

## A case of tuberculous meningitis and the role of perivascular spaces in lymph cell migration in the brain

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### Abstract

Meningitis and encephalitis are inflammatory diseases in which acute and chronic inflammatory cells infiltrate leptomeninges, especially the arachnoid, and migrate through the subarachnoid space and by diapedesis, in order to extend around blood vessels and into the brain parenchyma. To what extent migrated/resident inflammatory cells participate to these interactions, or what are exactly the initial steps by which these cells reach the brain interstitium, it is not yet completely known. Recent years have brought new insights into the description of water flow circuits in the brain, suggesting that the cerebrospinal fluid enters the brain within the perivascular spaces of arteries, while interstitial fluid drains along perivascular venous sector. Moreover, it has been showed that vascular basement membranes have a complex multi-layered architecture that originates with epithelial, endothelial, smooth muscle cells and glial cells, and that the virtual space between these layers might be in fact an essential component of these perivascular spaces. Starting from a patient that presented with active pulmonary tuberculosis and with consecutive purulent-hemorrhagic meningitis and encephalitis, we have characterized here the compartments in which immune cells can be found in the brain tissue. Besides the classical histopathological description, what was of interest here, was that we identified for the first time mononucleated inflammatory cells that seemed to be present in pockets of the vascular basement membranes, small spaces devoid of red blood cells. Although this is mere a morphological observation, future high-resolution studies should clarify if this is a possible route for the immune cells entering the brain.

**Keywords:** meningitis, encephalitis, perivascular spaces, basement membranes, lymphocyte migration.

### Introduction

Tuberculosis (TB) is a chronic granulomatous infection caused by *Mycobacterium tuberculosis* (MTB). TB is the tenth cause of death around the world, and pulmonary TB is the most common form, although there many extrapulmonary forms (up to 20% of all forms), such as those involving the pleura, lymph nodes, meninges, genitourinary tract, abdominal viscera, joints and bones or skin [1]. The diagnosis of TB is ascertained by the isolation of MTB microorganisms in cultures from patient's biological material. However, there are also paucibacillary samples taken from difficult to explore areas, thus lowering the accuracy of the extrapulmonary TB diagnosis, so altogether a negative results cannot fully rule out the diagnosis of TB [2].

For greater diagnosis accuracy, repeated tests are recommended. For example, in patients with TB of the urinary tract, between three to six urine exams are required for a probability of about 80–90% to obtain a positive culture [3]. Also, examination of the cerebrospinal fluid (CSF) by repeated lumbar puncture increases the sensitivity of the diagnosis, as studies have demonstrated that culture positivity is approaching a maximum possible level after taking at least four CSF serial samples [4].

Of all forms of TB, tuberculous meningitis accounts for 1–2% and is the most severe form of the disease, being met especially in children and young people with immunodeficiency syndromes [4]. The neurological deficits with which survivors remain are due to infarctions in areas irrigated by these arteries [5]. Paralysis of the cranial nerves may result either from compression due to exudate or infarction [6]. Diagnosis of tuberculous meningitis can be difficult because of the symptomatology which can simulate other neurological pathologies and laboratory tests which may not be very sensitive [7]. The quickest initiation of the treatment is lifesaving because many patients with this form of TB remain with severe disabilities [8].

Infection of the central nervous system (CNS) with MTB occurs under the form of a subacute or chronic meningitis. Tuberculomas may also be present, and sometimes can be interpreted as space replacement lesions [6]. Tuberculous meningitis may be the only manifestation of TB or may occur at the same time as pulmonary or disseminated disease [9]. Another consequence is the occurrence of vasculitis in the vertebro-basilar territory, the middle cerebral artery and the Willis arterial circle [10]. The most common complication of these diseases is

communicating hydrocephalus, which appears secondary to obstruction of CSF in basal cisterns. Tuberculous meningitis can cause dural venous thrombosis [6].

Pathologically, meningitis and encephalitis in general are characterized by the presence of both acute and/or chronic inflammatory infiltrate into the leptomeninges (mostly) and the brain parenchyma, with the presence of a granulomatous reaction with epithelioid cells, with inflammatory cells scattered around in the neuropil, around blood vessels (a pattern called perivascular cuffing) or in dilated perivascular spaces.

The objective of this study was to characterize the compartments in which migrating inflammatory cells reside/act at the level of the CNS.

### ☐ Case presentation

In this paper, we present the case of a 71-year-old female patient admitted in the Emergency Department of the Clinical Neuropsychiatry Hospital of Craiova, Romania, for diffuse headache, dizziness, gait and balance disorders, night sweats with sudden onset of symptoms three days before presentation. There was no significant personal and heredo-collateral history, with no previous patient transcript data. A written informed consent was obtained from the family regarding the publication of the data.

At neurological examination, the patient presented amblyopia with both eyes, dizziness, diffuse headache, nausea and vomiting. During hospital time, the patient presented fever, asthenia, inappetence, mild nuchal rigidity. Biological examinations have seen elevated erythrocyte sedimentation rate (ESR) values, and eye exam has revealed papillary alterations. Next, the patient has a native computed tomography (CT), with post-contrast imaging (Figure 1). Both showed on oval formation surrounded by edema in the right parietal lobe with the deletion of subarachnoid spaces on the right side.

Radiological findings of the lungs reveal a nodular formation in the left upper lobe with diffuse surrounding opacification, image suspected for pulmonary TB.

The lumbar puncture was performed and this revealed a clear, hypertensive fluid, increased level of lymphocytes (740/mm<sup>3</sup>), Pandy's intensely positive reaction with 120 mg/dL albumin, low glucose level (30 g%), a biological exam that suggested cerebral involvement.

The patient's condition worsened, with loss of consciousness and cranial nerve palsies, so that she was transferred to the Intensive Care Unit of the Emergency County Hospital of Craiova.

The patient died seven days after presentation due to bronchopneumonia/respiratory failure on a compromised immune system. At necropsy, the lateral part of the right brain hemisphere showed a thick purulent exudate deposition within the leptomeninges and in the upper cortical tissue, with minimal hemorrhagic areas. The exploration of the superior lobe of the right lung confirmed the presence of pulmonary TB and showed a few caseous nodules ranging in diameter between 0.5–1 cm, and surrounded by a condensed parenchyma with purulent exudate appearing spontaneous on a cut-section. The pancreas showed diffuse hemorrhagic infiltration, and all other organs were congested.

After macroscopic examination, tissue was fixed and processed for paraffin embedding in the Department of Pathology, Emergency County Hospital of Craiova, and prepared for immunohistochemistry (IHC) in the Research Center for Microscopic Morphology and Immunology, University of Medicine and Pharmacy of Craiova. After fixation in 10% neutral buffered formalin, all the tissue fragments were sectioned with a microtome, and sections stained with Hematoxylin–Eosin (HE) for basic histopathological diagnosis. Brain sections were further processed for IHC using the anti-collagen IV [mouse, Dako (Glostrup, Denmark), code M0785, 1:50], anti-leukocyte common antigen [LCA, cluster of differentiation 45 (CD45)] (mouse, Dako, code M0701, 1:100), or the anti-smooth muscle actin (mouse, Dako, code M0851, 1:100) primary antibodies. After antigen retrieval by microwaving in citrate buffer, pH 6, and peroxidase blocking, the primary antibodies were incubated on the sections overnight, at 4°C. The next day, after thorough washing, an anti-mouse goat secondary antibody linked with Horseradish peroxidase (HRP) was added on the slides for 30 minutes (Nichirei Bioscience, Tokyo, Japan), the enzyme was visualized with 3,3'-Diaminobenzidine tetrahydrochloride hydrate (DAB) (Dako), and the slides were coverslipped with a xylene-based medium (Sigma-Aldrich, St. Louis, MO, USA).

On microscopy, the lung reconfirmed the active disease, showing a generalized purulent and mononuclear mixed alveolitis, with large confluent caseating necrotic areas surrounded by chronic granulomatous reaction, with epithelioid cells and Langhans giant cells (Figure 2). There was important fibrosis and moderate mononuclear inflammatory cells in the portal spaces of the liver, with associated steatosis, and with large areas of cytoateolysis in the pancreas. On the microscopic examination of the brain in the region of the lateral sulcus, the leptomeninges and the upper cortical layers exhibited a polymorphonuclear and mononuclear exudate, with fibrin co-existence, hemorrhages and caseous necrotic areas. No Langhans giant cells could be found here, but towards the middle cortical layers, the affected region was surrounded by numerous reactive astrocytes. Septic emboli could be identified in larger subcortical vessels. The inflammatory exudate was not, however, limited to the meninges and surrounding cortex, but extended around vessels throughout the regional cortex, without involving the white matter beneath.

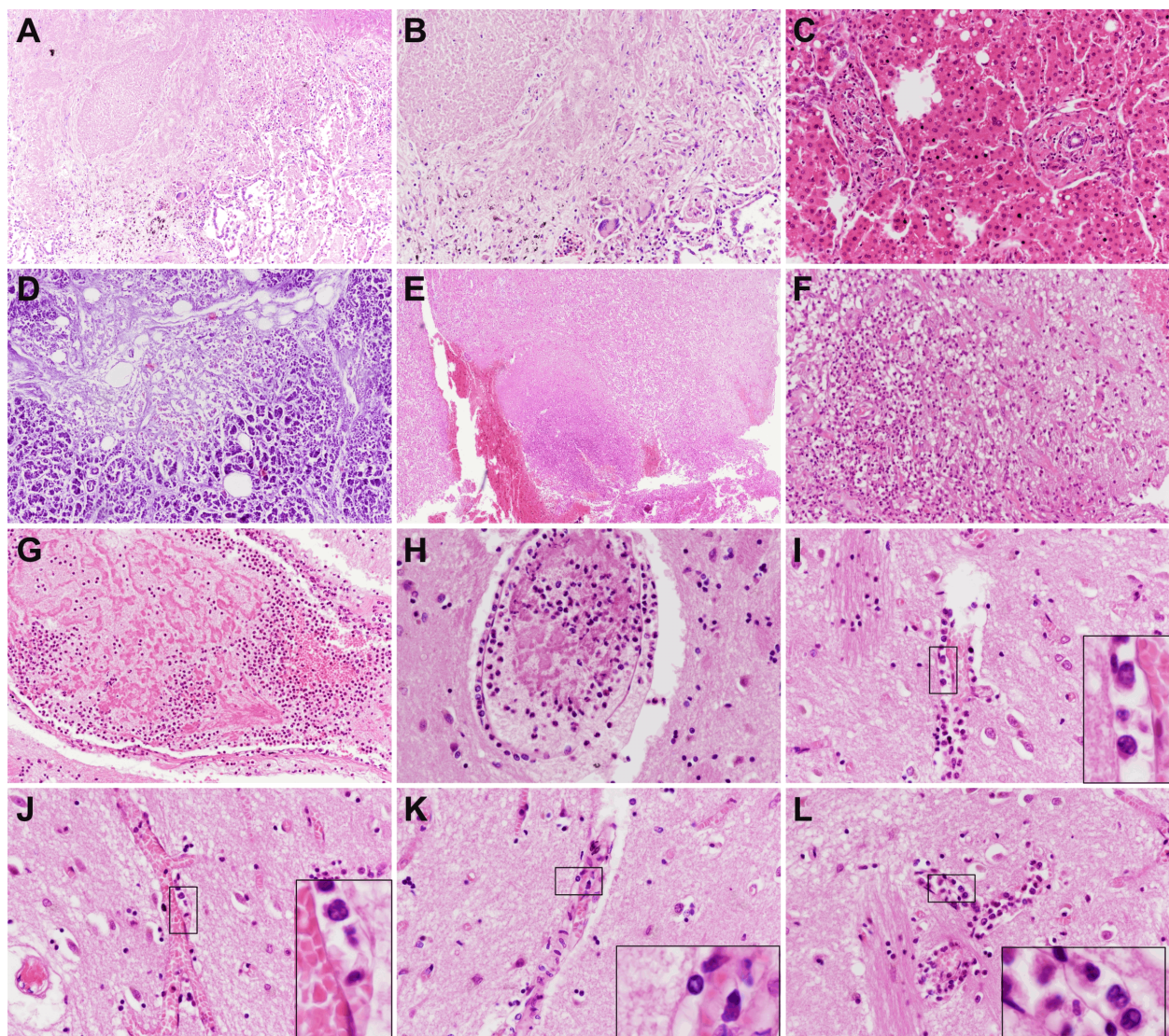
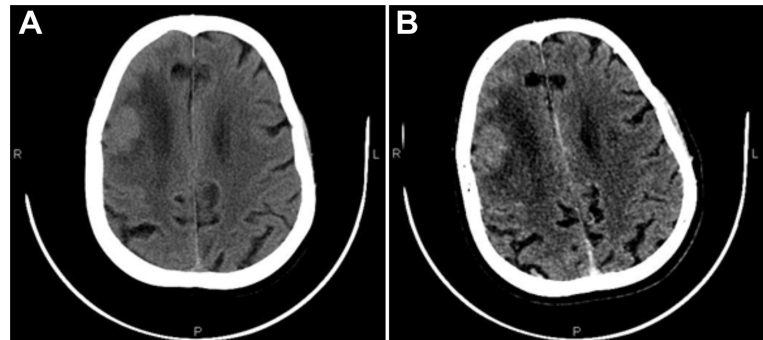
What was interesting, however, was that the mononuclear inflammatory cells were not present only around the vessels, in the brain parenchyma or the dilated perivascular spaces, but in what there seems to be deduplicated and dissected vascular walls, spaces that in most of the cases did not contain birefringent material that could have been identified as red blood cells (Figure 2). Sometimes, endothelial cells' nuclei allowed a clear demarcation of the vascular lumen and ascertained that the lymph cells were not located in the luminal areas of the vessels. Without extravasated red blood cells, and without enlarged perivascular spaces, sharp demarcation lines suggested deduplicated vascular basement membranes.

In an attempt to clarify this observation, we further performed IHC for collagen IV, and in multiple instances,

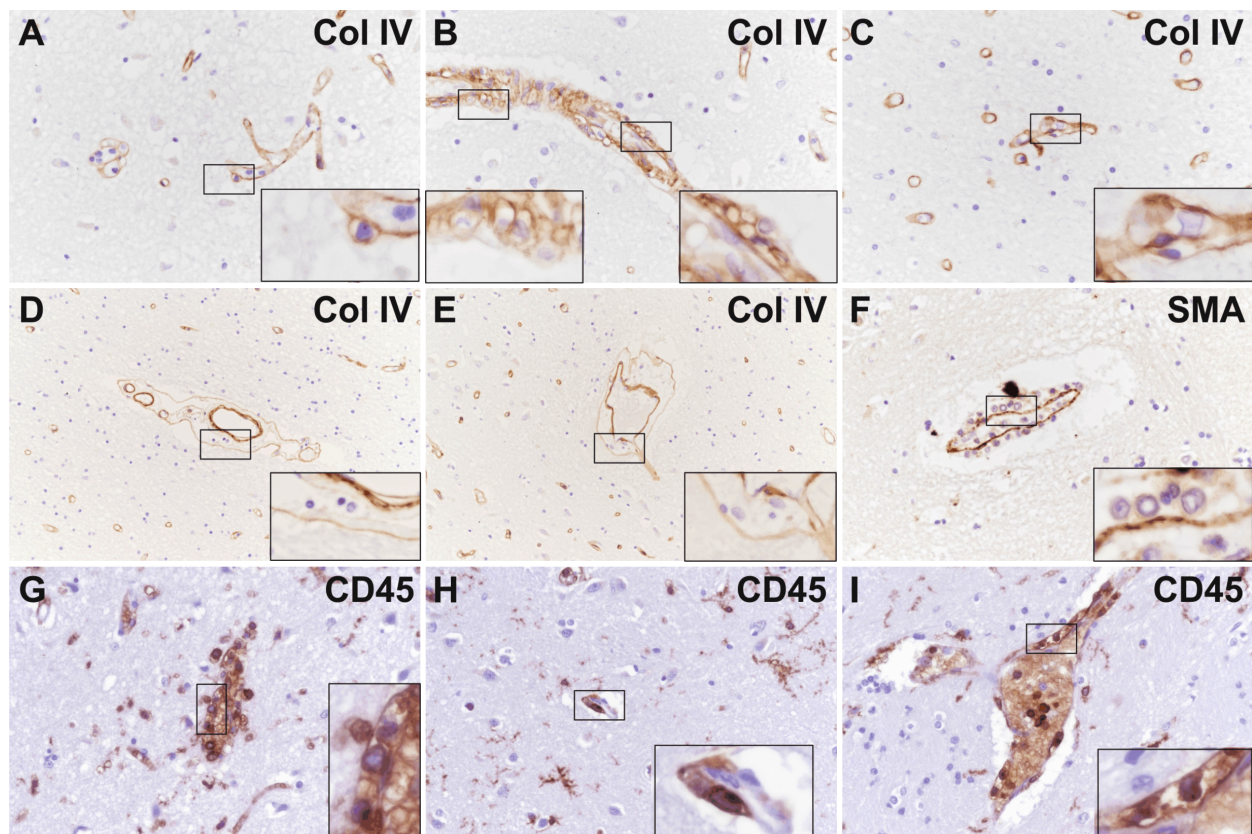
this clearly delineated vacuolated and saccular spaces in the thickness of the vessel walls surrounded by collagen IV and containing round lymph cell-looking nuclei (Figure 3). Dilated perivascular spaces also contained lymph cells, and small vessels without a clear-cut dilated perivascular space

also showed this phenomenon. Immunostaining for smooth muscle actin also confirmed that these nuclei appear to dissect the muscle wall, that these cells were not smooth muscle cells, and that these spaces were distinct from the perivascular spaces.

**Figure 1** – Native CT imaging (A) and post-contrast (B) reveal a discrete native hyperdense region with reduced iodophilia at the level of the right parietal lobe, with moderate subarachnoid labeling on the right frontoparietal lobes, with a granular appearance and the disappearance of the right subarachnoid spaces.



**Figure 2** – Histopathology of the case reveals a caseating granulomatous reaction in the lung (A and B) with epithelioid and Langhans giant cells (B), with moderate fibrosis and scant lymph cells infiltrate into the portal spaces (C), and with cytotesteatonecrosis in the pancreas (D). In the CNS, there was a mononuclear-hemorrhagic meningitis with abscesses extending in the upper part of the cortex (E), with surrounding gliosis (F) and the presence of septic emboli (G). Mononuclear cells and hemorrhage exudate was present in the enlarged perivascular spaces (H), but in many instances, mononucleated cells seemed to be trapped in between thin and dedublated vessel walls, rather than just on the abluminal side of the vessel wall (I–L), and the enlarged insets in the respective images. HE staining:  $\times 50$  (A and E);  $\times 100$  (D);  $\times 200$  (B, C, F and G);  $\times 400$  (H–L).



**Figure 3 – Immunohistochemistry of the CNS revealed indeed mononucleated cells being trapped between deduplicated basement membranes (and without the presence of red blood cells), as revealed by anti-collagen IV antibody staining (A–E) and anti-smooth muscle actin antibody (F). Anti-CD45 antibody confirms that the trapped nuclei are indeed inflammatory cells (G–I);  $\times 200$  (D and E);  $\times 400$  (A–C, F–I).**

Lastly, we were interested to show that indeed these cells were of inflammatory origin, so we performed an anti-CD45 antibody immunostaining. The antibody labeled a plethora of resident microglia and migrated immune cells within the parenchyma and within the vessel lumen, but also clear-cut cells' nuclei in the walls of the vessels, in the clefts and spaces described above, showing again that these entrapped cells were indeed mononucleated inflammatory cells.

## Discussions

Tuberculous infections, regardless of location, begin by inhalation of bacilli in distal airways in the form of airborne particles, called droplet nuclei. From the airways, MTB passes in the pulmonary interstitium, toward the local lymph nodes and to remote areas *via* the bloodstream. Bacilli elicit the formation of epithelioid granulomas near ventricles or subarachnoid space during hematogenous dissemination [6]. In post mortem studies in patients diagnosed with TB, granulomas are found in the CNS, even among patients without a suspected disease at that level. In their studies, Rich & McCordock observed that all granulomas adjacent to the subarachnoid space opened in this space [11]. The rupture of these granulomas (known as a “Rich focus”) leads to the occurrence of an important inflammatory response, these being the initiating event of tuberculous meningitis. The immune response of the patient is triggered by this rupture, resulting in the accumulation of tuberculous exudates at the basal brain. Histologically, this exudate contains neutrophils, bacilli,

erythrocytes and mononuclear cells [6]. Subpial exudate is located in different areas of the CNS, especially on the anteromedial surface of the temporal lobes, the superior areas of the cerebellum, the inferomedial surface of the frontal lobes and the floor of the third ventricle. From these areas, the exudate extends to interpeduncular and pontomesencephalic cistern [12].

Tuberculous meningitis is the rarest extrapulmonary localization of TB (with an incidence of 5–15%), and the most severe. Especially in developing countries, tuberculous meningitis has high mortality and morbidity rates, the second largest incidence in the world being found in China [13].

The first description of tuberculous meningitis was provided by the Scottish physician Robert Whytt (1714–1766), in a series of 20 patients, titled “On the Dropsy on the Brain” [14]. In 1–5% of patients diagnosed with TB, TB meningitis co-occurs; of these cases, the highest mortality is for patients infected with human immunodeficiency virus (HIV) (40–58%) and in those uninfected with HIV it raises up to 19–28% [15–18]. Half of the cases of tuberculous meningitis lead to death or severe disabilities [19]. Diagnosis and rapid treatment of the disease are important to reduce the mortality and complications of tuberculous meningitis [20]. The diagnosis of tuberculous meningitis remains a challenge due to the patient's non-specific clinical symptoms. Laboratory tests do not have a higher sensitivity. The result of culture testing in the CSF is delayed and CSF acid-fast smear has a relatively low sensitivity. In developing countries, recent diagnostic methods, such as enzyme-linked immuno-

sorbent assay (ELISA), are not widely available [21]. One of the most effective methods of rapid diagnosis is to identify bacilli with Ziehl–Neelsen staining [22].

Among the risk factors of tuberculous meningitis are alcoholism, muscle dystrophy, immunosuppressive diseases, defects of cell mediated immune mechanism, diabetes mellitus, chronic hepatitis, cirrhosis and corticotherapy [23].

Imaging is essential in diagnosing this disease, which has multiple imaging characteristics; the most frequent radiographic change detected on a CT is the obliteration of basal reservoirs by isodense exudates or slightly hyperdensities. In the early stages of the disease, magnetic resonance imaging (MRI) is better than a CT scan for diagnosis [24].

The differential diagnosis includes non-infectious inflammatory diseases of the meninges, other fungal, viral or bacterial infections but also intracranial neoplasms [13]. The inflammatory response from tuberculous meningitis is associated with a number of complications including paralysis of the cranial nerves, cerebrovascular disease, infarction and hydrocephalus [20].

Perivascular spaces in the brain were initially described by Virchow & Robin as being spaces that linked directly the extracellular space and the subarahnoidian space. They originate in meningeal epithelial cells that surround the penetrant cerebral vessels as they enter the brain and they are replaced by internal *glia limitans* only at the level of the capillaries [25]. Experiments utilizing labeled India ink and albumin injected into the brain parenchyma showed that these solutes enter in a perivascular pathway that drains the interstitial fluid until the cervical and nasal lymph nodes [26–28]. Injections of labeled tracers into both the CSF and the neuropil suggested that the CSF enters the brain within the perivascular spaces of arteries, while interstitial fluid drains along perivascular venous sector [29]. The *glia limitans* at this level has an essential role, as the aquaporin 4 (AQP4) channels located on the astrocytes end-feet have been deemed responsible for the movement of water at this interface in both physiological and pathological conditions, with its inhibition reducing the cytotoxic brain edema that occurs after an ischemic stroke [30–32].

More closer studies, utilizing confocal and two-photon microscopy revealed, however, that labeled tracers co-localized with the basement membranes of capillaries and with the tunica media of arterioles [33]. Vascular basement membranes are complex structures produced not only by endothelial cells, but also by meningeal and smooth muscle cells, so that the basement membranes are not in fact single sheets of organized filamentous glycosylated proteins, but are in fact a complex entanglement that isolate structurally and functionally the luminal side of the vessel from the brain parenchyma [34].

It has been postulated that immune complexes can become entrapped in the vascular basement membrane, but not complete immune cells [35]. Here, we showed for the first time that the influx of immune cells into the brain in a case of meningitis with encephalitis also traps lymph cells into the thickness of the vascular basement membrane, besides the classical cuffing around the blood vessels. This was not a rare event and we could frequently

identify inflammatory mononucleate cells between layers of collagen IV or smooth muscle actin without the presence of red blood cells in these spaces. Although this is a morphological observation, electron microscopy studies are further needed to establish the exact disposition of these mononucleated cells in relationship with the vascular wall, and also functional *in vivo* studies will be necessary to show where these cells do enter in this space and what their further dynamic is.

## Conclusions

We show here, to our knowledge for the first time, that in the brain, inflammatory cells migrate in the thickness of the vascular basement membranes, by dedubulating the complex layers of collagen and between smooth muscle cells.

## Conflict of interests

None to declare.

## Authors' contribution

Gabriela-Camelia Roșu, Dan Nechita and Iulia Diana Stanca equally contributed to the manuscript.

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