Arch Biol Sci. 2021;73(2):197-204 https://doi.org/10.2298/ABS201210014S

Age-related changes of choroid plexus morphology, vascularization and epithelial proliferation

Golub D. Samardzija[1], Bojana M. Andrejic-Visnjic[2,*], Matilda A. Djolai[2], Jelena R. Ilic-Sabo[2], Jelena P. Amidzic[2], Zoran Nikin[1], Natali Z. Rakocevic[3], Nikola N. Aleksic[3], Borislav E. Dolamic[4] and Biserka B. Vukomanovic-Djurdjevic[5]

[1]*Department of Pathology, Faculty of Medicine, University of Novi Sad*, Hajduk Veljkova 3, Novi Sad, Serbia
[2]*Department of Histology and Embryology, Faculty of Medicine, University of Novi Sad*, Hajduk Veljkova, Novi Sad, Serbia
[3]*Faculty of Medicine, University of Novi Sad*, Hajduk Veljkova 3, Novi Sad, Serbia
[4]*Department for Specialistic-Consulting Work, Health Center Novi Sad*, Bulevar cara Lazara 75, Novi Sad, Serbia
[5]*Department of Histology, Military Medical Academy, University of Defence*, Crnotravska 17, 11000 Belgrade, Serbia

***Corresponding author**: bojana.andrejic-visnjic@mf.uns.ac.rs

Received: December 10, 2020; **Revised**: March 15, 2021; **Accepted**: March 16, 2021; **Published online**: March 23, 2021

Abstract: The choroid plexus (ChP) is essential for brain homeostasis by regulating the secretion of the cerebrospinal fluid (CSF). Despite a substantial body of work on the pathologically alterations of the ChP, there is lack of data concerning the naturally occurring morphological changes asso-ciated with ageing. In this study, we investigated 30 human ChP tissue samples that were divided into 3 groups according to age. Morphometric analysis of psammoma bodies (PBs) on hematoxylin and eosin (H&E)-stained samples and immunohistochemical analysis of ChP were performed (using antibodies to transmembrane phosphoglycoprotein protein CD34 and nuclear protein Ki-67). Amyloid deposits were detected using Congo red staining. Middle-aged and older individuals exhibited a significantly higher numerical density (ndPB) mostly as increased immature forms, which led us to question the proposed nomenclature. The proliferation rate of the ChP epithelium did not show significant difference between groups. The vascular area was markedly decreased and accompanied by amyloid deposition in blood vessel walls. While the deposits were limited to middle-sized blood vessels in the middle-aged group, in the older group deposits were also present around small vessels. The identified major morphological alterations of ageing ChP tissue provide further understanding of disfunctions of the blood-cerebrospinal fluid barrier that underlie neurodegenerative disorders.

Keywords: choroid plexus; ageing; psammoma body; vascularization; amyloid

INTRODUCTION

The choroid plexus (ChP) is a highly specialized organ, localized in the ventricular system of the brain [1]. Its primary role is secretion of cerebrospinal fluid (CSF), which is essential for proper brain tissue homeostasis. The ChP is one of the most understudied areas of neuroanatomy and neurobiology [2,3]. Some authors [4] do not consider that the ChP is the main source of the CSF (the so-called classical hypothesis of CSF hydrodynamics) based on several arguments as follows: (i) this hypothesis originates from Dandy's experiment that was carried out at the beginning of the 20th century on a single dog. The methodology of the study is not reproducible and repeated attempts did not give the same result [5]; (ii) treatment of hydrocephalus by surgically removing the ChP did not produce the expected results [4]; (iii) ChP removal in animal experiments did not show any significant change in the production or composition of CSF in comparison to the control group [6-8]. In a review article it was hypothesized that the main mechanism of CSF production is through rapid fluid exchange between the capillaries within the brain parenchyma [9,10], i.e., between adjacent capillaries with high and low hydrostatic pressure, suggesting that the basis of CSF production is interstitial fluid [4]. This new hypothesis is not accepted in wider circles, and we, like

most researchers dealing with this topic, believe that although pioneering experiments utilized questionable methodologies, recent research has provided enough evidence to support the classical hypothesis.

Histologically, the ChP is composed of the *lamina propria*, originating from the connective tissue of the *pia mater*, and of a single layer of epithelial cells, *lamina epithelialis*. The connective tissue is highly vascularized and loose with abundant capillaries. As the ChP is the "transition zone" between peripheral blood and the central nervous system (CNS), it protects the brain by forming the blood-CSF barrier [1,2]. A distinctive feature of epithelial cells are the well-developed brush-type borders, i.e., microvilli on the apical epithelial surface that protrude into the CSF and increase the surface area of the choroidal epithelial, enabling rapid and efficient delivery of the CSF [3]. Histological analysis of the ChP almost always reveals acellular, laminar structures in the connective tissue, the so-called psammoma bodies (PBs). The nature and the mechanism of their occurrence are not fully elucidated, but it is generally accepted that they represent dystrophic calcifications [11-13]. Some authors differentiate between immature and mature forms of PBs [12].

Because of the increase in the number of the elderly in the world's population, much attention is given to the study of ageing and age-related diseases, including neurodegenerative disorders. The most prevalent neurodegenerative disorder, Alzheimer's disease (AD), is characterized by progressive loss of cognitive abilities. It is associated with brain atrophy, changes in blood-CSF barrier (BCSFB) [14, 15], abnormal CSF production [16] and amyloid deposits [17]. There are numerous studies of brain and ChP tissue changes in patients with AD and other neurodegenerative diseases. Changes in the ChP, CSF and BCSFB resulting from ageing have been observed in animal models [18,19].

Given that ageing results in changes that are not *per se* a disease [20,21], these alterations in humans need to be clearly described and quantified to clearly define expected changes in physiological tissue for different ages. Experiences in everyday pathohistological diagnostics underline the significance and necessity of these data, without which it is difficult to differentiate between age-related changes and abnormalities that imply pathological process, particularly during the early stages of disease. Therefore, the focus of our research was a pathohistological, morphological and morphometric analysis of dystrophic calcifications in the form of psammoma bodies, changes in ChP vascularization, depositions of amyloid and an examination of changes in the proliferative capacity of the choroid epithelium.

MATERIALS AND METHODS

Sample collection

Paraffin blocks (n=70) with ChP tissue from the lateral ventricles were collected retrospectively from the archives of the Department of Pathology, and the study was carried out with the approval of the institutional Scientific Ethics Committee of the Clinical Center of Vojvodina, Novi Sad, Serbia. All 70 paraffin blocks were cut to 5-μm thick histological sections and stained routinely with H&E. After preparation of the slides, the following inclusion criteria for the study were applied: (i) a medical history with no records of any neurological or psychiatric diseases; (ii) autopsy findings that pathological changes were not identified in ChP tissue; (iii) that ChP tissue was technically suitable for further histochemical and immunohistochemical staining. Based on these criteria, 30 samples were included in the study and divided into 3 groups according to age, and in agreement with the World Health Organization recommendations [10]: (Y) young individuals: ≤40 years old (n=10); (M) middle-aged individuals: 41-64.9 years old (n=10); (O) older individuals: ≥65 years old (n=10).

Morphometric analysis of psammoma bodies

Morphometric analysis was performed on HE-stained slides by computer software Fiji-Image J, on 10 randomly selected and photographed areas per slide (Leica MC190 HD camera, Germany) under 100× magnification. On each photograph we measured (i) the surface area of the ChP (saChP); (ii) the numbers of total (tPB), mature (mPB) and immature (imPB) psammoma bodies (Supplementary Fig. S1); (iii) surface area (saPB) of psammoma bodies; (iv) the diameter (diPB) of all psammoma bodies. Mature and immature PBs were differentiated by the concentric lamellar appearance of mature forms or the presence

of amorphous core of immature PBs [22]. The numerical density (ndPB, PB/mm^2) and surface area fraction (safPB, %) of the PBs were determined.

Histomorphometric analysis of ChP blood vessels and epithelial proliferation

Vascularization assessment and morphometry was performed on tissue sections immunohistochemically stained (according to manufacturer's instructions) with CD34 antibody (Thermo Fisher Scientific, USA). Ten photographs of randomly selected areas of CD34-stained slides were taken. Using Fiji-Image J software, we measured the surface areas of the blood vessels (saBV) and the ChP (saChP) in each photograph and then calculated the fraction of the blood vessel surface area (safBV) as the percentage of the saBV within the saChP. Tissue sections stained with Congo red were examined under polarized light to detect amyloid depositions. In specimens in which amyloid deposits were observed, the size of the affected vessel (small, medium or large blood vessel) was noted, and the number of deposits (mild, moderate or extensive). The criteria for the size of blood vessels and the amount of amyloid deposits were as follows: "small blood vessel" – capillaries and vessels present near the surface epithelium of the ChP, wall composed of endothelium and basal membrane; "medium blood vessel" – three-layered wall of the vessel, further removed from the epithelium, located in a branched areas of the ChP; "large blood vessel" – blood vessels within the deepest unbranched areas of the ChP, far-removed from the epithelial surface; "mild deposits" – not visible with H&E staining but detected with Congo red staining as sparse deposits, barely noticeable under polarized light: "moderate deposits" – visible with H&E and Congo red staining under polarized light, not extending throughout the entire circumference of the blood vessel; "extensive deposits" – extending through the entire circumference of the blood vessel. Epithelial proliferation was estimated based on the immunohistochemical expression of Ki-67 (Thermo Fisher Scientific, USA). We evaluated epithelial proliferation qualitatively (negative or positive nuclear staining), and semi-quantitatively, based on the approximate number of cells stained as follows: very low (<10%), low (11-30%), moderate (31-50%) and high (≥51% of cells).

Table 1. Morphometric characteristics of psammoma bodies within the choroid plexus tissue

	Young (<40) MV±SD	**Middle-aged** (40-65) MV±SD	**Older** (>65) MV±SD	P value
diPB (μm)	136.73±33.52	141.84±36.4	128.29±44.53	>0.05
saPB (μm^2)	1385.26±231.56	1597.85±124.58	1325.36±153.44	>0.05
ndPB (PB/mm^2)	7.35±13.13*	18.49±15.61	29.97±34.79	<0.05
safPB (%)	2.51±3.91*	6.47±7.63	6.06±4.08	<0.05

* – Statistical significance between young individuals compared to middle-aged and older individuals. diBP – psammoma body diameter, saPB – surface area of psammoma body, ndPB – numeric density of psammoma bodies, number of psammoma bodies per mm^2 of ChP surface; safPB – surface area fraction of psammoma bodies, fraction of surface area of ChP occupied by psammoma bodies.

Statistical analysis

Data were statistically analyzed in IBM SPSS Statistics 23. Mean values of the measured and calculated parameters in different groups were analyzed by ANOVA with the level of significance set at P<0.05 and P<0.01.

RESULTS

Morphometric analysis of psammoma bodies

The mean values of diPB and saPB were greatest in the middle-aged group and lowest in older individuals. Statistically, differences between the analyzed groups were not significant (P>0.05) (Table 1). According to the maturity of the psammoma bodies, we observed that the young group had significantly fewer imPB (55.58%) compared to middle-aged (67.07%) and older individuals-(62.84%) (P<0.05). The difference between the middle-aged and older groups was not statistically significant. The numerical density (ndPB) in the young group compared to the middle-aged and older groups was significantly lower (P<0.05). The surface area fraction (safPB) showed a similar distribution among groups, with significantly lower values in young individuals compared to the middle-aged and older groups (P<0.05) (Table 1).

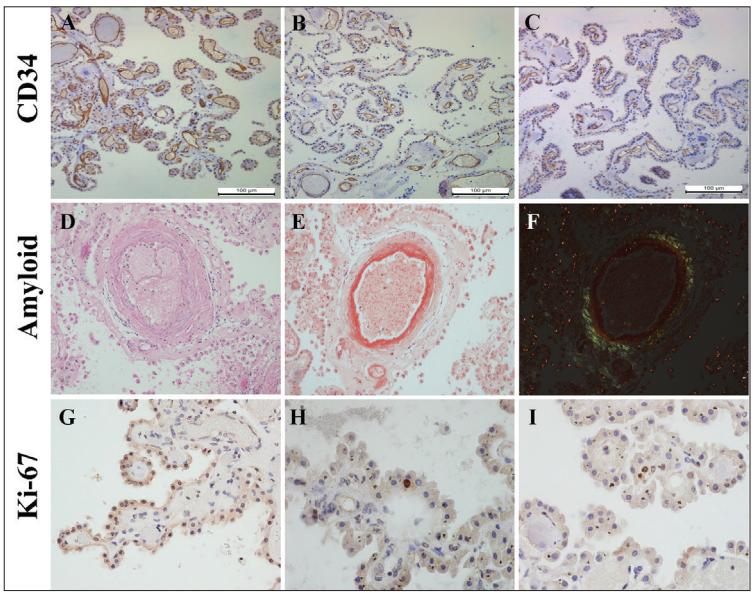

Fig. 1. Histological analysis of the ageing ChP. Vascularization of the ChP assessed by immunochemical staining for CD34, magnification 100×; **A** – young; **B** – middle-aged; **C** – older individuals. Amyloid deposition within the wall of the blood vessel in middle-aged and older individuals; **D** – H&E, magnification 20×; **E** – Congo red, magnification 20×; **F** – Congo red under polarized light, magnification 20×.; epithelial proliferation assessed by the number of Ki67 positive nuclei, magnification 60×; **G** – young; **H** – middle-aged; **I** – older individuals.

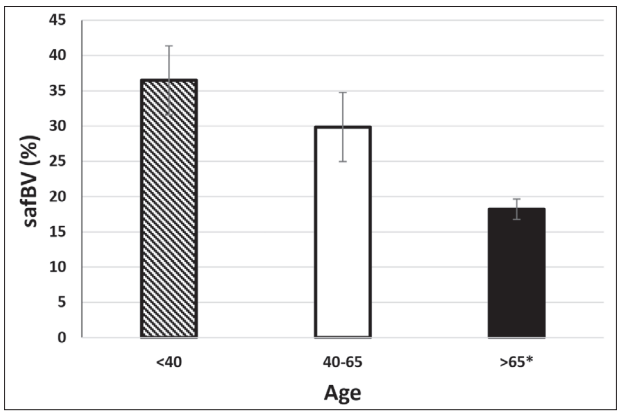

Fig. 2. Surface area fraction of blood vessels within the ChP. safBV – surface area fraction of blood vessels; * – statistically significant decrease of the safBV in older individuals (P<0.05).

Histomorphometric analysis of ChP blood vessels

Our results indicate that about one-third of the saChP – 36.48% in the young (Fig. 1A) and 29.86% in the middle-aged (Fig. 1B) groups, corresponded to the vascular space. The ChP of older individuals displayed a significant reduction of the safBV (P<0.01), occupying less than one-fifth (18.21%) of the ChP (Fig. 1C, Fig. 2). Although amyloid deposition was not detected in the ChP of young individuals, it was observed within the walls of blood vessels in the middle-aged and older groups; in both groups, amyloid deposits were detected within the medium-sized blood vessels in moderate amounts, exhibiting a non-circumferential distribution (Fig. 1D, E, F). However, accumulation in the small blood vessels was only observed in older individuals. Epithelial proliferation, evaluated through Ki-67 expression, was very low (<10% of epithelial cells) in middle-aged (Fig. 1H) and older individuals (Fig. 1I), while in young individuals, 62% of specimens displayed a low proliferation rate (11-30% of epithelial cells) (Fig. 1G). Despite nonspecific cytoplasmic staining, nuclear Ki-67 staining was visible as mild to moderate in younger individuals' ChP. Interestingly, the ChP of the middle-aged and older groups had rare, single cells with strong nuclear positivity to Ki-67. Differences did not show statistical significance.

DISCUSSION

Although not in itself a disease [20], ageing is characterized by an accumulation of molecular and cellular damage, resulting in a progressive decline in physiological functions of all organ systems [23]. One scientific challenge is to develop clear distinctions between these age-related changes from pathological alternations that underlie diseases [20].

PBs are dystrophic calcifications regarded as benign consequences of ageing in ChP stroma [11-13, 24]. However, they can also be found in tumors of other tissues that share some of the features with the ChP, such as fenestrated capillaries. These types of capillaries enable the entrance of noxious substances or leukocytes, whose presence, or inflammation itself, may induce stromal reaction and the formation of PBs [12].

In our study, young individuals had a significantly lower number of tPBs, ndPB and safPB compared to the middle-aged and older groups, while significant

differences between the middle-aged and older group were not observed. This could indicate that after the age of 40, still unidentified factors accumulate and allow for an increase in the number of PBs. Most research has focused on the total number of PBs regardless of their structure. Although the two forms of PBs (with an amorphous and laminated appearance) are referred to as mature and immature bodies [12], we do not claim that the name is completely justified. We established that the number of immature forms increased sharply in middle-aged individuals compared to the young group. The designated terms suggest that immature forms evolve over time into mature forms. Therefore, it would be expected that the number of imPB in the elderly decreases, which was not observed in our study. Another group of authors uses different terminology, according to which ChP stroma shows varying degrees of collagen compaction, focally intensely sclerotic and often calcified. The authors differentiated between these hyalinized and somewhat irregular structures (referred to as "concretions") from PBs by their well-delineated, spherical, laminated and often larger appearance [25]; keeping in mind definitions of so-called concretions and immature PBs, it can be concluded that they are in fact the same thing. The determination of the surface and diameter of PBs revealed no statistically significant difference between the age groups. Thus, it can be assumed that after their formation, PBs do not change in size. Although there are undoubtedly two forms of bodies, we question whether they truly represent the stage of development or qualitative changes and are perhaps the consequence of their different composition. The observed age-related changes of PBs mineral composition [12] along with a significant increase in their immunoreactivity for amyloid light chains [26,27] point to continuous qualitative changes and the dynamic nature of PBs. Moreover, the CPE basal membrane is often attenuated, discontinuous, thickened, or duplicated in proximity to the concretions [25]. This finding of altered basal membrane in the proximity of concretions (immature PB-like structures) favors our claim that PBs (no matter how we call them) disrupt the integrity and function of BCSFB, leading to the development of more pronounced degenerative disorders.

The choroid plexus is a highly vascularized structure with a superficial monolayer of choroid plexus epithelial (CPE) cells. CPE cells secrete the CSF and form the BCSFB, which restricts the entry of molecules and pathogens from the blood [28, 29] and controls the trafficking of nutrients as well as the clearance of toxic molecules, drugs and amyloid from the CSF [3]. During ageing or disease, the epithelium becomes atrophic, along with thickening of the basement membrane and stromal fibrosis [22], enzymatic activity is reduced, and CSF secretion decreases by 50% [29], leading to subnormal brain activity. In our study, the proliferation of CPE cells was not affected by ageing or the increased presence of PBs. However, based on our results, we cannot assess the impact of ageing on CPE cell function.

Some studies mentioned thicker arterial walls [29], but the underlying reason was not investigated. In our research, we confirmed both qualitative age-related changes of ChP blood vessels and quantitative changes in vascularization. Amyloid deposits were documented in the walls of medium-sized and small blood vessels. These deposits cause thickening of the vessel walls but may go unrecognized after H&E staining or appear as fibrin deposits or mucoid degeneration. A reduced vascular network (vascularization) of the ChP was documented through significantly decreased safBV in the ChP in middle-aged and older individuals. The decrease in safBV could be the result of the reduction in the number of blood vessels or their decreased diameter. The reduced diameter might be the consequence of thickening of the vessel wall due to deposits of fibrin, mucus, calcification or amyloid, as in our study, and could result in decreased blood flow. It is known that the ChP has a high local blood flow rate (5-10 times greater compared with that of other tissues) [30], but we cannot say for certain whether decreased vascularization impacts blood flow to a sufficient extent to cause health difficulties. To the best of our knowledge, no other study has demonstrated both qualitative and quantitative changes of ChP vasculature in ageing.

As opposed to the lack of description of age-related changes in ChP blood vessels, several studies and review articles stated that ageing is linked to the changed composition and reduced volume of CSF [29,31,32]. While these changes were explained solely by changes in CPE and BCSFB, we believe that the reduced vascular network documented in our research could also play an important role. Possible

explanations for this reduction could be a diminished secretion of growth factors in aged CPE cells, such as vascular endothelial growth factor (VEGF) and transforming growth factor-beta (TGF-β) [32], and increased activation of stromal matrix metalloproteinases (MMPs) [15]. In our research, reduced vascularity of the ChP coincided with an increase in the number of PBs. According to some authors, PBs may have significantly higher biological activity and function than previously assigned [33]. Several studies showed that a high number of PBs [22,13,34] or concretions [25] are associated with abnormalities of ChP tissue. We therefore postulate that the observed sharp increase in the number of PBs after the age of 40 might be an additional important factor that fosters the reduction of ChP vascularization, subsequently disrupting the integrity and function of BCSFB and leading to the development of more pronounced degenerative disorders.

Everything stated so far supports the importance of our findings regarding the increase in the number of PBs after the age of 40 as an indicator of possible BBB and BCSFB damage. In addition, our research is the only one to link this increase with reduced vascularization of the ChP.

Studies on sheep have revealed age-related ChP changes and BCSFB disruption, and authors believe that these might contribute to age-related cognitive decline and neurodegenerative disease in humans [19,35]. Alzheimer's disease, the most common progressive neurodegenerative disorder [29], is diagnosed pathohistologically by amyloid β (Aβ) plaques and neurofibrillary tangles in the brain. In an AD model, a cascade of detrimental events leads to morphological changes in the ChP, similar to those in ageing: stromal fibrosis and amyloid deposits, thickened basement membrane, epithelial atrophy, dysregulation of CPE cell tight junctions and loss of BCSFB integrity [15,17]. Aged and/or damaged CPE and BCSFB enable stromal infiltration of soluble amyloid molecules, which precedes clinical signs of AD [15,19,35]. Researchers have identified specific CPE transporters that bind Aβ and facilitate its removal. Interestingly, these receptors were found to be decreased in CSF in ageing and AD [35]. In late onset AD (LOAD), which shows morphological changes similar to ageing, the clearance of Aβ peptide is significantly decreased [17]. In light of these conclusions, the question arises whether ageing is in itself the beginning of AD, and whether in some individuals, changes progress slowly throughout life without manifest clinical or pathohistological indicators.

The described animal model of AD revealed that the presence of soluble amyloid molecules increased MMP gene expression in the ChP connective tissue, CPE and MMP activity in CSF [15]. The main role of MMP is the degradation of the extracellular matrix, providing an optimal medium for the formation of PBs. Our results showed an increased number of total and immature PBs in middle-aged and older individuals, and that the same age groups had amyloid deposits in blood vessels. This strongly suggests that ageing leads to BCSFB dysregulation, amyloid deposition in blood vessel walls and probably some soluble amyloid molecules in ChP stroma, resulting in MMP induced tissue damage and an increased formation of PBs.

Funding: This manuscript was supported by the Ministry of Science and Technical Development of the Republic of Serbia (Grant No. 41012).

Acknowledgements: The realization of this work required an inter-institutional effort. The authors are grateful to all those who made it possible.

Author contributions: GS, JIS, JA and ZN collected and interpreted the samples. GS, BAV, BVD and BD designed and wrote the manuscript. BD, NR and NA designed the figures. MD and BVD critically revised the manuscript.

Conflict of interest disclosure: The authors declare that they have no potential conflicts of interest with respect to the research, funding, authorship or publication of this article.

REFERENCES

1. Ross MH, Pawlina W. Histology. 8th ed. Philadelphia: Lippincott Williams & Wilkins; 2018.
2. Lun MP, Monuki ES, Lehinen MK. Development and functions of the choroid plexus cerebrospinal fluid system. Nat Rev Neurosci. 2015;16(8):445-57. https://doi.org/10.1038/nrn3921
3. Spector R, Keep RF, Robert Snodgrass S, Smith QR, Johanson CE. A balanced view of choroid plexus structure and function: Focus on adult humans. Exp Neurol. 2015;267:78-86. https://doi.org/10.1016/j.expneurol.2015.02.032
4. Oreskovic D, Klarica M. The formation of cerebrospinal fluid: nearly a hundred years of interpretations and misinterpretations. Brain Res Rev. 2010;64(2):241-62. https://doi.org/10.1016/j.brainresrev.2010.04.006

5. Milhorat TH. Choroid plexus and cerebrospinal fluid production. Science. 1969;166(3912):1514-6. https://doi.org/10.1126/science.166.3912.1514
6. Milhorat TH. The third circulation revisited. J Neurosurg. 1975;42(6):628-45.
7. Milhorat TH. Structure and function of the choroid plexus and other sites of cerebrospinal fluid formation. Int Rev Cytol. 1976;47:225-88. https://doi.org/10.1016/s0074-7696(08)60090-x
8. Milhorat TH, Hammock MK, Fenstermacher JD, Rall DP, Levin VA. Cerebrospinal fluid production by the choroid plexus and brain. Science. 1971;173(3994):330-2. https://doi.org/10.1126/science.173.3994.330
9. Bulat M, Klarica M. Fluid filtration and reabsorption across microvascular walls: control by oncotic or osmotic pressure? Period. Biol. 2005;107(2):147-52.
10. Bulat M, Lupret V, Orešković D, Klarica M. Transventricular and transpial absorption of cerebrospinal fluid into cerebral microvessels. Coll Antropol. 2008;32(1):43-50.
11. Lipper S, Dalzell JC, Watkins PJ. Ultrastructure of psammoma bodies of meningioma in tissue culture. Arch Pathol Lab Med. 1979;103:670-5.
12. Živković VS, Stanojković MM, Antić MM. Psammoma bodies as signs of choroid plexus ageing-a morphometric analysis. Vojnosanit Pregl. 2017;74(11):1054-9. https://doi.org/10.2298/vsp160321205z
13. Jovanović I, Ugrenović S, Vasović Lj, Petrović D, Cekić S. Psammoma bodies - Friends or foes of the ageing choroid plexus. Med Hypotheses. 2010;74:1017-20. https://doi.org/10.1016/j.mehy.2010.01.006
14. Balusu S, Brkic M, Libert C, Vandenbroucke RE. The choroid plexus-cerebrospinal fluid interface in Alzheimer's disease: more than just a barrier. Neural Regen Res. 2016;11(4):534. https://doi.org/10.4103/1673-5374.180372
15. Brkic M, Balusu S, Van Wonterghem E, Gorlé N, Benilova I, Kremer A, et al. Amyloid β oligomers disrupt blood - CSF barrier integrity by activating matrix metalloproteinases. J Neurosci. 2015;35(37):12766-78. https://doi.org/10.1523/jneurosci.0006-15.2015
16. Redzic ZB, Preston JE, Duncan JA, Chodobski A, Szmydynger-Chodobska J. The choroid plexus-cerebrospinal fluid system: from development to aging. Curr Top Dev Biol. 2005;71:1-52. https://doi.org/10.1016/s0070-2153(05)71001-2
17. Bolos M, Antequera D, Aldudo J, Kristen H, Bullido MJ, Carro E. Choroid plexus implants rescue Alzheimer's disease-like pathologies by modulating amyloid-β degradation. Cell Mol Life Sci. 2014;71(15):2947-55. https://doi.org/10.1007/s00018-013-1529-4
18. Serot JM, Foliguet B, Béné MC, Faure GC. Choroid plexus and ageing in rats: a morphometric and ultrastructural study. Eur J Neurosci. 2001;14(5):794-8. https://doi.org/10.1046/j.0953-816x.2001.01693.x
19. Chen RL, Kassem NA, Redzic ZB, Chen CP, Segal MB, Preston JE. Age-related changes in choroid plexus and blood-cerebrospinal fluid barrier function in the sheep. Exp Gerontol. 2009;44(4):289-96. https://doi.org/10.1016/j.exger.2008.12.004
20. Goal A: Better understand the biology of aging and its impact on the prevention, progression, and prognosis of disease and disability [Internet]. The National Institute on Aging; [cited 2021 Feb 14]. Available from: https://www.nia.nih.gov/about/aging-strategic-directions-research/goal-biology-impact
21. Naja S, Makhlouf MMED, Chehab MAH. An ageing world of the 21st century: a literature review. Int J Community Med Public Health. 2017;4:4363-9. https://doi.org/10.18203/2394-6040.ijcmph20175306
22. Jovanović I, Ugrenović S, Vasović L, Čukuranović R, Stoiljković N. Morphometric characteristics of choroid plexus epithelial cells in cases with significantly different psammoma bodies' presence. Microsc Res Tech. 2009;72(1):32-41. https://doi.org/10.1002/jemt.20642
23. Ageing and health. [Internet]. World Health Organization; [cited 2021 Feb 14]. Available from: https://www.who.int/news-room/fact-sheets/detail/ageing-and-health
24. Levine S. Choroid plexus: target for systematic disease and pathway to the brain. Lab Invest. 1987;56(3):231-3.
25. Moore GW, Laule C, Leung E, Pavlova V, Morgan BP, Esiri MM. Complement and humoral adaptive immunity in the human choroid plexus: roles for stromal concretions, basement membranes, and epithelium. J Neuropathol Exp. 2016;75(5):415-28. https://doi.org/10.1093/jnen/nlw017
26. Jovanović I, Ugrenović S, Vasović L, Stojanović I. Immunohistochemical and morphometric analysis of immunoglobulin light-chain immunoreactive amyloid in psammoma bodies of the human choroid plexus. Anat Sci Int. 2014;89(2):71-8. https://doi.org/10.1007/s12565-013-0201-2
27. Jovanović I, Stefanović N, Antić S, Ugrenović S, Djindjić B, Vidović N. Morphological and morphometric characteristics of choroid plexus psammoma bodies during the human aging. Ital J Anat Embryol. 2004;109(1):19-33. https://doi.org/10.1002/jemt.20442
28. Balusu S, Brkic M, Libert C, Vandenbroucke RE. The choroid plexus-cerebrospinal fluid interface in Alzheimer's disease: more than just a barrier. Neural Regen Res. 2016;11(4):534. https://doi.org/10.4103/1673-5374.180372
29. Serot JM, Béné MC, Faure GC. Choroid plexus, aging of the brain, and Alzheimer's disease. Front Biosci. 2003;8 (suppl):s515-21.
30. Vandenbroucke RE. A Hidden Epithelial Barrier in the Brain with a Central Role in Regulating Brain Homeostasis. Implications for Aging. Ann Am Thorac Soc. 2016;13Suppl5:S407-10. https://doi.org/10.1513/annalsats.201609-676aw
31. Damkier HH, Brown PD, Praetorius J. Cerebrospinal fluid secretion by the choroid plexus. Physiol Rev. 2013;93(4):1847-92. https://doi.org/10.1152/physrev.00004.2013
32. Kaur C, Rathnasamy G, Ling EA. The choroid plexus in healthy and diseased brain. J Neuropathol Exp Neurol. 2016; 75(3):198-213. https://doi.org/10.1093/jnen/nlv030
33. Das DK. Psammoma body: a product of dystrophic calcification or of biologically active process that aims at limiting the growth and spred of tumor? Diagn Cytoplathol. 2009;37(7):534-41. https://doi.org/10.1002/dc.21081

34. Jovanović I, Ugrenović S, Antić S, Stefanović N, Mihajlović D. Morphometric and some immunohistochemical characteristics of human choroids plexus stroma and psammoma bodies. Micros Res Tech. 2007;70(7):616-27. https://doi.org/10.1002/jemt.20442
35. Chen RL, Athauda SB, Kassem NA, Zhang Y, Segal MB, Preston JE. Decrease of transthyretin synthesis at the blood-cerebrospinal fluid barrier of old sheep. J Gerontol A Biol Sci Med Sci. 2005;60(7):852-8. https://doi.org/10.1093/gerona/60.7.852

Supplementary Material

The Supplementary Material is available at: http://www.serbiosoc.org.rs/NewUploads/Uploads/Samardzija%20et%20al_6043_Supplementary%20Material.pdf