

## CANINE CEREBELLAR CORTICAL ABIOTROPHY IN TWO MIXED BREED LITTERMATES

del Amo AN<sup>1</sup>, Diessler ME<sup>2,3</sup>, Massone AR<sup>2</sup>, Idiart JR<sup>2</sup>, Portiansky EL<sup>2</sup>

1-Small Animal Clinics, 2-Institute of Pathology, 3-Histology and Embriology.  
School of Veterinary Sciences, La Plata Nacional University.

**Abstract:** Neuronal abiotrophy is an inherited premature degeneration of neurons due to an abnormality of the cell metabolism, which often begins in the first months of life. Onset of signs is progressive and occurs weeks or even years later. The aim of the work was to describe 2 cases of canine abiotrophy in mixed breed littermates. Two 45-day-old male and female puppies from a litter of four were examined due to muscle tremors and ataxia. Affected puppies exhibited ataxia, wide based stance, loss of balance, vocalization and head tilt to the left side. Postural reactions were slowed down and there was loss of menace response. When inability to stand worsened, euthanasia and necropsy were performed. Samples were collected for histopathological studies. Number of Purkinje cells was calculated by means of morphometric analysis. Lectin histochemistry was performed in affected and normal tissues. Reduction in cerebellar size was observed. Comparing to control tissues, a marked decrease in Purkinje cells was found. They were eosinophilic and swollen. Differences in cellular count (normal/death) in normal and affected cerebellum were significant. None of the lectins tested showed affinity for cytoplasmic components. Differential diagnoses were excluded owing to course, clinical, pathological, image analysis and lectin histochemical findings.

**Key words:** ataxia - cerebellum - abiotrophy - Purkinje cells

## ABIOTROFIA CEREBELAR CORTICAL EN DOS CANINOS MESTIZOS HERMANOS

**Resumen:** La abiotrofia neuronal es una degeneración prematura de neuronas debida a una anomalía hereditaria del metabolismo celular, que comienza en los primeros meses de vida. La aparición de los signos ocurre semanas o incluso años más tarde, y el déficit es progresivo. El objetivo del trabajo fue describir 2 casos de abiotrofia en caninos hermanos mestizos. Un macho y una hembra de 45 días de una camada de 4 fueron examinados debido a temblores y ataxia de los 4 miembros. Los cachorros enfermos exhibían ampliación de la base de sustentación, pérdida del equilibrio, vocalización, inclinación de la cabeza hacia la izquierda, reacciones posturales enlentecidas y respuesta a la amenaza disminuida. Cuando fueron incapaces de mantenerse en estación se llevaron a cabo la eutanasia e inmediata necropsia. Se tomaron muestras para estudios histopatológico. Los análisis morfológico (número de células de Purkinje) y lectinohistoquímico se llevaron a cabo en tejidos afectados y en un control normal. Se observó reducción del tamaño cerebelar y significativa disminución del número de células de Purkinje, que se observaron tumefactas y eosinófilas. Las lectinas no mostraron afinidad por componentes citoplasmáticos. Los diagnósticos diferenciales fueron excluidos debido al curso, examen clínico y análisis histopatológico, lectinohistoquímico y de imágenes.

**Palabras claves:** ataxia - cerebelo- abiotrofia - células de Purkinje

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**Dirección para correspondencia:** A del Amo, Cátedra de Clínica de Pequeños Animales. Facultad de Ciencias Veterinarias. Universidad Nacional de La Plata. CC 296, (B1900AVW) La Plata. Argentina.

**E-mail:** adelamo@fcv.unlp.edu.ar

## INTRODUCTION

"Abiotrophy" means lack of a life-sustaining factor, but it does not refer to the specific nature of the metabolic disturbance. Neuronal abiotrophy is a primary premature degeneration of neurons (1, 2). This disorder is due to an intrinsic inherited development abnormality of the cell metabolism causing its death (3).

Channelopathies followed by exocytotoxicity and apoptosis of neurons has been suggested as a mechanism of disease (4). Nucleotide repeats, an aberration causing spinocerebellar ataxias in humans, might be involved in abiotrophy (5). Nevertheless, the pathogenesis remains obscure (1, 2).

Examples of human abiotrophic diseases (Alzheimer and Parkinson's diseases) have been considered to reflect accelerated neuronal aging. In the abiotrophies described in animals, neurological deficit is postnatal, progressive and does not result from an acquired injury either, but often begins in the first months of life (1). In dogs, cerebellar ataxia has been described in many breeds (6, 7, 8). Affected dogs present neurological normality at birth or at the time of ambulation. Onset of signs occur weeks, months or even years later (1, 5, 9). Cerebellar deficit is progressive or reaches static periods before worsening. Sometimes the condition stabilizes, but this is not common (1, 2, 6). Clinical manifestations are: cerebellar and truncal ataxia, head tremor, symmetrical hypermetria, spasticity, broad-based stance, loss of balance and dysmetria (10). Symmetric or asymmetric reduced cerebellar size is the main lesion found later in the course of abiotrophy. Microscopically, degeneration and loss of Purkinje neurons in an otherwise normally developed cerebellum is observed (2). The following case describes the cerebellar abiotrophy found in two mixed breed puppies.

## MATERIALS AND METHODS

Two 45-day-old male and female puppies in a litter of four were presented due to muscle tremors while walking and ataxia of 3 days duration. Dogs were evaluated every four days during the following twelve days. When inability to stand worsened, euthanasia and necropsy were performed. Samples were collected for histopathological studies. Control samples from a 2-months-old normal dog were processed. The number of Purkinje cells was calculated by means of morphometric analysis. For this purpose, at least ten histological images were captured from each sample using an analogical video camera connected to the computer containing an image analysis software (ImagePro Plus, v4.5, Media Cybernetics, USA). Data was analyzed using the Student's t test. Lectin histochemical labeling was performed to demonstrate lysosomal stor-

age products in the neurons or rule out this entity. Affected and normal tissues were labeled using: *Dolichos biflorus* (DBA), *Glycine max* (SBA), *Triticum vulgaris* (WGA), Succinyl-WGA (WGAs), *Ulex europaeus*-I (UEA-1), *Concanavalina ensiformis* (Con A), *Arachis hypogaea* (PNA), and *Bandeirea simplicifolia* lectin I (BSL-I) (Lectin kit Biotinylated BK-1000, Vector laboratories, Inc. Burlingame, CA.)

## RESULTS

Affected puppies were normal shortly before the time of consultation. Physical examination revealed an abnormal gait with ataxia of the four limbs, hypermetric steps, muscle tremors which worsened with excitement, wide based stance, loss of balance and vocalization. Ataxia was asymmetric, with periodic falling to the left. Affected animals hardly stand. Postural reactions were slowed down and there was a progressive loss of menace response and head tilt to the left side. Paresis, weakness, circling, alteration of spinal reflexes, vision or mental status were not observed. Eating and drinking were normal.

**1A.** Cerebellum of the male dog. The left lobe is markedly reduced. **B.** Abiotrophic cerebellum showing a marked decrease of Purkinje cells number and degeneration of the remaining cells. HE. Bar = 10µm.

**1A.** Cerebelo del cachorro macho. El lóbulo izquierdo está marcadamente reducido. **B.** Cerebelo con abiotrofia mostrando una marcada disminución del número de las células de Purkinje y degeneración de las restantes. HE. Bar = 10µm.

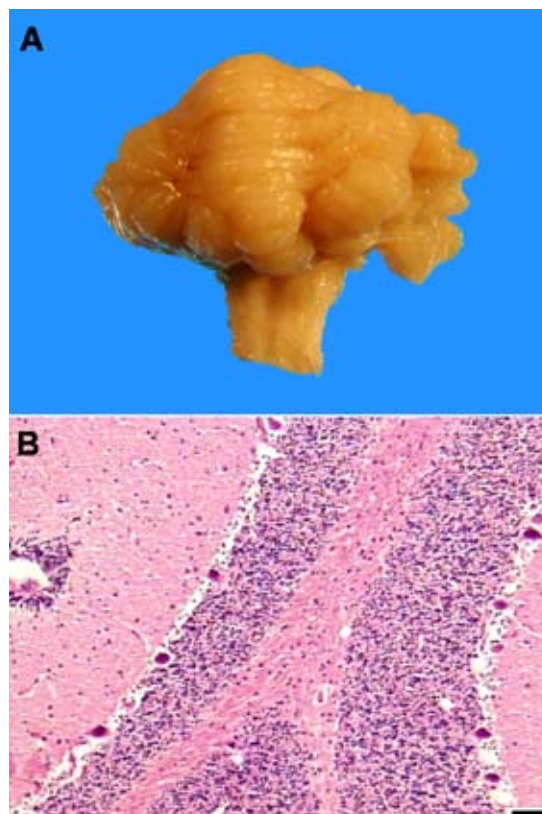


Table 1. Number of alive/dead cells in the cerebellum of a normal puppy and of those suffering abiotrophy.

Tabla 1. Número de células vivas/muertas en el cerebelo de un cachorro normal y de aquellos que padecían abiotrofia.

Puppies	Alive cells	Dead cells	p value (a)
Abiotrophy	83 (41.50)	117 (58.50)	0.1079
Normal	160 (80.00)	40 (20.00)	0.0015 *
p value (b)	0.0028 *	0.0076 *	

Values indicate the amount of dead or alive cells over 200 counted cells. The corresponding percentage is shown in parenthesis. The Student's t test was applied. (a) alive vs. dead cells (b) abiotrophy vs. normal.

\* Significant.

Table 2. Number of alive/dead cells in the cerebellum of a normal puppy and of those suffering abiotrophy per unit length.

Tabla 2. Número de células vivas/muertas en el cerebelo de un cachorro normal y de aquellos que padecían abiotrofia por unidad de longitud.

Puppies	Alive cells	Dead cells	Total count	p value (a)
Abiotrophy	6 (42.86)	8 (57.14)	14	0.0989
Normal	14 (82.35)	3 (17.65)	17	0.0004 *
p value (b)	0.0002 *	0.0138 *		

Values indicate the amount of dead or alive cells over 1000  $\mu$ m length considered. The corresponding percentage is shown in parenthesis. The Student's t test was applied. (a) alive vs. dead cells (b) abiotrophy vs. normal. \* Significant.

At necropsy, reduction in cerebellar size was observed. The vermis, declive and folium were as narrow as the tuber, lateral lobes were small and the folia of the dorsal surface were not very obvious. The cerebellum of the male presented a markedly reduced left lateral lobe (A). Degeneration of Purkinje cells was observed. They were dark, eosinophilic and swollen, and some nuclei were absent (B). Differences in cellular count in normal and affected cerebellum were significant. None of the lectins showed affinity for cytoplasmic components.

## DISCUSSION

Littermates were presented after a 3 days history of a clinical picture consistent with a diffuse cerebellar lesion. Head tilt was a paradoxical central vestibular sign attributed to a damaged flocculonodular lobe. A congenital disorder was suspected due to the age of the animals. These clinical signs raised some differential diagnosis, namely cerebellar hypoplasia, cerebellar abi-

otrophy, multisystem neuronal abiotrophy and storage diseases. Cerebellar hypoplasia usually appears at birth without progression. Conversely, these puppies were normal in appearance until 42 days of age and deteriorated progressively. This supports the diagnosis of a progressive disorder. Furthermore, in both affected cerebella all layers were completely organized, but there were loss or degenerative changes of Purkinje cells, unlike lesions found in typical hypoplasia. Multisystem neuronal abiotrophy was ruled out because of the absence of neurological deficit other than the cerebellar signs. Abnormal storage products were not observed during histopathological and lectin histochemical analysis. Evolution, clinical, gross, histopathological, lectin histochemical, and morphometric findings were indicative of cerebellar abiotrophy.

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