

2023

## Journal Club: Glymphatic System in Neurodegenerative Diseases

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### Recommended Citation

Lott RG, Zonna X, Wlasowicz S. Journal Club: Glymphatic System in Neurodegenerative Diseases. *Advances in Clinical Medical Research and Healthcare Delivery*. 2023; 3(2). doi: 10.53785/2769-2779.1156.

ISSN: 2769-2779

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## Journal Club: Glymphatic System in Neurodegenerative Diseases

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### Abstract

Neurodegenerative diseases such as Alzheimer's Disease impact a multitude of individuals worldwide. The neural Glymphatic system is a relatively newly discovered cellular transport pathway within the brain that has been implicated in neurodegenerative diseases. This article serves to provide a journal club review of current literature on the Glymphatic system to raise awareness of this system and its potential for future treatment and prevention of neurodegenerative conditions.

### Keywords

Neurology, Glymphatic, Alzheimer's, Parkinson's, Neurodegenerative, AQP4, Aquaporin-4, aquaporin, tau protein, amyloid protein

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### Conflict of Interest Statement

The authors disclose no conflict of interest.

### Cover Page Footnote

The authors of this article contributed equally and should all be considered co-first authors.

## REVIEW

# Journal Club: Glymphatic System in Neurodegenerative Diseases

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## Abstract

Neurodegenerative diseases such as Alzheimer's Disease impact a multitude of individuals worldwide. The neural Glymphatic system is a relatively newly discovered cellular transport pathway within the brain that has been implicated in neurodegenerative diseases. This article serves to provide a journal club review of current literature on the Glymphatic system to raise awareness of this system and its potential for future treatment and prevention of neurodegenerative conditions.

**Keywords:** Neurology, Glymphatic, Alzheimer's, Parkinson's, Neurodegenerative, AQP4, Aquaporin-4, Aquaporin, Tau protein, Amyloid protein

## 1. Introduction

Neurodegenerative diseases are a prominent cause of impairment in the elderly population. Specifically, Alzheimer's dementia (AD) has been estimated to impact nearly 6.07 million people in the United States in 2020 and this number is expected to rise exponentially as the 'baby boom' generation ages.<sup>1</sup> As the prevalence of AD increases, its economic and social burden will rise concurrently. Today, AD is estimated to cost the US Economy \$321 billion and an additional \$271 billion in unpaid caregiving.<sup>2</sup> The substantial burden of AD and other neurodegenerative diseases necessitates an investigation into new modalities which may be utilized to treat this impairing and expensive disease process. Current research has found that the glymphatic system, a waste-clearing system in the brain, may be a newly discovered, common final pathway of many neurodegenerative conditions.<sup>3</sup> By maximizing the efficiency of this system, scientists may be able to prevent the buildup of toxic metabolites in the brain. This journal club serves to provide a review of the current literature on the glymphatic system and its potential for a mechanism by which neurodegenerative diseases could be prevented and treated.

## 2. Literature review

**Study 1:** Zeppenfeld DM, Simon M, Haswell JD et al. Association of Perivascular Localization of Aquaporin-4 With Cognition and Alzheimer Disease in Aging Brains. *JAMA Neurol.* 2017; 74(1):91–99. <https://doi.org/10.1001/jamaneurol.2016.4370>.

Zeppenfeld et al. attempted to answer if the expression or localization of astroglial water channel aquaporin-4 (AQP-4) was altered in patients with advanced age or with Alzheimer's disease (AD).<sup>4</sup> Their study consisted of 79 patients from a local aging and AD center and either had no known neurological disease (control group) or had a clinical history of AD established by neurological evaluation at the center. Brain autopsy was performed on all participants and expression of AQP4 and localization was evaluated by Western blot and immunofluorescence. Participants were grouped into either AD and greater than 60 years old, no AD and less than 60 years old (Young), and no AD and greater than 60 years old (Aged).

In a linear regression analysis, there was no association between postmortem interval and global AQP4 immunoreactivity or perivascular AQP4 localization. Histopathological examination found

Accepted 17 March 2023.  
Available online ■■■

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<https://doi.org/10.53785/2769-2779.1156>  
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greater A-beta plaque density in AD compared with young or aged. General cerebrovascular pathology (VIS), which took into multiple factors, was significantly greater in aged and AD individuals compared to the young group.

In the young group, AQP4 expression measured by immunofluorescence double labeling was uniform through the cortical layers. Intense AQP4 expression was maintained near the cortical surface in the aged group, but AQP4 expression became discontinuous below cortical layer II. Multiple linear regression that corrected for the influence of age showed that increased AQP-IR was significantly associated with increasing A-beta plaque density. Additionally, the authors found that periventricular AQP4 localization was reduced in the AD patient population. They again found that when corrected for the influence of age in multiple linear regression, increased AQP-IR was significantly associated with increasing A-beta plaque density.

When evaluating AQP4 expression or localization in the aging brain, analysis showed a significant association between increased age and AQP4-IR. However, there was no association between age and alterations in perivascular AQP4 localization. Perivascular AQP4 localization values were similar between aged and AD individuals. A logistic regression analysis showed that increased age and decreased perivascular AQP4 were significantly associated with AD. The authors also found that increasing vascular pathology and increasing global AQP4-IR are general features of the aging brain, but the preservation of perivascular AQP4 localization was a significant predictor of preserved cognitive function.

Ultimately the authors concluded that loss of perivascular AQP4 localization is a factor that makes the aging brain vulnerable to A-beta aggregation and neurodegeneration. They suggest targeting AQP4 localization may provide a therapeutic approach to AD upstream of A-beta plaque or neurofibrillary tangle formation.

**Study 2:** Xu, Z., Xiao, N., Chen, Y. et al. Deletion of aquaporin-4 in APP/PS1 mice exacerbates brain A $\beta$  accumulation and memory deficits. *Mol Neurodegeneration*. 2015; 10: 58 <https://doi.org/10.1186/s13024-015-0056-1>.

This study examines the role of clearance of interstitial solutes from the CNS via the perivascular system as an etiology for AB amyloid accumulation, deposition, and Alzheimer's Dementia progression.<sup>5</sup> The authors examined Aquaporin 4, a water channel that is located in perivascular astrocytes to maintain fluid movement in the Central nervous system. This was performed by analyzing the spatial and memory skills of mice that were wild type as well as

AQP4-/- mice that had a nonfunctional Aquaporin 4 water channel. APP/PS1 mice were also studied as transgenic mice that produce excess AB protein and are often used to study the pathogenesis and success of therapeutic interventions in Alzheimer's Dementia.<sup>6</sup> The final mouse that was studied was the AQP4-/- APP/PS1 which knocked out the AQP4 gene on the transgenic mouse.

The mice were assessed using the Morris water maze spatial learning test over a period of 6 days.<sup>7</sup> The wild-type mice decreased latency for escape over the course of the training. The AQP4-/- mice excelled during the first day of the training but exhibited decreased performance over the latter half of the training. The APP/PS1 mice were found to have increased course latency and swimming speed as compared to the wild-type mice and the APP/PS1 mice with the AQP4-/- knockout performed the lowest out of the 4 groups. This group also showed the greatest variation among swimming patterns.

Analysis of AB deposition showed increased amyloid in the APP/PS1 knockout mice as compared to the APP/PS1 controls. The AB deposition was present in the cortex and hippocampus in the APP/PS1 knockout mice. An ELISA found that the AQP4 knockout APP/PS1 mice had significantly increased soluble and insoluble levels of AB1-40 and AB1-42 protein levels. AQP4 knockout was not found to affect the proteins of production or degradation of AB as determined by Western blotting. Immunohistochemistry was performed to determine the effect of AQP-4 on reactive gliosis and found that the quantities of glial fibrillary acidic protein (GFAP) were significantly increased in the APP/PS1 mice as opposed to the AQP4-/- APP/PS1 mice although no relationship was established for ionized calcium binding adaptor molecule 1 (Iba-1). These markers are indicators of neuroinflammation following glial cell activation. The researchers describe a cascade of events once the AQP4 gene is deleted leading to decreased perivascular clearance, AB accumulation, and angiopathy as well as diminished reactive gliosis, astrocyte atrophy, and neuroinflammation that leads to cognitive impairment.

They concluded that the AQP-4 knockout exacerbates the deficits in mice that are susceptible to AD such as APP/PS1 mice that are prone to over-accumulation of AB. The reduced clearance of AB in the AQP-4 deleted mice reduces perivascular transport and leads to accumulation. AQP4 modulation thus may be a possible treatment modality for AD due to its ability to upregulate clearance of harmful proteins.

**Study 3:** Ishida K, Yamada K, Nishiyama R et al. Glymphatic system clears extracellular tau and

protects from tau aggregation and neurodegeneration. *J Exp Med.* 2022; 219(3). Doi: <https://doi.org/10.1084/jem.20211275>.

This study examined the effect of the glymphatic system and Aquaporin-4 water channels on perivascular clearance of tau protein from the interstitial fluid to the cerebrospinal fluid and then eventually being eliminated through the Dural venous sinuses.<sup>8</sup> The study was performed by examining the clearance of injected human tau in the CSF of wild-type mice and AQP4 knockout mice through fluorescence.

Mice with a nonfunctioning AQP4 gene exhibited significantly increased levels of tau in the brain 48 h post-injection compared to the wild-type mice. AQP4 knockout mice had impaired clearance of tau from the ISF to the CSF. The mice with nonfunctional AQP4 were also found to have delayed clearance from the CSF to the deep cervical lymph nodes and showed significantly increased amounts of Tau in the CSF. This indicates that defective lymphatic clearance of tau is also a factor of AQP4 dysfunction in addition to reduced perivascular transport. This increased tau accumulation was observed over time using PS19 transgenic mice that are used to study tau pathologies due to their increased accumulation of this protein. It was found that the PS19 X AQP4 knockout mice had significant levels of tau in the CSF as well as in the hippocampus after 6 months compared to PS19 X wild type. This change was exacerbated at the 9-month mark and there were significant amounts of phosphorylated tau in the PS19 X knockout mice.

Brain regions affected included the hippocampus, cerebral cortex, thalamus, and amygdala. The brains of the PS19 X AQP4 knockout mice exhibited atrophy of the cerebral cortices, hippocampi, and ventricular dilation consistent with neurodegeneration. The results indicated that the PS19 X knockout mice had significantly decreased neuron quantities in the dentate granule cells as well as the piriform cortex, but this was not seen in the AQP4 KO mice. The researchers surmised that the AQP4 channel predominantly affects neurodegeneration in the setting of increased tau concentrations. They concluded that further investigation is needed to determine the pathophysiology of altered tau clearance leading to enhancement of tau accumulation.

**Study 4:** Kress BT, Iliff JJ, Xia M et al. Impairment of paravascular clearance pathways in the aging brain. *Ann Neurol.* 2014; 76(6):845–61. <https://doi.org/10.1002/ana.24271>.

Cerebral waste removal is mediated by paravascular clearance pathways between the cerebrospinal fluid and the interstitial spinal fluid.<sup>9</sup> In this

study, researchers investigated the impact of aging on the paravascular clearance pathways in mice. The researchers utilized three study groups to examine glymphatic pathway function, cerebral arterial pulsatility, and aquaporin-4 (AQP-4) transporter polarization across differently aged brains: young mice (2–3 months old), middle-aged mice (10–12 months old), and old mice (18–20 months old).

Glymphatic pathway function was examined by infusing a fluorescent radiotracer substance into the cerebral spinal fluid (CSF) of the three study groups and evaluating the infusion with imaging. These researchers found that after 30 min, there was a significant reduction in tracer penetration of middle-aged and old mice as compared to young mice. Furthermore, interstitial substance clearance was measured in the three test groups via an injection of 125I-Amyloid  $\beta$ 1–4. Clearance of this substance was significantly impaired in middle-aged and old mice when compared to young mice. This indicates that overall glymphatic pathway function is impaired in older mice brains.

Cerebral arterial pulsatility plays a role in CSF recirculation within the brain. Thus, these researchers also examined cerebral arterial pulsatility in differently aged brains via 2-photon microscopy measurements of the cerebral vasculature. These researchers found that the penetrating arteries of young mice were significantly more pulsatile than those of old mice while the pulsatility of both groups' ascending veins had no significant difference. These data indicated that vascular pulsatility alterations with age may be implicated with age-related impairments in CSF clearance.

Lastly, these researchers found that AQP-4 transport proteins have impaired polarization in the aging brain, and the loss of this polarization is associated with glymphatic pathway impairment. These researchers measured the expression and polarization of AQP-4 within the mouse brain in various areas within the cortex. Researchers then injected CSF tracers and mapped the differences in perivascular CSF tracer penetration in the brain. Overall, there was a marked reduction in CSF tracer penetration in the brain of old mice when compared to young mice. A linear regression on paired data from cortical regions of interest was then performed which demonstrated a significant impairment of CSF tracer penetration in areas with loss of cortical AQP-4 function in both the young and the young or the old brains. It must be noted that these researchers found evidence that other pathways are involved in glymphatic clearance and that these data provide correlative evidence that supports the



hypothesis of AQP-4 polarization impairment being involved with age-related impairment of glymphatic pathways.

Overall, a 40% impairment in the clearance of 125I-Amyloid  $\beta$ 1–4 was found in the old mice as compared to the young mice. Additionally, a 27% reduction in arterial pulsatility and a widespread loss of AQP-4 polarization was found in old mice in conjunction with the impaired clearance of 125I-Amyloid  $\beta$ 1–4. This research demonstrates on a molecular level that there are aging-associated changes in the regulation of CSF.

**Study 5:** Si X, Wang T, Wang Z et al. Neuroimaging evidence of glymphatic system dysfunction in possible REM sleep behavior disorder and Parkinson's disease. *NPJ Parkinsons Dis.* 2022; 8(1):54. <https://doi.org/10.1038/s41531-022-00316-9>.

In a 2022 cohort study, researchers studied the relationship between glymphatic impairment and a-synuclein accumulation that facilitates neurodegeneration in Parkinson's disease (PD) in addition to REM sleep behavior disorder (RBD).<sup>10</sup> Human subjects meeting the criteria for either of these diseases were selected as well as healthy controls. Patients with PD withdrew therapy the morning of the MRI and all patients were analyzed using diffusion tensor image analysis along the perivascular space (DTI-ALPS). This allows for the study of glymphatic function by measuring the diffusion of the perivascular space with MRI to ascertain the fluid shifts of the central nervous system without injections of toxic contrast such as gadolinium.<sup>11</sup>

129 healthy controls, 119 RBD, and 168 PD patients were studied during this trial. The ALPS index in the healthy control group was higher than in both the PD and RBD groups. The ALPS index of the RBD group was also significantly increased compared to the PD group indicating patients with Parkinson's Disease as having the highest degree of glymphatic transport impairment. These results were determined by various statistical tests. While using Spearman's correlation analysis, researchers determined that there was a significant negative correlation between the ALPS index and the RBD Questionnaire – Hong Kong (used for disease screening) in the RBD subgroup indicating that increased glymphatic impairment led to increased disease severity. There were also negative correlations between the ALPS index and Unified Parkinson's Disease Rating Scale (UDPRS III) in the Parkinson's with excessive daytime sleepiness (PD-EDS) and PD with symptomatic RBD (PD-sRBD) subgroups but were absent from the PD group. Since sleep has been found to be an activator of glymphatic function, it is plausible that sleep

dysfunction can exacerbate the inhibition of the glymphatic system in these patients and may accurately depict the current severity of the disease state.

Lastly, a longitudinal DTI-ALPS analysis was performed but there was not determined to be a statistically significant difference in PD patients at baseline versus at follow-up. The demographics and chronic medical comorbidities were also analyzed, and it was determined that smoking, hypertension, and hyperlipidemia were significantly different between the 3 groups. Post hoc testing identified healthy controls exhibiting the highest levels of education duration and the lowest incidence of smoking among the 3 groups. The researchers hypothesize that a-synuclein augments glymphatic dysfunction demonstrated by the ALPS index in these patients, leading to the deposition and accumulation of more a-synuclein as a positive feedback loop. In terms of the ALPS index not significantly predicting severity in the PD patient group, it is believed that since the majority of the participants in this group were in the early stages of the disease there was not enough variation to detect a significant difference. They conclude by proposing DTI-ALPS as a potential method for diagnosis and prognostication of patients with neurodegenerative diseases.

### 3. Conclusion

The glymphatic system is fundamental in the waste clearance of the central nervous system as proven by animal and human models. Further studies of the glymphatic system are necessary to better understand the role of this system and its potential for therapeutic intervention in neurodegenerative conditions. This journal club review provides an overview of the current literature regarding the complex glymphatic system to increase awareness of a new, evolving topic in neurologic medicine.

#### Conflict of interest

The authors of this text report no conflict of interest.

#### Acknowledgments

All authors should be considered first authors due to equal distribution of work.

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