e29738 (2017). PMID: 28971799

Antila, S., Karaman, S., Nurmi, H., Airavaara, M., Voutilainen, M. H., Mathivet, T., Chilov, D., Li, Z., Koppinen, T., Park, J. H., Fang, S., Aspelund, A., Saarma, M., Eichmann, A., Thomas, J. L. & Alitalo, K. Development and plasticity of meningeal lymphatic vessels. J Exp Med 214, 3645-3667 (2017). PMID: 29141865

Bedussi, B., van der Wel, N.N., de Vos, J., van Veen, H., Siebes, M., VanBavel, E. & Bakker, E.N. Paravascular channels, cisterns, and the subarachnoid space in the rat brain: A single compartment with preferential pathways. J Cereb Blood Flow Metab 37, 1374-1385 (2017). PMID: 27306753

Dobson, H., Sharp, M. M., Cumpsty, R., Criswell, T. P., Wellman, T., Finucane, C., Sullivan, J. M., Weller, R. O., Verma, A. & Carare, R. O. The perivascular pathways for influx of cerebrospinal fluid are most efficient in the midbrain. Clin Sci (Lond) 131, 2745-2752 (2017). PMID: 29021222

Holter, K. E., Kehlet, B., Devor, A., Sejnowski, T. J., Dale, A. M., Omholt, S. W., Ottersen, O. P., Nagelhus, E. A., Mardal, K. A. & Pettersen, K. H. Interstitial solute transport in 3D reconstructed neuropil occurs by diffusion rather than bulk flow. Proc Natl Acad Sci U S A 114, 9894-9899 (2017). PMID: 28847942

Ma, Q., Ineichen, B. V., Detmar, M. & Proulx, S. T. Outflow of cerebrospinal fluid is predominantly through lymphatic vessels and is reduced in aged mice. Nat Commun 8, 1434 (2017). PMID: 29127332

Ratner, V., Gao, Y., Lee, H., Elkin, R., Nedergaard, M., Benveniste, H. & Tannenbaum, A. Cerebrospinal and interstitial fluid transport via the glymphatic pathway modeled by optimal mass transport. Neuroimage 152, 530-537 (2017). PMID: 28323163

Santonja, C., Medina-Puente, C., del Castillo, C. S., Úbeda, A. C. & Rodríguez-Pinilla, S. M. Primary effusion lymphoma involving cerebrospinal fluid, deep cervical lymph nodes and adenoids. Report of a case supporting the lymphatic connection between brain and lymph nodes. Neuropathology 37, 249-258 (2017). PMID: 27862361

Smith, A. J., Yao, X., Dix, J. A., Jin, B. J. & Verkman, A. S. Test of the 'glymphatic' hypothesis demonstrates diffusive and aquaporin-4-independent solute transport in rodent brain parenchyma. Elife 6, pii: e27679 (2017). PMID: 28826498

Wang, M., Ding, F., Deng, S., Guo, X., Wang, W., Iliff, J. J. & Nedergaard, M. Focal solute trapping and global glymphatic pathway impairment in a murine model of multiple microinfarcts. J Neurosci 37, 2870-2877 (2017). PMID: 28188218

2018

Da Mesquita, S., Louveau, A., Vaccari, A., Smirnov, I., Cornelison, R. C., Kingsmore, K. M., Contarino, C., Onengut-Gumuscu, S., Farber, E., Raper, D., Viar, K. E., Powell, R. D., Baker, W., Dabhi, N., Bai, R., Cao, R., Hu, S., Rich, S. S., Munson, J. M., Lopes, M. B., Overall, C. C., Acton, S. T. & Kipnis, J. Functional aspects of meningeal lymphatics in ageing and Alzheimer's disease. Nature 560, 185-191 (2018). PMID: 30046111

Eide, P. K., Vatnehol, S. A. S., Emblem, K. E. & Ringstad, G. Magnetic resonance imaging provides evidence of glymphatic drainage from human brain to cervical lymph nodes. Sci Rep 8, 7194 (2018). PMID: 29740121

Kumar, N. N., Lochhead, J. J., Pizzo, M. E., Nehra, G., Boroumand, S., Greene, G. & Thorne, R. G. Delivery of immunoglobulin G antibodies to the rat nervous system following intranasal administration: Distribution, dose-response, and mechanisms of delivery. J Control Release 286, 467-484 (2018). PMID: 30081144

Ringstad, G., Valnes, L. M., Dale, A. M., Pripp, A. H., Vatnehol, S. S., Emblem, K. E., Mardal, K. A. & Eide, P. K. Brain-wide glymphatic enhancement and clearance in humans assessed with MRI. JCI Insight 3, e121537 (2018). PMID: 29997300

Semyachkina-Glushkovskaya, O., Abdurashitov, A., Dubrovsky, A., Pavlov, A., Shushunova, N., Maslyakova, G., Navolokin, N., Bucharskaya, A., Tuchin, V. & Kurths, J. The interaction between the meningeal lymphatics and blood-brain barrier. Proc SPIE 10495, Biophotonics and Immune Responses XIII, 104950J-1-104950J-5, doi: 10.1117/12.2289406 (2018). PMID: -

Verheggen, I. C. M., Van Boxtel, M. P. J., Verhey, F. R. J., Jansen, J. F. A., Backes, W. H. Interaction between blood-brain barrier and glymphatic system in solute clearance. Neurosci Biobehav Rev 90, 26-33 (2018). PMID: 29608988

2019

Ahn, J. H., Cho, H., Kim, J. H., Kim, S. H., Ham, J. S., Park, I., Suh, S. H., Hong, S. P., Song, J. H., Hong, Y. K., Jeong, Y., Park, S. H. & Koh, G. Y. Meningeal lymphatic vessels at the skull base drain cerebrospinal fluid. Nature 572, 62-66 (2019). PMID: 31341278

Caunca, M. R., De Leon-Benedetti, A., Latour, L., Leigh, R. & Wright, C. B. Neuroimaging of cerebral small vessel disease and age-related cognitive changes. Front Aging Neurosci 11, 145 (2019). PMID: 31316367

Hsu, M., Rayasam, A., Kijak, J. A., Choi, Y. H., Harding, J. S., Marcus, S. A., Karpus, W. J., Sandor, M. & Fabry, Z. Neuroinflammation-induced lymphangiogenesis near the cribriform plate contributes to drainage of CNS-derived antigens and immune cells. Nat Commun 10, 229 (2019). PMID: 30651548

Meng, Y., Abrahao, A., Heyn, C. C., Bethune, A. J., Huang, Y., Pople, C. B., Aubert, I., Hamani, C., Zinman, L., Hynynen, K., Black, S. E. & Lipsman, N. Glymphatics visualization after focused ultrasound-induced blood-brain barrier opening in humans. Ann Neurol 86, 975-980 (2019). PMID: 31525269

2020

Naganawa, S., Ito, R., Taoka, T., Yoshida, T. & Sone, M. The space between the pial sheath and the cortical venous wall may connect to the meningeal lymphatics. Magn Reson Med Sci, 19, 1-4 (2020). PMID: 31611540

Ringstad, G. & Eide, P. K. Cerebrospinal fluid tracer efflux to parasagittal dura in humans. Nat Commun 11, 354 (2020). PMID: 31953399

Song, E., Mao, T., Dong, H., Boisserand, L. S. B., Antila, S., Bosenberg, M., Alitalo, K., Thomas, J. L. & Iwasaki, A. VEGF-C-driven lymphatic drainage enables immuno-surveillance of brain tumours. Nature 577, 689-694 (2020). PMID: 31942068

SI Table S1 – Antibody specifications

Primary Ab target	Clone	Vendor	Cat#	Dilution
Ms Podoplanin	PDPN/1433	enQuire BioReagents	10630-MSM1-P1	1:4000
Ms CD45	130	BD Pharmingen	555480	1:1000
Rbt CD3	SP7	Abcam	Ab16669	1:1000
Ms CD8	C8/144B	Dako	M7103	1:1000
Rbt FOXP3	5H10L18	Thermo Fisher Scientific	700914	1:200
Rbt ICAM1	EP1442Y	Abcam	Ab53013	1:1000
Rbt VCAM1	EPR5047	Abcam	Ab134047	1:700
Rbt Stabilin2		Abcam	Ab121893	1:500
Rbt CD62L		Abcam	Ab18959	1:200
Rbt VEGFR3		Abcam	Ab27278	1:500
Rbt LYVE1		Millipore	Ab2988	1:10 000
Rbt von Willebrand Factor		Vector	VP-V686	1:5000
Ms CD163	10D6	NeoMarkers	Ms1103-S0	1:100

List of Primer sequences used for PCR:

- PDPN
  - Forward: AGAGCAACAACTCAACGGGA
  - Reverse: TGTAGTCTCAGTGTCATCTTC
- LYVE1
  - Forward: TGGGGATCACCCTTGTGAG
  - Reverse: AGCCATAGCTGCAAGTTTCAAA
- VEGFR3
  - Forward: GCACTGCCACAAGAAGTACCT
  - Reverse: GCTGCACAGATAGCGTCCC
- PROX1
  - Forward: GGATGTTGAGTATTCAGTGGTGC
  - Reverse: CTGGGAAATTATGGTTGCTCCT
- ICAM1
  - Forward: CGTGGGGAGAAGGAGCTGAA
  - Reverse: CAGTGCGGCACGAGAAATTG
- VCAM1
  - Forward: TGGGCTGTGAATCCCCATCT
  - Reverse: GGGTCAGCGCGTGGAATTGGTC
- SELL (CD62L)
  - Forward: CAGCCCTCTGTTACACAGCTT
  - Reverse: GCCCATAGTACCCCACATCA
- HPRT1
  - Hs_HPRT_SG
    Qiagen QuantiTect Primer Assay (Catalog number QT00059066)

Reference

1. J. B. Tuke, The Morisonian Lectures, Delivered before the Royal College of Physicians of Edinburgh: Session 1894. *Edinb Med J* **39**, 673-683 (1894).