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Clinicopathological Features of Globoid Cell Leucodystrophy in Cats

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Summary
Clinical and pathological findings consistent with globoid cell leucodystrophy (GLD) were evaluated in two domestic shorthaired cats, aged 3 and 4 months. Both showed neurological signs mainly characterized by progressive pelvic limb ataxia, paraplegia with loss of deep pain perception in the pelvic limb, and intentional tremors of the thoracic limbs. Pathological changes affecting the central and peripheral nervous systems were characterised by diffuse, bilateral and symmetrical myelin loss, and marked astrogliosis. In the leucodystrophic areas there was perivascular accumulation of large PAS-positive, non-metachromatic macrophages (globoid cells), with intracytoplasmic accumulation of crystalloid tubular aggregates. Peripheral nerves showed demyelinating features with thin myelin sheaths, myelin splitting, and ballooning; the nerve fibres had bizarre shapes due to the presence of pale inclusions in the Schwann cells. GLD in cats shares clinical and pathological features with the disease described in other animals and human beings. The neurological signs differed from those of other feline inborn neurometabolic diseases and cerebellar hypoplasia.

Globoid cell leucodystrophy (GLD), i.e., Krabbe’s disease in human neurology, is a rare inherited neurometabolic disease that results from a deficiency of lysosomal hydrolase galactosylceramidase (galactocerebroside-β-galactosidase; GALC) activity. The disease is characterized by symmetrical demyelination, loss of oligodendrocytes, and accumulation, especially perivascularly, of large phagocytic cells (globoid cells) containing non-sudanophilic, non-metachromatic, periodic acid-Schiff (PAS)-positive material (Suzuki and Suzuki, 2002). Galactosylceramide, which cannot be degraded because of the underlying GALC deficiency, elicits infiltration of haematogenous macrophages, which phagocytize undegradable galactosylceramide and are transformed into the characteristic globoid cells. Early and extensive loss of oligodendrocytes has been considered a consequence of the accumulation of psychosine (galactosylsphingosine), which is also a substrate for the GALC enzyme. Indeed, oligodendrocyte progenitor cell death via an apoptotic mechanism has been demonstrated (Zaka and Wenger, 2004).

The disease has been reported in several dog breeds (Summers et al., 1995; McDonnell et al., 2000), sheep (Pritchard et al., 1980), rhesus monkeys (Macaca mulatta) (Baskin et al., 1998), and in a naturally occurring mutant mouse (twitcher mouse), which represents a laboratory model of human GLD (Kobayashi et al., 1980). In the feline species the disease has been described only in two inbred domestic shorthaired kittens (Johnson, 1970), and in one domestic longhaired kitten (Sigurdson et al., 2002). The present report
describes the clinicopathological and ultrastructural findings of two additional cases of GLD in cats.

Cat 1, a 3-month-old male domestic shorthaired cat, was referred to the clinic for small animals at the Faculty of Veterinary Medicine, Pisa University, with a history of progressive pelvic limb ataxia first noted at 4 weeks of age. The kitten was one of three normal kittens with no known history of any other disease. All three kittens were housed indoors and fed a balanced commercial diet. Physical examination revealed an alert and vital kitten, but neurological examination revealed that the animal was paraplegic with absence of deep pain perception in the pelvic limbs. The thoracic limbs were dysmetric and tremors of the body and head were observed. There was an absence of vestibular eye movements and decreased pupillary light reflexes. Menace response was moderately depressed bilaterally.

Cat 2, a 4-month-old male domestic shorthaired cat, was referred to the Veterinary Clinic “Colle Salario”, Rome, with clinical signs similar to those of the previous case, first noted at 6 weeks of age. Physical examination revealed an alert and vital kitten with moderate atrophy of the pelvic limb muscles. The animal showed paraplegia with absence of patellar reflex and deep pain perception in the pelvic limbs. The thoracic limbs were dysmetric and showed intentional tremors. Spinal reflexes and deep pain perception were normal. The movements of the head were normal and only a mild reduction of menace response to the right was observed. The kitten was fed with a commercial cat food and no known history of toxin exposure was reported. No familial history was available.

Both kittens maintained a normal mental state throughout the course of the disease. The neurological localization was consistent with a diffuse or multifocal lesion of the brain and spinal cord, with possible involvement of the peripheral nerves of the pelvic limbs. Results of a complete blood count, serum biochemical analyses, urinalysis, and faecal examination were within reference ranges in both cats. The animals were seronegative for infections with feline leukaemia virus, feline immunodeficiency virus, and feline coronaviruses. Radiographs of the vertebral column were normal. No definitive diagnosis was made after clinical examination, but in view of a poor prognosis the owners requested euthanasia.

In both cats, the entire central nervous system, selected peripheral nerves, quadriceps muscles and samples of major organs were fixed in phosphate-buffered 4% formalin solution. Tissue samples were processed by routine methods for histology, and sections were stained with haematoxylin and eosin (HE), periodic acid-Schiff (PAS), Luxol fast blue (LFB), crystal violet for Nissl substance, toluidine blue, and Bielschowsky silver stain. Selected brain sections were immunolabelled by the peroxidase—anti-peroxidase (PAP) method with a polyclonal antibody against glial fibrillary acidic protein (GFAP; DakoCytomation, Glostrup, Denmark) diluted 1 in 100. Samples of fixed nervous tissue were frozen in isopentane precooled in liquid nitrogen and cut with a cryostat. Frozen sections were stained with Oil Red O (ORO) for neutral lipids. Samples of nervous tissue and peroneal nerves were fixed in glutaraldehyde 2.5% in 0.1 M cacodylate buffer at 4°C, post-fixed in osmium tetroxide, dehydrated, and infiltrated and embedded in Spurr resin. Semi-thin sections were stained with toluidine blue, and ultra-thin sections were stained with uranyl acetate and lead citrate. Samples of peroneal nerve were post-fixed in 1% osmium tetroxide, dehydrated in acetone and then soaked overnight in Spurr resin from which the accelerator had been omitted. Approximately 100 single fibres, teased from different fascicles under a dissecting microscope, were examined.

On post-mortem examination no gross changes were observed. Histological lesions were restricted to the central and peripheral nervous systems and were almost identical in both cases. Bilateral and symmetrical myelin loss, of variable intensity, was observed at different levels within the neuraxis; there was, however, sparing of some white matter structures, such as arcuate subcortical fibres, internal and external capsule, anterior and posterior commissure, alveus, cerebral peduncles, superior and middle cerebellar peduncles, white matter of the cerebellar foliae, pyramids, and the fasciculus proprius of the spinal cord. The most severely affected regions were deep white matter areas, such as the corona radiata, corpus callosum, optic tracts, optic radiation, fimbria, fornix, inferior cerebellar peduncles, and subcortical white matter of the cerebellum. In these regions, the leucodystrophic process was active and conspicuous aggregates of large, rounded macrophages (globoid cells) were observed, mainly perivascularly. The globoid cells measured 10 to 35 μm in diameter and had abundant cytoplasm, which was faintly eosinophilic, strongly PAS-positive (Fig. 1), ORO-negative, and non-metachromatic. Nuclei were round to oval with finely dispersed chromatin and a single prominent nucleolus. Frequently, the globoid cells were...
multinucleated with eccentric, flattened, and closely packed nuclei. Numerous globoid cells were scattered throughout the white matter tissue, which showed occasional foci of malacia. In cat 2, multiple basophilic, PAS-positive calcospherites, 5 to 15 μm in diameter, often with a lamellar pattern, were observed within the necrotic areas of the subcortical white matter of the cerebellum. Occasional focal perivascular infiltrates of lymphocytes were intermixed with the globoid cell collections in the corona radiata and medulla oblongata of cat 1. In both cases, the active leucodystrophic process was accompanied by an extensive astrocyte reaction, with the presence of gemistocytic astrocytes. In some regions of the neuraxis, such as the gyral white matter of the cerebrum, white matter at all levels of the spinal cord and spinal nerve roots, the infiltration by globoid cells was less pronounced; nevertheless, loss of myelin was demonstrated by LFB staining. Particularly evident in cat 2, hypertrophy and hyperplasia of astrocytes were marked in these areas and often the reactive astrocytes were binucleated or were arranged in pairs or small groups (Fig. 2). Astrogliosis and proliferation of microglial cells were also observed in the deeper layers of the cerebral cortex and diffusely in the thalamus, midbrain, and grey matter of the spinal cord. Transverse sections of cranial nerve roots revealed myelin loss, infiltration by globoid cells, and fibrosis. Examination of longitudinal sections of the spinal cord and caudal brain stem with silver staining did not reveal significant neuroaxonal changes, except for axonal necrosis in the spinocerebellar tracts of the medulla oblongata. Marked astrogliosis and neuronal loss with some shrunken and hypereosinophilic neurons were observed in the olivary and cerebellar nuclei (Fig. 3). Neurons of the cerebral cortex, hippocampus, brain stem, cerebellar cortex and spinal cord were normal, and no significant changes were observed in the cells of the choroid plexus, ependymal layer or pineal gland. Ultrastructural examination of the globoid cells disclosed moderately electron-dense, straight or slightly curved tubular intracytoplasmic inclusions of crystalloid appearance, admixed with myelin debris (Fig. 4). Semi-thin sections of peroneal nerves showed numerous fibres with abnormally thin myelin sheaths, accompanied by multifocal myelin splitting and ballooning of the myelin sheath. “Onion bulb” formations were not observed. The nerve fibres often had a bizarre shape due to the presence of pale inclusions in the Schwann cells (Fig. 5). A few globoid cells were detected and, at the ultrastructural level, showed typical
intracytoplasmic inclusions. Teased fibre preparations showed diffuse segmental demyelination and normal axons. Myelin ovoids were not observed. Examination of quadriceps muscles revealed moderate denervation atrophy with presence of atrophic angular fibres and mild fibrosis. Intramuscular nerve branches showed extensive endoneural fibrosis, and myelin loss.

Fig. 2. GLD in cat 2. Gyral white matter; marked astrogliosis with groups of hypertrophied astrocytes. HE. ×400. Inset: binucleated astrocytes (indicating astrocytic proliferation) express GFAP. PAP, haematoxylin counterstain. ×400.

Fig. 3. GLD in cat 2. Olivary nucleus; extensive astrogliosis, neuronal loss, and shrunken eosinophilic neurons with pyknotic nuclei (arrowheads). Scattered globoid macrophages are evident. HE. ×320.
The histopathological and ultrastructural findings in the central and peripheral nervous system were consistent with GLD. The disease is characterized by severe myelin degeneration and necrosis throughout the central nervous system, with typical PAS-positive, non-sudanophilic, non-metachromatic, globoid-type macrophages, often clustered around blood vessels.

Fig. 4. GLD in cat 1. Globoid cell macrophage demonstrating intracytoplasmic hollow tubular inclusions with fibrillar walls (arrowheads). Degenerating myelin structures are also evident. Electron microscopy. ×11 000.

Fig. 5. GLD in cat 2. Peroneal nerve; the thickness of the myelin sheath is markedly reduced in many nerve fibres, and some of them show bizarre shapes with pale inclusions in the Schwann cells (arrowheads). Toluidine blue. ×500.
Although the neuropathological picture of GLD is highly characteristic, a definitive diagnosis requires biochemical assays to demonstrate the enzymatic deficit or a genetic test to disclose mutations. This latter test is available for the dog in some laboratories (Victoria et al., 1996) but is not applicable to the cat because the feline GALC gene has not yet been cloned.

In cats, GLD was diagnosed in two female kittens humanely destroyed at 6.5 and 8.5 weeks of age (Johnson, 1970). These cases, which resulted from inbreeding, were characterized by posterior ataxia, ascending incoordination, and occasional generalized tremors. In a female domestic longhaired cat, initial signs were body tremors and tetraparesis at 8 weeks of age (Sigurdson et al., 2002). The cat died at 21 weeks of age having developed pelvic limb paralysis, muscle atrophy and respiratory distress. In the present study, the neurological signs were similar, but also included loss of pain perception and cranial nerve involvement, suggesting a more severe form or an advanced stage of the disease. In all three cats described by others (see above), histopathology was morphologically and topographically similar to that observed in the present study, but neither ultrastructural findings nor peripheral nerve lesions were described. Perivascular cuffs of lymphocytes, as observed in cat 1, have been described in the white matter of human GLD patients and in the twitcher mouse during the early stage of demyelination; successively they disappeared, despite continuous demyelination. These findings may indicate either a specific involvement of immunological factors in the pathogenesis of the disease, or a non-specific reaction to degenerating tissue components (Ohno et al., 1993). Occasional foci of mononuclear inflammatory cell infiltration of the leptomeninges were found in one of the cats described by Johnson (1970). Multiple necrotic foci with calcium deposits, as observed in cat 2, were described by Suzuki and Suzuki (2002) in neuropathological reports of late-onset human cases. Loss of neurons in the dentate nucleus and the inferior olivary nuclei further characterizes human GLD but has not been previously reported in domestic animals. Peripheral nerve pathology was particularly severe in our cats, but qualitatively comparable with the changes described in canine GLD, in which reduction in width of myelin lamellae, infiltration by globoid cells, and typical inclusions in both globoid and Schwann cells have been documented (Kurtz and Fletcher, 1970).

In conclusion, GLD in cats is consistently an early-onset and severe disease, pathomorphologically identical with the forms described in human infantile patients, domestic animals, and in the twitcher mouse model. The clinical signs, which resemble those of the canine disease, are characterized by early posterior dysmetria and ascending incoordination, and generalized tremors. These signs, associated with paraplegia and loss of deep pain perception, as observed in the present study, distinguish GLD from other feline inborn neuro-metabolic diseases and cerebellar hypoplasia. Clinicians and pathologists should be alerted by these findings, so that fresh tissue samples may be obtained for specific tests, such as culture of skin fibroblasts and enzymatic analyses, with the aim of characterizing the disease in cats more fully.

References
Baskin, G. B., Ratterree, M., Davison, B. B., Falkenstein, K. P., Clarke, M. R., England, J. D., Vanier, M. T., Luzi, P., Rafi, M. A. and Wenger, D. A. (1998). Genetic galactocerebrosidase deficiency (globoid cell leukodystrophy, Krabbe disease) in rhesus monkeys (Macaca mulatta). Laboratory Animal Science, 48, 476–482.
Johnson, K. H. (1970). Globoid leukodystrophy in the cat. Journal of the American Veterinary Medical Association, 157, 2057–2064.
Kobayashi, T., Yamanaka, T., Jacobs, J. M., Teixeira, F. and Suzuki, K. (1980). The Twitcher mouse: an enzymatically authentic model of human globoid cell leukodystrophy (Krabbe disease). Brain Research, 202, 479–483.
Kurtz, H. J. and Fletcher, T. F. (1970). The peripheral neuropathy of canine globoid-cell leukodystrophy (Krabbe type). Acta Neuropathologica (Berl.), 16, 226–232.
McDonnell, J., Carmichael, K. and McGraw, R. (2000). Preliminary characterization of globoid cell leukodystrophy in Irish Setters. Journal of Veterinary Internal Medicine, 14, 340.
Ohno, M., Komiyama, A., Martin, P. M. and Suzuki, K. (1993). MHC class II antigen expression and T-cell infiltration in the demyelinating CNS and PNS of the twitcher mouse. Brain Research, 625, 186–196.
Pritchard, D. H., Naphine, D. V. and Sinclair, A. J. (1980). Globoid cell leucodystrophy in polled Dorset sheep. Veterinary Pathology, 17, 399–405.
Sigurdson, C. J., Basaraba, R. J., Mazzaferro, E. M. and Gould, D. H. (2002). Globoid cell-like leuocodystrophy in a domestic longhaired cat. Veterinary Pathology, 39, 494–496.
Summers, B. A., Cummings, J. F. and de Lahunta, A. (1995). Veterinary Neuropathology, Mosby-Year Book, St Louis, pp. 220–221.
Suzuki, K. and Suzuki, K. (2002). Lysosomal diseases—Krabbe’s disease. In: *Greenfield’s Neuropathology*, Vol. 1, 7th Edit., D. I. Graham and P. L. Lantos, Eds, Arnold, London, pp. 679–682.

Victoria, T., Rafi, M. A. and Wenger, D. A. (1996). Cloning of the canine GALC cDNA and identification of the mutation causing globoid cell leukodystrophy in West Highland White and Cairn terriers. *Genomics*, 33, 457–462.

Zaka, M. and Wenger, D. A. (2004). Psychosine-induced apoptosis in a mouse oligodendrocyte progenitor cell line is mediated by caspase activation. *Neuroscience Letters*, 358, 205–209.

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