EUROPEAN COLLEGE OF VETERINARY PATHOLOGISTS
COMPREHENSIVE PATHOLOGY

ANSWER KEY

A. Abstract

You are a reviewer for the Veterinary Pathology journal and the following abstract is submitted for your review:

JJ Smart and MM Sharp. Department of Veterinary Pathology, University of Pretty Stains, Microscopia, Patholand.

Comprehensive study of diagnostic and prognostic immunohistochemical markers for canine oral melanoma

Oral melanoma is a common canine cancer with a historically poor prognosis. **Most of these tumors are amelanotic and resemble spindle cell tumors** therefore **special stains and immunohistochemical stains are essential** for making diagnosis. Recent evidence suggests that some dogs survive for more than a year after initial diagnosis. It is important to correctly identify these tumors as well as to discover special markers that directly correlated with prognosis. Definitive diagnosis of canine oral melanocytic neoplasms is based on detection melanin in melanocytes by **argyrophilic** stain; however not all oral melanomas are positive. The authors investigated a panel of immunohistochemical markers previously reported to be **specific** for canine melanocytes in 25 canine oral melanocytic neoplasms—namely, **MART-1, S-100,** tyrosinase-related proteins 1 and 2 (TRP-1 and TRP-2), and **NSE**. Ten well-differentiated cutaneous soft tissue spindle cell sarcomas were used as negative controls. **MART-1, TRP-1, and TRP-2 were 75% sensitive** and 100% specific for the diagnosis of canine oral amelanotic melanocytic neoplasms. S-100 and NSE showed high **specificity** but were less **sensitive**; that is, **they also labeled a proportion of the soft tissue spindle cell sarcomas**. The likelihood of correctly diagnosing canine oral amelanotic melanocytic neoplasms was dramatically higher when argyrophilic staining **in combination with one of the IHC stains** (MART-1, TRP-1 and TRP-2) was used. Cell proliferation marker Ki67, tumor suppressor gene *p53* and **oncogene *E-cadherin*** expression were used as prognostic indicators in the same set of 25 oral melanomas among which were **3 from dogs with extended survival past 1 year after diagnosis**. The dogs that survived for more than a year had lower Ki67 index (<10/HPF) and **decreased E-cadherin expression**; however there was no difference in p53 expression. We conclude that **longer survival for dogs with oral melanomas depends on** decreased E-cadherin expression and that use of these IHC stains

together with one of melanoma specific stains (MART-1, TRP-1 or TRP-2) **should be used for all diagnostic cases in which oral melanoma is suspected.**

1. Your decision (rejection, minor/major revision): (20 points)
Major revision or rejection

2. Justify your decision and correct any wrong statements and facts: (80 points)
Although the abstract contains new information about gene expression in association with prognosis in addition to assessment of more commonly used IHC stains for diagnostic use, this abstract contains multiple incorrect statements and unsubstantiated conclusions based on the data presented in the abstract. Major revision is suggested.
Here is the list of points that are incorrect or questionable:

1. Title is misleading. Application of 3 diagnostic IHC markers for a series of 25 cases does not constitute comprehensive study. Futhermore, analysis of prognostic markers using 3 cases with prolonged survival is not sufficient for making conclusions regarding prognosis. (8 pts)

2. Most of the oral melanomas are **amelanotic or contain scant pigment**. (5 pts)

3. Melanomas may resemble **spindle cell sarcomas, carcinoma, or lymphoma.** (8 pts)

4. Special stains and/or IHC stains would not be needed in cases when pigmentation is evident and junctional activity of neoplastic cells is seen. (5 pts)

5. Suggested histochemical stain for melanocytic tumors is **Fontana-Masson** (not argyrophilic). (8 pts)

6. More appropriate designation for MART-1 would be Melan-A. MART – 1 is a gene, the product of which is Melan-A, protein that is melanocyte antigen recognized by T lymphocytes. (5 pts)

7. **NSE and S100 are not stains specific for melanomas**; these also stain spindle cell sarcomas of neural origin and several other tumor types (including neuroendocrine and smooth muscle). (8 pts)

8. It seems highly unlikely that reported sensitivity of 75% was indeed the same for all three markers (Melan A, TRP-1 and TRP-2). If it was so then there are several issues with this

a) previous studies have reported much higher sensitivity for Melan A (over 90%). Why the diagnostic sensitivity was lower? (5 pts)

b) further in the abstract there is a recomendation that one of these stains (Melan A, TRP-1 or TRP-2) is used for increased diagnostic accuracy, however with sensitivity of 75% the diagnostic accuracy will not be very good. The recommendation seems to be unsubstantiated. (5 pts)

9. If NSE and S-100 labeled portion of spindle cell tumors used as controls and all oral melanomas, then it means that these markers have **high sensitivity but low specificity.** (8 pts)

10. **E-cadherin is tumor suppressor gene**. Decreased expression in 3 oral melanomas from dogs with increased survival needs to be investigated futher in higher number of cases. E-cadherin is responsible for adhesion and, generally, **loss of adhesion** leads to separation of tumor cells from each other. **Reduction in E-cadherin is one of features associated with increased malignancy in epithelial tumors.** (10 pts)

11. The data presented in the abstract in not sufficient to substantiate recommendation at the end of the abstract. (5 pts)

B. Data analysis

Neuroinflammation in amyotrophic lateral sclerosis (ALS)

Background

Amyotrophic lateral sclerosis (ALS), is a form of motor neuron disease, with loss of motor cortex, brainstem and spinal cord neurons, particularly in spinal cord ventral horns. Recent reports have proposed an active role of non-neuronal cells, namely astrocytes and microglial cells in the pathogenesis of the disease.

While the pathogenesis of ALS is unknown, a subset of humans suffering ALS possess a mutant superoxide dismutase gene (SOD1). Using a mouse model of ALS, the $SOD1^{G93A}$ mouse which posses a mutant SOD1 gene, you design a number of studies to test the hypothesis that astroglial and microglial activation can be correlated with neuronal degeneration at different phases of the disease.

Experiment 1

$SOD1^{G93A}$ mice, which typically develop overt disease at 90-days of age and become moribund 30 days later, were divided into 4 groups representing different phases of disease: presymptomatic (**PRESYM**: aged approx 60 days) symptomatic (**SYM**: median age males 85+/- 3days, females 86 +/- 3 days) end-stage (**ES**: median age, makes 108+/-4, females 104+/-3), and moribund (**MB**: median age, males 121+/-4, females 131 +/- 4). A well-documented ALS scoring system, based on clinical and task evaluations, was used to group the animals and aged-matched C57BL/6 non-transgenic littermates were used as wild-type (**WT**) controls.

At sacrifice animals were transcardially perfused and brains and spinal cords were post-fixed. Sections were stained with a rabbit anti GFAP (1:2500 dilutions) and sections visualised with a Nikon Eclipse E800 fluorescent microscope. Staining intensity was assessed using a Metamorph image analysis system and statistical analysis was performed using a one-way ANOVA test comparing between each diseased and wild-type grouping. Statistical significance was achieved when the p value was less than or equal to 0.05.

Results are presented in **Figure 1**

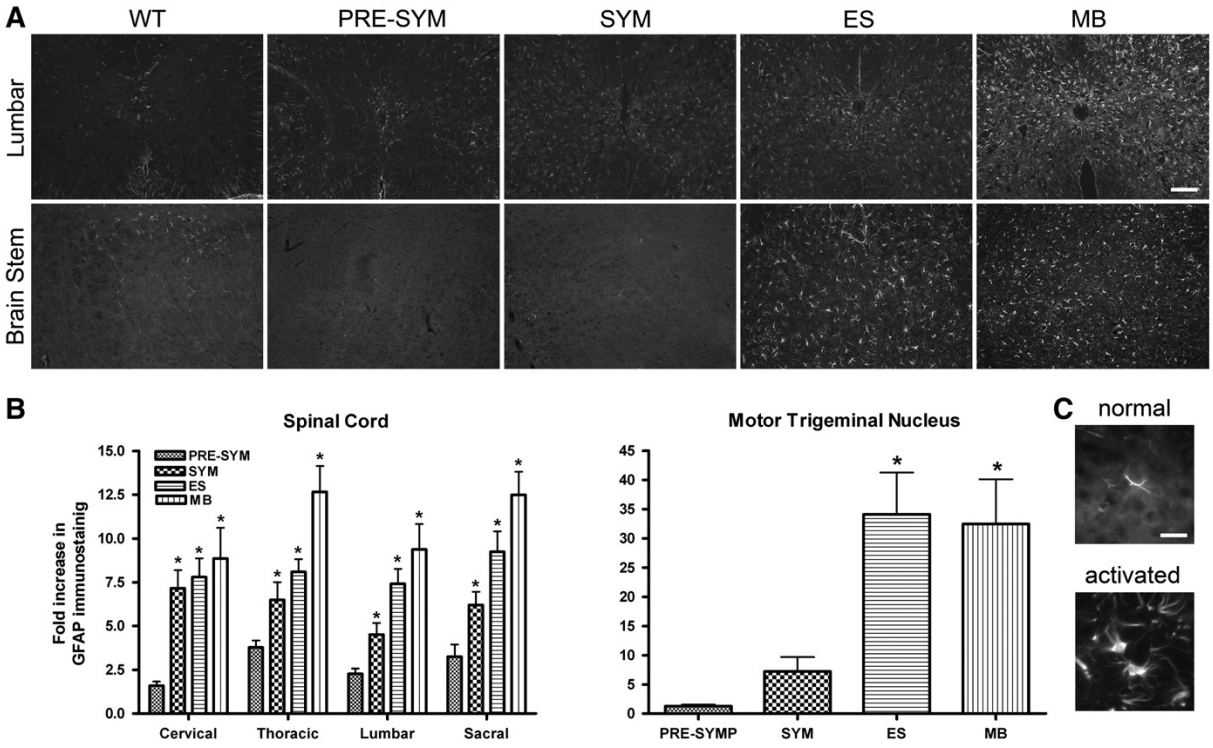


Figure 1: GFAP immunostaining and quantification

A. Expression of GFAP in the lumbar spinal cord and brain stem of SOD1^{G93A} mice at various stages of disease (scale bar 100µm)
B. Quantification (by metamorph analysis) of GFAP staining intensity at different levels of spinal cord and lateral medulla (location of MT nucleus) of SOD1^{G93A} mice at various stages of disease in comparison to wildtype controls. Values, which represent fold-increases compared to controls, represent means +/- SEM from 10 animals per stage.
C. High magnification showing the morphology of normal and activated astrocytes (scale bar 16µm)

Question 1: Summarize and Analyse the results.

Summary

Fig1 A shows an **increase in GFAP staining in cord and brainstem (1pt)** at the **end stage (1pt)** and **moribund stag**es **(1pt)** of disease in comparison to the earlier stages and the controls. Appears **stronger in brainstem at endstage** in comparison to lumbar cord.

Fig 1B shows increases in GFAP expression in **all levels of spinal cord and the trigeminal nucleus from presymptomatic stages (1pt)**. This increases **correlated positively with disease progression (1pt)** and in the spinal cord this becomes **statistically significant** in the **symptomatic** (5-7 fold increases), **end-stage** (7-9 fold increases) and **moribund stages** (8-12 fold increases) of disease **(1pt),** whereas in the **trigeminal nucleus it is statistically significant** at the **end stage and moribund stage (1pt)** with 30-33 fold increases

Fig 1C highlights that an **activated cells expresses more GFAP**

Analysis

The **progression (1pt)** of ALS in $SOD1^{G93A}$ mice is associated with a gradual **increase (1pt)** in GFAP expression suggesting **activation (hyperplasia/hypetrophy) (1pt)** (as of yet we have not evaluated cell counts)

TOTAL MARKS: 10 points

Experiment 2

Having all of the above samples available for further evaluation, sections were also stained against F4/80 which detects the presence of microglial cells.

Results are illustrated in **Figure 2.**

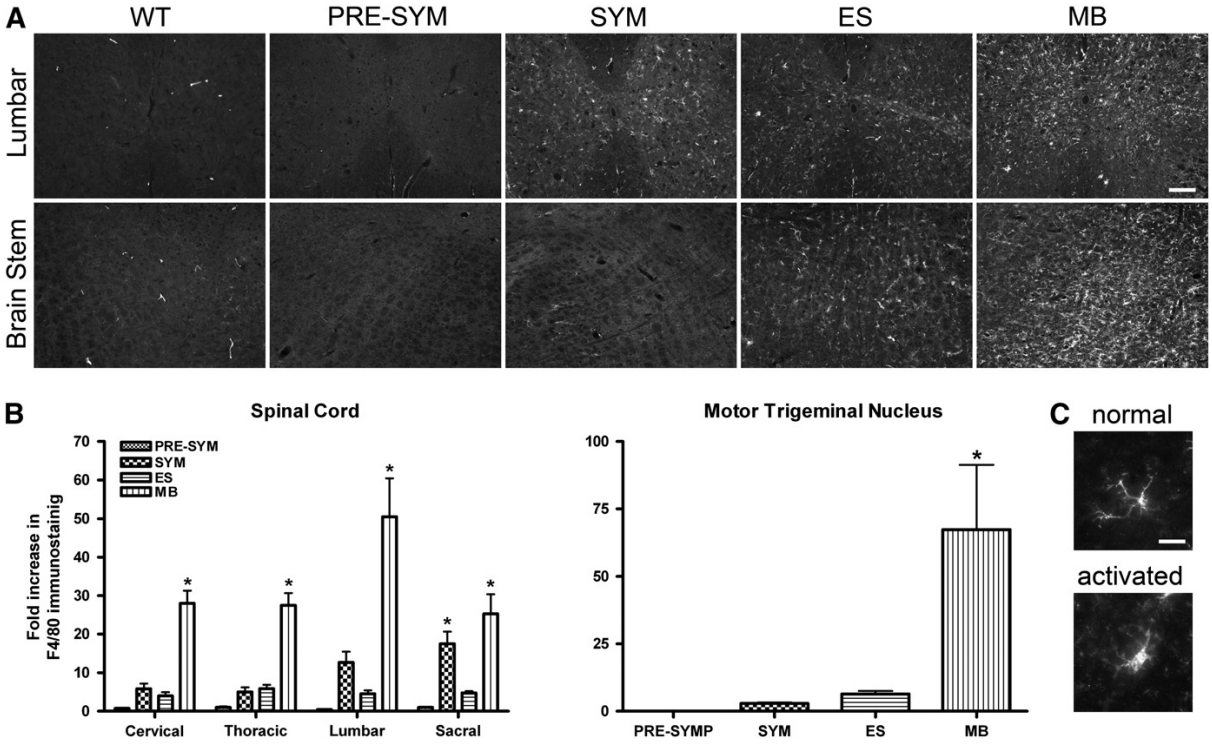


Figure 2: F4/80 immunostaining and quantification

A. Expression of F4/80 in the lumbar spinal cord and brain stem of SOD1^{G93A} mice at various stages of disease (scale bar 100µm)
B. Quantification (by metamorph analysis) of F4/80 staining intensity at different levels of spinal cord and lateral medulla (MT Nucleus) of SOD1^{G93A} mice at various stages of disease in comparison to wildtype controls. Values, which represent fold-increases compared to controls, represent means +/- SEM from 10 animals per stage.
C. High magnification showing the morphology of normal and activated microglia (scale bar 16µm)

Question 2: Summarize and Analyse the results.

Summary

Fig2 A shows an **increase in F4/80 staining in the cord at the symptomatic, end stage and moribund stages of disease (1pt)** in comparsion to the earlier stages and the controls. It also appears strongest at the moribund stage. Within the **brainstem staining was first detected at the end-stage time (1pt)**period. Apart from at the symptomatic stage, where the signal appears stronger in cord samples, there appears to be **no appreciable difference in the strength of the signal between the cord and brainstem.**

Fig 2B shows increases **in F4/80 expression in all levels of spinal cord and the trigeminal nucleus from presymptomatic stages (1pt)**. This increase in the spinal cord expression could **not be positively correlated with progression (1pt)** of disease but achieved **statistical significant in all levels of the spinal cord in moribund stage (1pt)** (30-50 fold increases) and at the symptomatic stage in sacral cord (20 fold rise).
A **positive correlation** (**1pt)** with expression and disease progression was observed in the TM nucleus and **statistical significance was achieved at the moribund stage (1pt)** with 60 fold increases observed.
Fig 1C highlights that an activated microglial cells expresses more F4/80 **(1pt)**

Analysis

The progression of ALS in $SOD1^{G93A}$ mice is associated with increased F4/80 expression suggesting activation **(1pt)** (hyperplasia /hypertrophy of microglia (as of yet we have not evaluated cell counts). In contrast to astrocyte activiation, **microglial activation appeared to intensify later in the course of disease (1pt)** following astrocyte activation.

TOTAL MARKS: 10 points

Experiment 3

Using Metamorph Image Analysis systems GFAP astrocytes and F4/80 miroglial cells were counted in the ventral horn of lumbar spinal cord at different stages of disease.

Results are summarized in **Table 1.**

	WT	**Pre-SYM**	**SYM**	**ES**	**MB**
GFAP	2±0.2	4±0.4	39±2.8*	99±5.1*	118±7.8*
F4/80	3±2.3	2±0.4	37±6.8*	44±2.6*	128±4.8*

Table 1: Cells counts of astrocytes and microglia in the ventral horn of lumbar spinal cord at different stages of disease (*p <0.01 versus wildtype control mice)

Question 3: Summarize and analyse the data in Table 1.

Summary

Astrocyte cell numbers in the ventral horn of the lumbar spinal cord **increased significantly in the symptomatic, end stage and moribund states of ALS (1pt)**. There was also a **positive correlation with disease progression (2pt)** .

Similarily there was a statistically **significant increase in microglial cells in the symptomatic, end stage and moribund states (1pt)**, when compared to wildtypes. It is noteworthy to see almost a **triple fold increase in the moribund stage (2pt)** compared to the symptomatic and end stages.

Analyse

ALS **progression (1pt)** in $SOD1^{G93A}$ mice is associated with **significant increases in the number of astrocytes and microglial cells (2pt),** with the latter cell population tending to **proliferate more dramatically in the late stages of disease (1pt).**

TOTAL MARKS: 10 points

Experiment 4

It is well established that in ALS motor neuron death involves apoptosis and other additional mechanisms. The mutant SOD-1 gene is present in neurons, astrocytes and microglial cells and there is a proposal that this causes defects in glial cells causing them to respond differently and produce substances to induce neuronal death. In order to proceed with this theory a number of further experiments/analyses were performed.

In this experiment the alterations in GFAP and F4/80 cells documented in the earlier experiments were compared to neuronal cell degeneration (as evaluated by fluoro-Jade C expression) in sections of lumbar spinal cord (ventral horns) and motor trigeminal nucleus with results presented in **Figure 3.**

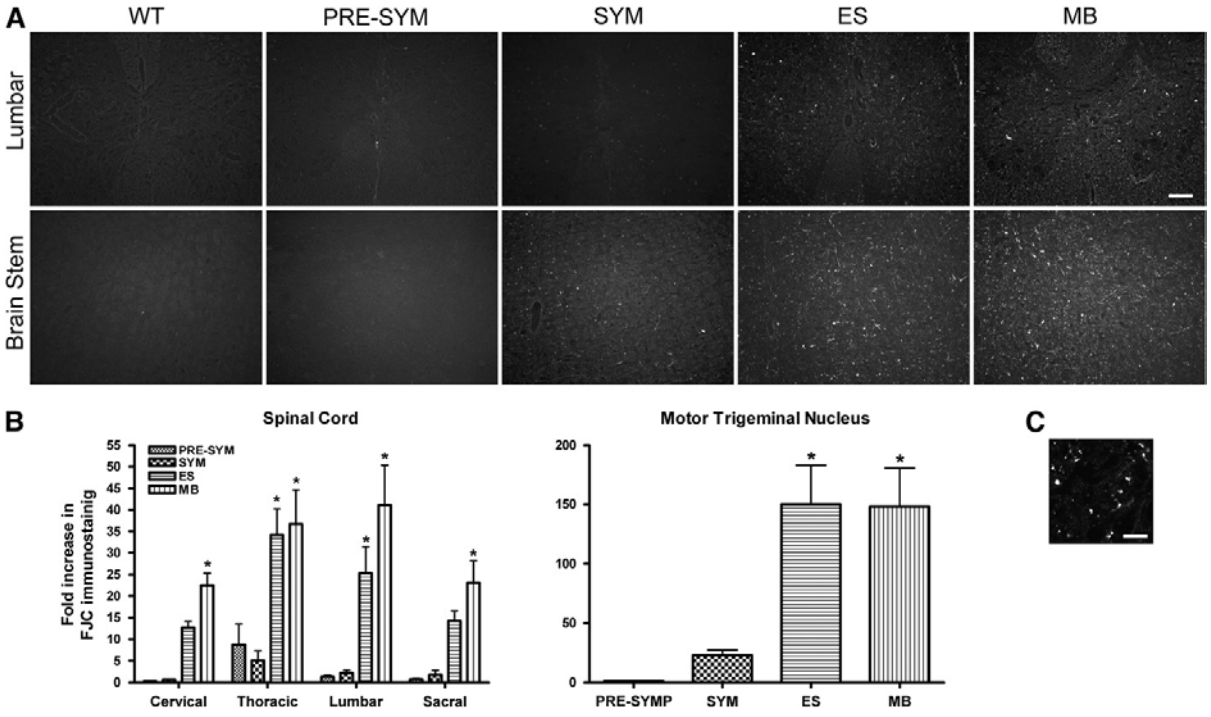


Figure 3: Fluoro-Jade C immunostaining and quantification

A. Expression of Fluoro-Jade C in the lumbar spinal cord and brain stem of SOD1^{G93A} mice at various stages of disease (scale bar 100µm)

B. Quantification (by metamorph analysis) of Fluoro-Jade C staining at different levels of spinal cord and lateral medulla (TG Nucleus) of SOD1^{G93A} mice at various stages of disease in comparison to wildtype controls. Values, which represent fold-increases compared to controls, represent means +/- SEM from 10 animals per stage.

C. High magnification showing positive FJC-stained cell (scale bar 16µm)

Question 4: Summarize and analyse the results of Figure 3.

Summary

Fig3A **FJC expression is observed in lumbar cord at end stage and moribund phase (1pt)** of disease and appears to increase in intensity with disease progression (**positive correlation)(1pt).** In **brainstem it is observed at symptomatic, end stage and moribund phases, again increasing in intensity (1pt)**.

Fig3B FJC expression **in cord is positive correlated with disease progression (1pt)** in cervical, lumbar and sacral cord and becomes **statistically significant (1pt) in the end-stages** of disease in thoracic and lumbar cord and by the **moribund stage** it is significant at all levels of cord where 20-40 fold increases are noted.

Similarily in the trigeminal nucleus FJC expression appears to **progress with disease (1pt)** and becomes a **significant expression in the end stage and moribund stages** where 150 fold increases are observed **(1pt).**

Fig 3C FJC staining highlights neurodegeneration

Analysis

ALS progression in SOD1^{G93A} mice is associated with, neurogeneration occurring **from the symptomatic stages (1pt)** of disease and **progressing (1pt)** to reach **significant levels by the end-stage and moribund stages of disease (1pt).**

TOTAL MARKS: 10 points

Question 5: Taking the results of all four experiments summarize the observations

ALS progression in SOD1^{G93A} mice is associated with significant increases in the **number (1pt)** and **activation status (1pt)** of astrocytes and microglial cells, with the **latter cell population tending to proliferate more dramatically in the late stages of disease (1pt)**. This occurs **concurrently with neuronal cell degeneration (1pt)**, which is also more **dramatic in the late stages of disease (1pt)**

TOTAL MARKS: 5 points

Experiment 5

It has been suggested that Nitric oxide (**NO**), produced by astrocytes, contributes to the pathogenesis of ALS through its reaction with superoxide anions to generate peroxynitrite, a potent oxidant and inhibitor of the mitochondrial electron transport chain. Peroxynitrite mediates tyrosine nitration to generate nitrotyrosine. Nitrotyrosine has been shown to be elevated in the CNS/CSF of human ALS patients.

In this experiment sections of lumbar spinal cord from all stages of disease in SOD1^{G93A} mice were stained for nitric oxide synthase (NOS) and for immunoreactivity to nitrotyrosine as a marker of peroxynitrite. Results are presented in **Figure 4**

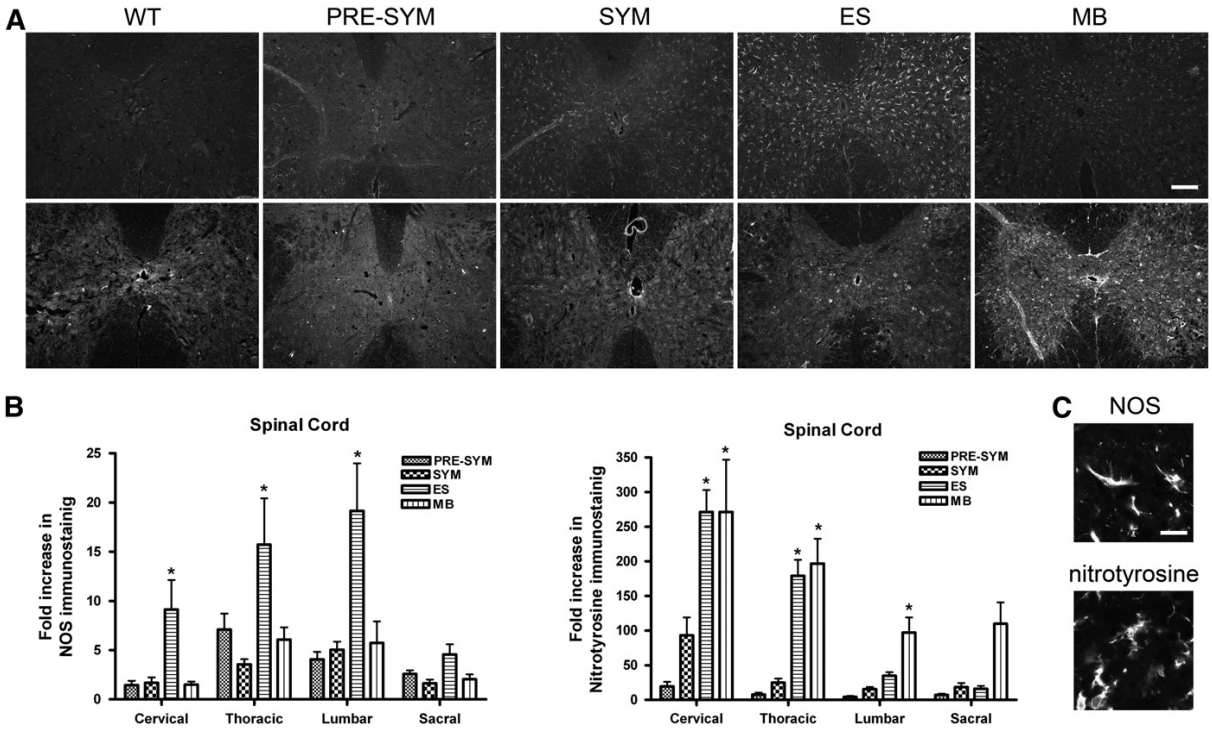


Figure 4: NOS and peroxynitrate expression in lumbar spinal cord

A. Expression of NOS (top row) and peroxynitrite (bottom row) in the lumbar spinal cord of SOD1^{G93A} mice at various stages of disease (scale bar 100µm)
B. Quantification (by metamorph analysis) of NOS and peroxynitrite staining intensity at different levels of spinal cord of SOD1^{G93A} mice at various stages of disease in comparison to wildtype controls. Values, which represent fold-increases compared to controls, represent means +/- SEM from 10 animals per stage.

C. High magnification showing positive NOS and nitrotyrosine-stained cell, which were confirmed as astrocytes (data not shown) (scale bar 16µm)

Question 6: Summarize and analyse the results of figure 4.

Summary

Fig4a NOS expression could be **visualised from the pre symptomatic stage (1pt)** of disease but the **greatest signal was observed at the end-stage (1pt)** of disease, reducing by moribund stage, so no positive correlation.

In regard to peroxynitrite expression, when comparing with wildtype it was difficult to assess significant differences between wt and expression at the pre-sym, symptomatic and end stages of disease. There appeared to a **greater level of expression at the moribund stage (1pt)**

Fig4b Metamorph analysis revealed elevated NOS expression **from the presymptomatic stages, at all levels of spinal cord (1pt)** with greatest expression (achieving **statistical significance in the end-stage of disease (1pt)** and a **reducing and none-statistical significant expression by the moribund stage (1pt).**

Elevations in expression of peroxynitrite were observed in **all levels of spinal cord, with subtle increases observed at the presymptomatic stages (1pt)** achieving more **statistically significant fold increases by the end stage and moribund stages (up to 250 fold un cervical cord)(1pt)**.

Fig4c: reveals NO and peroxynitrite expression in astrocytes

Analysis

Nitric oxide production from astrocytes is a feature of ALS disease, with greatest production in the **end stages of diseases (1pt)**. Nitric oxide production leads to the production of the potent antioxidant **peroxynitrite which is a prominent feature of the disease process particularly in the end and moribund stages (1pt).**

TOTAL MARKS: 10 points

Experiment 6

In order to investigate the role of microglial cells in ALS, a further experiment was undertaken.

Microglia in ALS possess the mutant SOD-1 gene. This confers inherent unique functions of these cells , particularly when they are activated. A review of ALS literature highlighted that two cytokines in particular are crucial in microglial cell activity, namely TNF-α and IL-6 and these were shown to be robustly upregulated in ALS. Furthermore such proinflammatory cytokines are known to induce neurodegeneration. Therefore an experiment was undertake to establish if differences in TNF-α and IL-6 production could be observed between activated wildtype derived and microglia from $SOD1^{G93A}$ mice.

Microglial cells were isolated from the brains of neonatal transgenic $SOD1^{G93A}$ mice and non-transgenic controls (n=3) and from similar mice aged 60 days of age. At this timepoint the SOD-1 mice were presymptomatic and previous studies had highlighted early stage microglial cell activation.

LPS (lipopolysaccharide), a recognised stimulator of microglia, was used to stimulate microglia derived from wildtype and $SOD1^{G93A}$ mice and these stimulated cells were analysed for the production of the known neurodegenerative-inducing cytokines.

For LPS stimulation tests incubated microglial cell cultures were exposed to 1µg.ml LPS or carrier control. After 24 hours of stimulation 100µl supernatant was collect for cytokine ELISA testing. Cell counts were assessed by WST-1 assay, cytokines (TNF-α and IL-6) were analysed by established ELISA methods. Statistical analysis was ANOVA, followed by Tukey's post-test. A P value of <0.05 was considered statistically significant.

Results are presented in **Figures 5 & 6.**

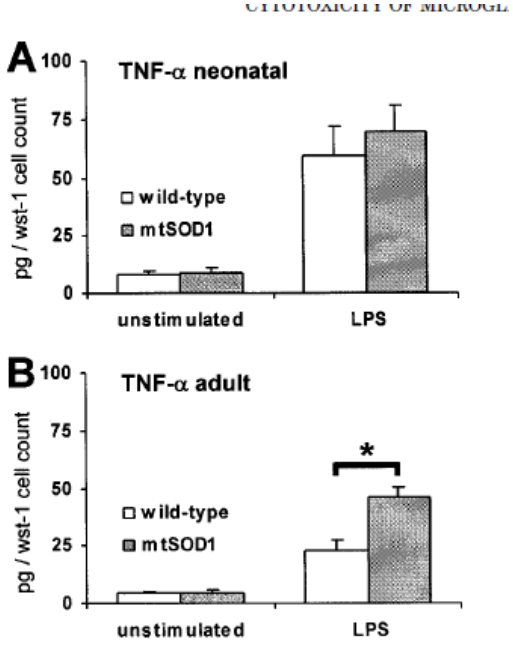


Figure 5: TNFα release from neonatal and adult microglial cells derived from wildtype and SOD1^{G93A} mice under unstimulated and LPS stimulated conditions. Concentrations normalised to cell counts, *P<0.05

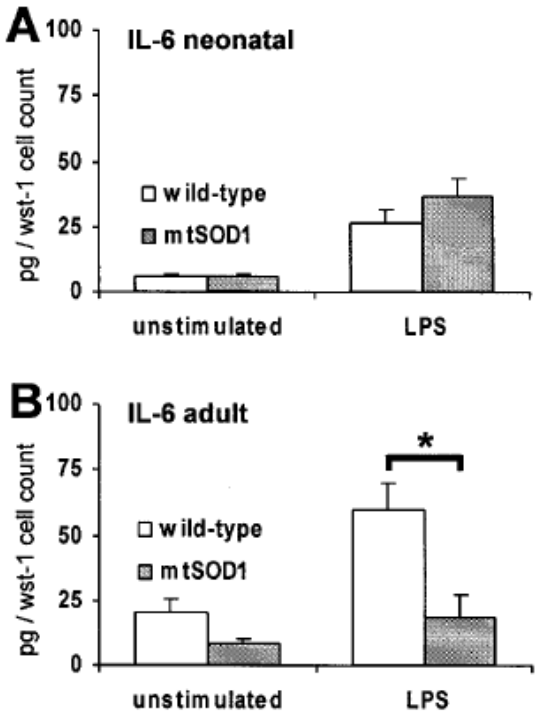


Figure 6: IL-6 release from neonatal and adult microglial cells derived from wildtype and SOD1^{G93A} mice under unstimulated and LPS stimulated conditions. Concentrations normalised to cell counts, *P<0.05

Question 7: Summarize and Analyse the data in figures 5 & 6.

Summary

Fig5a: **No significant difference (neonates) (1pt)** in TNFα concentrations produced in unstimulated microglia derived from WT and SOD1^{G93A} neonates **.**

While LPS resulted in increased TNFα contentrations in **stimulated microglia, there was again no significant differences (1pt)** between the wild-type and SOD1^{G93A} derived microglia from neonates

Fig5b: As for 5a, **No significant difference (adults, unstimulated) (1pt)** in TNFα concentrations in unstimulated microglia from 60 day old adults

LPS stimulated SOD1^{G93A} derived (**stimulted)adult cells did show a statistically significantly greater TNFα production in comparsion to wildtype (2pt)**

Fig 6a **No significant difference** between wildtype and SOD-1 neonatal cells , both in the **unstimulated (1pt)** and LPS **stimulated (1pt)** conditions, although LPS does induce increased IL-6 production in neonatal microglia.
Fig 6b In adult tissue, there was evidence that **wildtype unstimulated (1pt) microglia produced more IL-6 (or SOD-1 produced less) than SOD-1 cells and this was statistically significant (2pt)** when cells were **LPS stimulated**.

Analyse
In ALS, **activated (1pt)** (not unactivated) microglial cells (ie $SOD1^{G93A}$ derived) produce **more/excess TNFα (1pt)**and elevated TNF levels are known to contribute to neuronal death. Suprisingly in ALS, **activated and unactivated** (1pt) microglial cells (ie SOD-1 derived) **produce less IL-6 (1pt)**. Therefore the microglia are **unlikely to be the source of elevated IL-6** (1pt)in the disease.

TOTAL MARKS: 15

Question 8: Outline how your results in experiment 6 differ from what is proposed to happen in ALS.

Findings did not concur as
Literature/previous studies suggest elevations of TNF α and IL-6 occur in ALS and mediate neuronal degeneration/necrosis. In the present study stimulated $SOD1^{G93A}$ microglia from presymptomatic animals produce less IL-6 than wildtype microglia. **(5pts)**

TOTAL MARKS: 5 points

Question 9: Give two reasons why the results may not concur with what is proposed to happen in ALS.

Possible reasons include

1. The source of IL-6 is a different cell such as astrocyte (lots of evidence of this) **(3pt)**
2. Microglial cells removed for 60 day old ALS animals are already stimulated. Could the additional LPS stimulation have a negative effect? **(3pt)**
3. Could there be a more enhanced effect if other cells (such as astrocytes are present) **(3pt)**

TOTAL MARKS: 6 points

Question 10: Briefly outline an additional experiment you could undertake to support your reasoning.

so one could do the following

1. Screen astrocyte cultures via mRNA or cytokine assay **(4pts)**
2. Screen tissues by insitu for mRNA **(4pts)**
3. Look at dual cell populations or evaluate microglia in presence of astrocytic conditioned medium? (4pts)
4. Other options (4pts)

TOTAL MARKS: 4 points

Question 11: Summarize the complete data findings into a hypothesis on the pathogenesis of ALS

Mutated S0D-1 gene present in all cell types (neurons/microglia/astrocytes) **(3pts)**
Leads to an inherent instability in these cells making them more susceptible to activation of microglia and astrocytes (neuroinflammation) **(3pts)**
Activation of astrocytes potentially proceeds and may contribute to the stimulation of microglial cells **(3pts)**
Activated astrocytes produce NO..leading to neuronal degeneration & death **(3pts)**
Activated microglial cells produce excess TNFα leading to neuronal degeneration & death **(3pts)**

TOTAL MARKS: 15 points

Sources:
Weydt et al., (2004) Increased cytotoxic potential of microglia from ALS-transgenic mice. Glia, 48,179-182
Yang et al.,(2011) relationship between neuropathology and disease progression in the SOD1 ALS mouse. Experimental Neurology, 227 287-295

C. Forensic case

You are a pathologist at the University of Omniscience. One Friday afternoon, an owner delivers a 16 week old, male Golden Retriever puppy ("Ben") to you for examination. He has bought the dog 1 week ago and the clinical signs were apathy, severe, recurrent vomiting and diarrhea since the second day of ownership. A consultation with the breeder revealed that two other puppies from this litter (6 puppies in total) showed the same signs and were also in veterinary care. The owner of "Ben" consulted his veterinarian and the veterinarian performed a physical examination including ultrasonographic examination of the abdomen with the following results: apathy, slightly retarded growth, poor nutritional condition, moderate dehydration, pale mucous membranes, thickening of the intestinal mucosa, watery intestinal contents and small heterogeneous kidneys. Hematology and biochemistry results are as follows:

marker	"Ben"	reference range
hematocrit	0.15 l/l	0.37-0.55 l/l
red blood cell count	4.1 $x10^6$/µl	5.5-8.5 $x10^6$/µl
white blood cell count	8 $x10^3$/µl	6.0-12.0 $x10^3$/µl
neutrophils, segs	4.7 $x10^3$/µl	3.6-11.5 $x10^3$/µl
neutrophils, bands	0.1 $x10^3$/µl	0.0-0.3 $x10^3$/µl
lymphocytes	1.0 $x10^3$/µl	1.0-4.8 $x10^3$/µl
monocytes	0.8 $x10^3$/µl	0.15-1.35 $x10^3$/µl
eosinophils	0.04 $x10^3$/µl	0.01-1.25 $x10^3$/µl
platelets	4 $x10^5$/µl	2-9 $x10^5$/µl
total protein	4.0 g/dl	5.2-7.2 g/dl
albumin	1.9 g/dl	2.5-4.3 g/dl
glucose (GLU)	65 mg/dl	65-120 mg/dl
ALT	35 U/l	10-70 U/l
gamma GT	0.9 U/l	<1.2 U/l
blood urea nitrogen (BUN)	200 mg/dl	6-24 mg/dl
creatinine (CREA)	4.0 mg/dl	0.4-1.4 mg/dl
sodium (Na)	145 mmol/l	140 - 153 mmol/l
calcium (Ca)	12.4 mg/dl	9.5-12.0 mg/dl
potassium (K)	0.9 mg/dl	1.5 - 2.7 mg/dl
phosphorus (P)	10.3 mg/dl	3.3-6.8 mg/dl
chloride (Cl)	95 mmol/l	106 - 118 mmol/l

Additionally, fecal samples were sent for microbiological and virological examinations, but the results are not yet available. The dog was treated with antibiotics, anti-acids, anti-emetics, infusions and total intravenous nourishment. Despite intensive medical care, the dog died 1 day after initial presentation. Since two other puppies of this litter were also affected, the owner suspected that the dog was ill at the time of adoption and he wants his money refunded by the breeder.

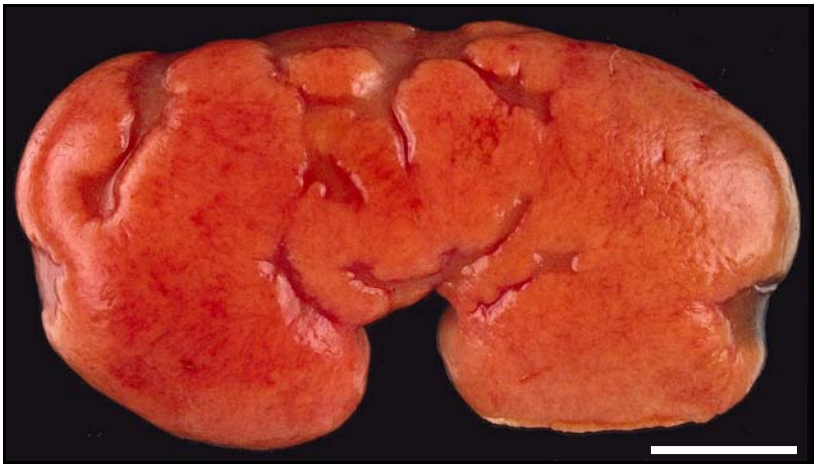
Question 1. Give a brief description/interpretation of the clinical and laboratory findings. (10 points)

- Emaciation
- Gastroenteritis
- Anemia
- Hypoproteinemia
- Hypalbuminemia
- Uremia
- Hypercalcemia
- Hypokalemia
- Hyperphosphatemia
- Hypochloremia
- Renal failure also accepted

You are enthusiastic and perform the necropsy on the Friday afternoon. You note the following macroscopic findings: 6 kg body weight, poor nutritional condition, ca. 40 ml ascites, ca. 20 ml hydrothorax. The other findings are depicted on the pictures below.

1.

2.



Bar = 0.5 cm

Question 2. Give a gross morphological diagnosis for the organs, shown above (15 pts).

1. Stomach: gastritis, multifocal, acute, severe, catarrhal to erosive (hemorrhagic or uremic gastritis also accepted)
2. kidney: renal dysplasia, multifocal (Nephropathy or renal fibrosis-5 points)

Question 3.
Which samples do you need for further clarification of your suspected diagnosis (Do not list more than 5) (15 pts)?

Histology from kidney, stomach, intestine, bone marrow (parathyroid glands, bone), determination of urea content using the aqueous humor of the anterior eye chamber; urine analysis (Combur9 or similar test)

Question 4. Give the histomorphological diagnosis for the slide obtained (no description) (20 pts).

Kidney: renal dysplasia, diffuse, severe
(nephropathy with tubular degeneration, mineralization and interstitial fibrosis- 10 points)

Question 5. Describe the pathogenesis of the laboratory abnormalities (30 pts).

anemia → erythropoietin deficiency due to renal failure
hypoproteinemia, hypalbuminemia → loss of albumin/protein via the kidney due to renal failure and via the gastrointestinal tract
uremia → due to renal failure with decreased capacity to excrete urea
hypercalcemia → due to excessive mobilization from the bone due to increased blood phosphate levels resulting in high levels of parathormone
hypokalemia → due to renal failure with resultant loss of reabsorption capacity in the kidney
hyperphosphatemia → due to renal failure leading to phosphate retention
hypochloremia → due to recurrent vomitus and loss of hydrochloric acid from the stomach

Question 6. Summarize your conclusions and explanations to the owner. (10 pts)

Congenital defect with asynchronous differentiation of renal tissue; inheritance suspected, familial accumulation of affected dogs; disease/defect present at time of adoption