Letter

The presence of shrunken neurons with pyknotic nuclei in the dentate nucleus is a common postmortem change associated with autolysis of the cerebellar granular cell layer

Bilge Dundar[1], Busranur Agac[2], Eyas Alzayadneh[3], Randy Tashjian[4], Kyle S. Conway[4]

[1] *Mayo Clinic, Department of Pathology, Rochester, MN, USA*

[2] *Washington University in St. Louis, Department of Neurology, St. Louis, MO, USA*

[3] *University of Iowa, Department of Pathology, Iowa City, IA, USA*

[4] *University of Michigan, Department of Pathology, Ann Arbor, MI, USA*

Corresponding author:
Kyle S. Conway · Neuropathology Section · University of Michigan · 2800 Plymouth Rd., Building 35 · Ann Arbor · MI 48109 · USA
kyconway@med.umich.edu

Submitted: 29 February 2024 · Accepted: 03 May 2024 · Copyedited by: Lauren Walker · Published: 24 May 2024

Keywords: Cerebellum, Autolysis, Dentate nucleus, Post-mortem, Granular cell layer

In 1937, G.E. Smyth observed that "isolated pyknotic cells and even small groups of three or four pyknotic shrunken cells are quite commonly seen in the normal dentate nucleus".[1] Although this change is commonly encountered in postmortem material, it is not well-described in the literature, possibly due to a presumption it is an artifactual change. While the cerebellum is well-known to be exquisitely sensitive to postmortem autolytic change, this change is described almost exclusively in the granular cell layer and not in the dentate nucleus (DN).[2-8]

To examine whether the presence of shrunken neurons with pyknotic nuclei in the DN represents a postmortem change or a true antemortem/perimortem injury, we retrospectively examined formalin-fixed, paraffin-embedded sections of cerebellum containing both granular cell layer and DN from randomly selected adult autopsies performed at the University of Iowa. We qualitatively examined the DN for eosinophilic and pyknotic neurons, then photographed five fields at 200 x magnification (equal to 0.42 mm^2 each), and manually measured soma diameter of all dentate neurons in these fields. Blinded to the findings in the DN, we qualitatively assessed the cerebellar granular cell layer autolysis (GCA) as absent, mild, moderate, or severe according to the scheme described by Sheedy et al. (2012).[7] For decedents who underwent full autopsy and had available clinical information, we obtained a limited selection of clinical and pathologic data from autopsy reports and patient records to assess for clinicopathologic associations with this change. Pathologic data obtained included the small vessel arteriolosclerosis in the cerebellar DN, the presence or absence of neurodegenerative pathology, and the presence or absence of global hypoxic-ischemic injury, as assessed in three locations: anterior cerebral artery / middle cerebral artery (ACA / MCA) watershed region, hippocampus, and cerebellar Purkinje cell layer (as either ischemic Purkinje cells, Purkinje cell loss, or Bergmann gliosis).

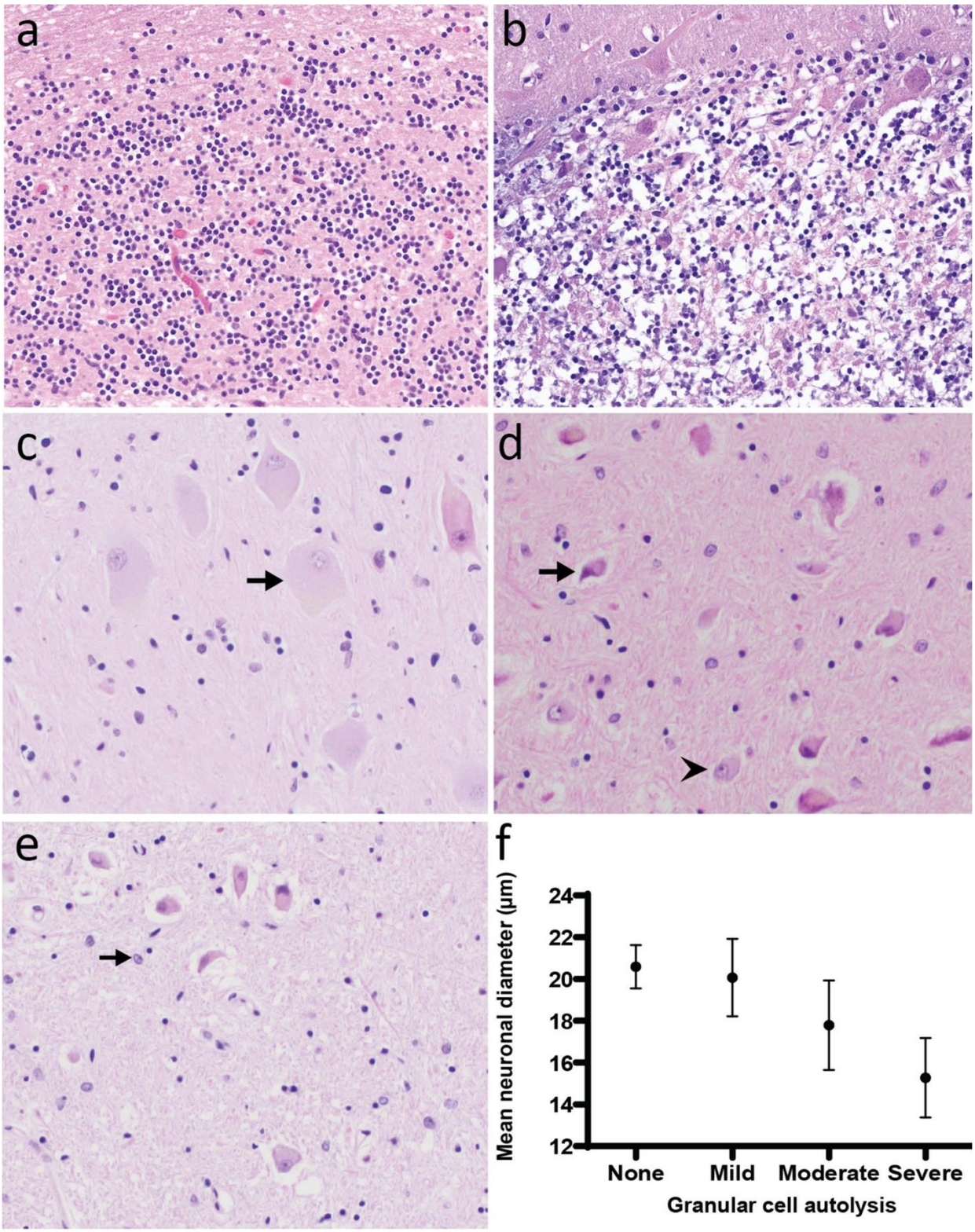

Figure 1. (a) Moderate GCA showing significant loss of viable granular-cell neurons. **(b)** Severe GCA showing significant loss of viable granular-cell neurons and extensive vacuolation. **(c)** Normal, unaffected dentate nucleus is composed of large neurons (arrow) with abundant cytoplasm and large nuclei with prominent nucleoli. **(d)** A case with moderate GCA showing shrunken, pyknotic neurons (arrow) with some relative preservation of smaller, intermediate neurons (arrowhead). **(e)** A case with severe GCA showing prominent neuronal change and the presence of scattered swollen astrocytes (arrow). **(f)** MND decreases in a linear fashion from mild through severe GCA (bars show 95 % confidence interval).

Our cohort consisted of 81 brains, including 28 brain-only autopsies (primarily obtained for neurodegenerative evaluation) and 53 full autopsies of hospital inpatient decedents. The mean age was 66.5 years (+/- 15.0 years), and 54 of the patients (67 %) were male. In 32 cases (40 %), the dentate neurons were identified as having hypereosinophilic cytoplasm and pyknotic nuclei. The mean neuronal diameter (MND) of dentate neurons across all cases was 19.3 microns (+/- 3.8 microns), and the presence of hypereosinophilia and pyknosis was associated with MND (Student's t-test, $p < 0.01$). Therefore, we chose MND as an endpoint for further analysis using univariate linear regression with MND as the dependent variable.

MND was inversely associated with the severity of GCA (beta = -1.65 microns per qualitative level of autolysis, $p < 0.01$) (Figure 1f). No significant relationship was identified between MND and hypoxic-ischemic injury, either globally or in specific sampled regions: ACA/MCA watershed region ($p = 0.21$, sampled in 76/81 cases, 93 %), hippocampus ($p = 0.35$, sampled in 80/81 cases, 99 %), or cerebellar Purkinje cell layer ($p = 0.43$, sampled in all cases). Age and the presence of a neurodegenerative disease were associated with an increased MND, and both acute liver failure and a history of diabetes were associated with a decreased MND (Table 1). Given these results, we evaluated laboratory markers associated with liver injury, including aspartate transaminase (AST), alanine transaminase (ALT), ammonia, and bilirubin. No single marker showed a significant association with MND, although these data were available only for a limited number of cases. For 26 patients, arterial blood gas (ABG) measurements were available, and pH was obtained as the most recent value in the last 48 hours of life. This value was not associated with MND ($p = 0.27$). By univariate logistic regression, at least moderate GCA was associated with acute liver failure (odds ratio = 6.34, $p = 0.04$) and most-recent pH (odds ratio = 1.55, $p = 0.04$) and was suggestive of an association with diabetes (odds ratio = 3.12, $p = 0.06$).

These findings help to better characterize the nature of this common postmortem finding. The strong association between decreased MND and increasing severity of GCA suggests that shrunken neurons with pyknotic nuclei in the DN are a postmortem change. The relative preservation of dentate neurons in cases of mild GCA suggests that GCA will typically precede changes to the DN neurons. The absence of an association with global hypoxic-ischemic injury argues against an ischemic component to the change. The positive association between age and the presence of neurodegenerative disease may reflect that these brains were typically obtained from outpatient settings with fewer metabolic disturbances.

Table 1: Clinical and pathologic associations with MND

	Beta	p-value
Demographics (n = 81)		
Age	**0.97**	**< 0.01**
Male	1.20	0.18
Neuropathologic features (n = 81)		
Granular cell layer autolysis (severity; 0 - 3)	**-1.65**	**< 0.01**
Postmortem interval (24 hours)	-0.65	0.12
Cerebellar arteriolosclerosis (severity; 0 - 3)	-0.03	0.94
Hypoxic-ischemic injury (present/absent)	-0.65	0.52
Neurodegenerative disease (present/absent)	**2.91**	**< 0.01**
Clinicopathologic features (n = 53)		
Acute liver failure (present/absent)	**-4.39**	**< 0.01**
Chronic liver disease (present/absent)	-1.93	0.15
Acute kidney injury (present/absent)	-1.15	0.26
Chronic kidney disease (present/absent)	1.44	0.22
Diffuse alveolar damage (present/absent)	-2.05	0.85
History of smoking (present/absent)	-1.03	0.31
History of hypertension (present/absent)	-1.54	0.19
Heart weight (per 100 grams)	0.00	0.30
History of diabetes (present/absent)	**-2.29**	**0.03**

The fact that both significant clinicopathologic relationships – acute liver injury and a history of diabetes – appear to be also associated with GCA suggests that these clinical states predispose decedents to cerebellar autolytic change. GCA has been associated with acid-base status,[2, 8] and a case of rapid granular-cell autolysis in the setting of acute metabolic insult has been described,[8] suggesting that acid-base disturbance may be the predisposing

factor. The fact that pH was associated with GCA in our data but not clearly associated with MND raises the possibility of another contributing component of premortem toxic/metabolic injury, but our conclusions are limited by a small number of cases with laboratory data regarding potential sources of such injury.

Qualitatively, swollen astrocytes consistent with Alzheimer's type 2 astrocytes were seen in severe cases, but we did not formally document this finding given the subjectivity of identifying these cells in the setting of autolytic change. While our data overall support the postmortem nature of this change, the presence of gliosis suggests a possible antemortem component in some cases. Assessing the presence of Alzheimer's type 2 astrocytes with markers such as p62[10] could help better establish whether their presence is associated with injury to dentate neurons. Similarly, assessing the cerebellum with markers of ischemic injury such as MAP2 could help further support the conclusion that this change is not ischemic in nature.[11] Finally, we did not specifically examine changes in neuronal morphology in the thalamus and inferior olivary nucleus, which were sampled in a limited number of cases. Given the significant projections between the DN and these structures, a further examination into whether they undergo similar morphologic changes may be warranted.

Our study is also limited by the heterogeneous and complex nature of dentate neurons, of which four morphologically distinct subtypes have been described. Our data do not establish whether this neuronal change is due to a global reduction in neuronal diameter or preferentially affects a particularly neuronal subtype.[9] Nonetheless, while single-dimension morphometric analysis of neuronal cell bodies is inherently limited, our data show that MND is a meaningful marker that reflects the practical setting in which the DN is evaluated in the routine practice of autopsy neuropathology.

Recognition of this postmortem change to neurons of the DN is important for several reasons. Care should be taken not to overinterpret this change as a manifestation of acute ischemic injury, particularly if it is an isolated finding. In cases without significant GCA or a poorly sampled granular cell layer, changes to the DN may imply an autolytic change that should be considered when determining tissue viability for additional studies. The diameter of DN neuronal cell bodies may provide a measurable proxy for cerebellar autolysis to assist in semi-quantitative assessment of GCA. Further investigation into a potential mechanistic relationship between acid-base status, metabolic injury, changes to the DN, and cerebellar autolysis is warranted.

References

1. Smyth, GE. The significance of lesions in the dentate nuclei apparently consecutive to disease of the frontal lobes. *Brain*. 1941;64(1):63-72.

2. Agarwal AN, Gilbert AR, Mais DD. Correlation of cerebellar granular layer autolysis with ante-mortem systemic acid-base status. *Cell Tissue Bank*. 2021;22(3):505-509. https://doi.org/10.1007/s10561-021-09900-4

3. Albrechtsen R. The pathogenesis of acute selective necrosis of the human cerebellar cortex. *Acta Neuropathol*. 1977;37:31-34. https://doi.org/10.1007/bf00684537

4. Finnie JW, Blumbergs PC, Manavis J. Temporal Sequence of Autolysis in the Cerebellar Cortex of the Mouse. *J Comp Pathol*. 2016;154(4):323-8. https://doi.org/10.1016/j.jcpa.2016.03.005

5. Krassner MM, Kauffman J, Sowa A et al. Postmortem changes in brain cell structure: a review. *Free Neuropathol*. 2023;4. https://doi.org/10.17879/freeneuropathology-2023-4790

6. J. O, Yutani C, Imakita M et al. Autolysis of the granular layer of the cerebellar cortex in brain death. *Acta Neuropathol*. 1986;70:75-78. https://doi.org/10.1007/bf00689517

7. Sheedy D, Harding A, Say M, Stevens J, Kril JJ. Histological assessment of cerebellar granule cell layer in postmortem brain; a useful marker of tissue integrity? *Cell Tissue Bank*. 2012;13(4):521-7. https://doi.org/10.1007/s10561-011-9265-1

8. Suarez-Pinilla M, Fernandez-Vega I. An acute metabolic insult highly increased postmortem cerebellar autolysis: an autopsy case. *Clin Neuropathol*. 2015;34(3):166-8. https://doi.org/10.5414/np300809

9. Ristanovic D, Milosevic NT, Maric DL. On the classification of normally distributed neurons: an application to human dentate nucleus. *Biol Cybern*. 2011;104(3):175-83. https://doi.org/10.1007/s00422-011-0426-x

10. Gelpi E, Rahimi J, Klotz S et al. The autophagic marker p62 highlights Alzheimer type II astrocytes in metabolic/hepatic encephalopathy. *Neuropathology*. 2020;40(4):358-366. https://doi.org/10.1111/neup.12660

11. Barranco R, Bonsignore A, Ventura F. Immunohistochemistry in postmortem diagnosis of acute cerebral hypoxia and ischemia. *Medicine (Baltimore)*. 2021; 100(25). https://doi.org/10.1097/md.0000000000026486