Dottorato di ricerca in Scienze Veterinarie

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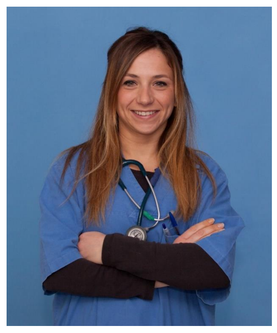

FRACTIONAL EXCRETION OF ELECTROLYTES IN DOGS WITH PRIMARY HYPOADRENOCORTICISM BEFORE AND AFTER TREATMENT

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Electrolytic abnormalities in dogs with primary hypoadrenocorticism (PH) have been widely described, while fractional excretion (FE) of urinary electrolytes (FEe) has not yet been evaluated. Furthermore, FEe could add useful information regarding the monitoring of the treatment.
The aim of this study were to evaluate FEe in dogs with PH. Study-population was grouped as follow: 1) dogs with acute adrenal insufficiency (AAI), dogs treated for PH (TD) 2) dogs with PH treated with desoxycorticosterone pivalate (DOCP) classified as well controlled ([Na] and [K] in the RI), under-controlled (hyponatremia and/or hyperkalemia) and over-controlled (hypernatremia and/or hypokalemia), respectively 3) dogs well controlled classified based on drug administration's timing: 9-15, 23-27 and 28-33 days after DOCP injection, respectively.
Only dogs with "typical" PH (hyponatremia and/or hyperkalemia) at the time of diagnosis were included. Healthy dogs (HD) were used as controls. Serum and urine chemistry were performed on combined samples using an automated analyser, and FEe was calculated. Nonparametric tests were used. Data are expressed as median and (range). P<0.05 was considered significant.
Seven dogs with AAI, 18 TD and 115 HD were enrolled; 76 follow-up from TD (13 DOCP, 5 fludrocortisone) dogs were evaluated. The main results are summarised in the following tables.

Variable	AAI	TD	HD
FENa (%)	2.64 (1.48-7.77)[a]	0.41 (0.04-1.91)[a]	0.25 (0.01-1.55)[a]
FECl (%)	3.76 (1.87-8.15)[a]	0.73 (0.07-21.85)[a]	0.54 (0.05-2.28)[a]
FEK (%)	18.53 (8.34-62.74)[a]	16.01(4.33-44.19)[b]	10.54 (2.23-45.20)[a,b]
FECa (%)	1.68 (0.68-8.11)[a]	0.35 (0.07-1.82)[a]	0.14 (0.03-0.66)[a]

Table I: Comparison of fractional excretion (FE) of urinary electrolytes in dogs with acute adrenal insufficiency (AAI), dogs treated for PH (TD) and healthy dogs (HD). *Data are reported as median and (range). [a,b] depict FE results that are significantly different among groups.*

Variable	DOCP Well controlled dogs	DOCP Under-controlled dogs	DOCP Over-controlled dogs
FENa (%)	0.43 (0.04-0.95)	0.29 (0.20-1.19)	0.4 (0.04-1.19)
FECl (%)	0.65 (0.07-1.57)	0.82 (0.33-0.95)	0.80 (0.16-2.68)
FEK (%)	16.14 (4.33-42.95)	12.45 (6.15-31.73)	17.10 (5.76-44.19)
FECa (%)	0.33 (0.07-1.60)[a]	0.11 (0.07-0.14)[a,b]	0.55 (0.17-1.82)[b]

Table II: Comparison of fractional excretion (FE) of urinary electrolytes in dogs with PH treated with DOCP classified as well controlled, under-controlle and over-controlled. Data are reported as median and (range). [a,b] depict FE results that are significantly different among groups.

Dogs with AAI have high FENa, FECl, FECa and they decrease after treatment. Further studies are necessary to clarify the clinical utility of the FEe in dogs treated for PH.

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COMPARISON OF DIFFERENT MONITORING METHODS IN DOGS WITH HYPERCORTISOLISM TREATED WITH TRILOSTANE

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ACTH stimulation test has been widely used and recommended as the monitoring method of choice for dogs with hypercortisolism (HC) receiving trilostane. However, it has never been validated for this purpose and recent data have shown a lack of correlation between ACTH stimulation test results and the clinical signs of dogs with HC treated with trilostane.
The aim of this study was to evaluate which of the previous investigated monitoring methods better correlate with a standardized and published clinical score (CS) obtained by an owner questionnaire and could represent the best method to monitor trilostane therapy.
Prospective multicentre study. Dogs with HC on treatment with trilostane twice daily for at least two weeks were blood sampled and categorized as unwell (sick or over-treated dogs), well and under controlled (no dose or dose increase required dogs respectively) based on the CS. The results of the CS were compared with: serum cortisol concentration pre-trilostane (T0), 3h-post-trilostane (T3) and 4h-post-trilostane (1h-post-ACTH) (T4) administration, plasma ACTH concentration pre-trilostane administration, plasma ACTH/cortisol (T0) ratio, serum haptoglobin concentration (Hp), chemistry variables (ALP, ALT, GGT), urinary cortisol/creatinine ratio and urine specific gravity (from urine of the evaluation's day and from the morning of the day before). Plasma ACTH and serum and urinary cortisol were measured with a chemilumescent assay (Immulite 2000).
76 re-evaluations of 37 dogs were included. Unwell dogs were excluded for further analysis. Haptoglobin was the parameter that better correlated with the CS (r=0.47),followed by ALT (r=0.34), T0 (r=0.33) and the UCCR average of the 2 urinary samples(r=0.33). ROC curve analysis identified a concentration of Hp of 150 mg/dl and a concentration of T0 of 4 µg/dl as useful value to discriminate well and under controlled dogs with a specificity of 91% and 78% respectively. (Fig. A and B)

Fig.A

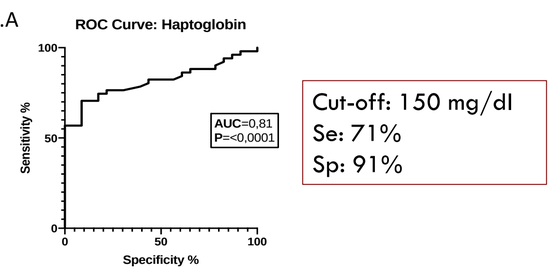
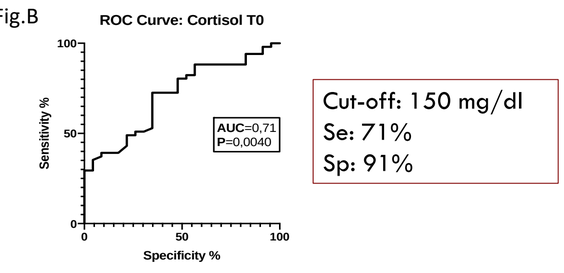


Fig.B



Receiver operating characteristics (ROC) curve for serum haptoglobin concentration (Fig.A) and cortisol concentrations at T0 (Fig.B) when used to discriminate between well and under controlled dogs dogs with HC. The area under the ROC curve (AUC) for haptoglobin was 0.81 and for cortisol T0 was 0.71.

Hp seems to be the best parameter to monitor trilostane therapy. However, Hp has likely limited capability to identify over-treated dogs; the concurrent evaluation of cortisol pre-trilostane may be useful in detecting an overdose of trilostane. The combined evaluation of Hp and T0 correctly categorized 85% of the cases and can be used as alternative monitoring method for dogs with HC under trilostane therapy.

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INCIDENTAL ADRENAL MASSES IN DOGS: IMMUNOHISTOCHEMICAL AND MOLECULAR FEATURES

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Incidental adrenal masses (AIs, adrenal incidentalomas) in dogs are being discovered with greater frequency due to the increased use and improved quality of imaging techniques. No studies had evaluated immunohistochemical (IHC) and molecular features of AIs in dogs.
The aim of the study was to describe the IHC and molecular characters of AIs in dogs and to investigate their utility in supporting the histopathological diagnosis. The second aim was to assess the role of Ki-67 PI and molecular markers as indicators of malignancy.
Tissue samples of canine AIs were collected from patients undergoing adrenalectomy between 2017 and 2019. Dogs were enrolled in the study if an adrenal mass was found in an imaging study performed for other reasons unrelated to adrenal disease. IHC detection of Steroidogenic factor-1 (SF-1), Melan-A (MART-1), 17α-hydroxylase/17,20-lyase (CYP17A1), Chromogranin A (CrA), Synaptophysin (SYP) and Ki-67 was performed. The mRNA expression levels of 5 target genes were analysed with quantitative RT-PCR (RT-qPCR): steroidogenic factor-1 (SF-1), pituitary tumour-transforming gene-1 (PTTG1), topoisomerase II alpha (TOP2A), GATA4 and GATA6.
Twenty dogs were enrolled in the study. Based on histopathological evaluation, 18 AIs were classified as adrenocortical tumours (ACTs) and 2 as pheochromocytoma (PHEOs). The 2 PHEOs showed a positive staining for Syn and CrA and negative staining for SF-1, MART-1 and CYP17A1. Regarding the ACTs, 2/18 (11.1%) had a positive staining for CrA and SYP and negative for SF-1, MART-1 and CYP17A1. 1/18 ACTs resulted positive only for CYP17A1. Of the other 15 (83.3%) ACTs, all were negative for CrA and SYP and positive for MART-1, 14/15 (93.3%) and 12/15 (80%) were positive for SF-1 and CYP17A1, respectively. To compare the values of Ki-67 PI, AIs were classified based on immunohistochemical results that have led to the diagnosis of 15 ACTs and 5 PHEOs. The median value of Ki-67 PI (%) was 3 (0.8-37.2) in ACTs and 20 (6.7-21.4) in PHEOs, respectively. The overall median Ki-67 PI (%) value was 5.5 (0.8-37.2). Gene expression was assessed in 14 AIs, 9 of which were ACTs and 5 PHEOs based on IHC evaluation. PTTG1 and TOP2A were overexpressed both in ACTs and PHEOs compared to normal adrenal cortex (NAC). The mRNA levels of SF-1, GATA4 and GATA6 were not significantly different between AIs and NAC.
Immunohistochemical evaluation (MART-1, SF-1, CrA and SYP) in addition to histopathological evaluation, appears to be useful for the correct diagnosis of canine AIs. 75% of AIs had a Ki-67 PI value that exceeds the threshold value previously defined to differentiate adrenocortical adenoma from carcinoma. Further studies are needed to assess the utility of Ki-67 PI and gene expression analysis as indicator of malignancy in AIs in dogs.

Preliminary data about "Adrenal Incidentalomas" project conducted at the Department of Clinical Sciences of Companion Animals, Faculty of Veterinary Medicine, Utrecht University (November 2018-May 2019).

Fig.A

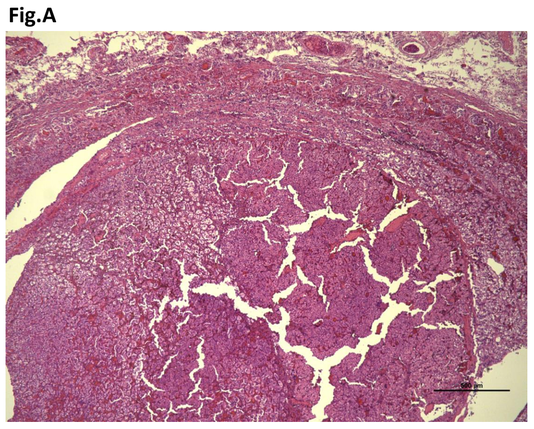


Fig.B

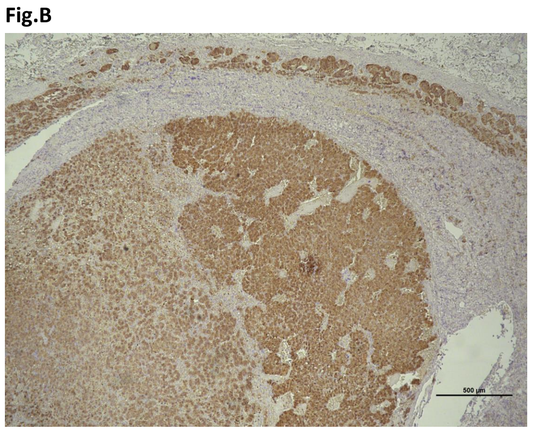


Fig.C

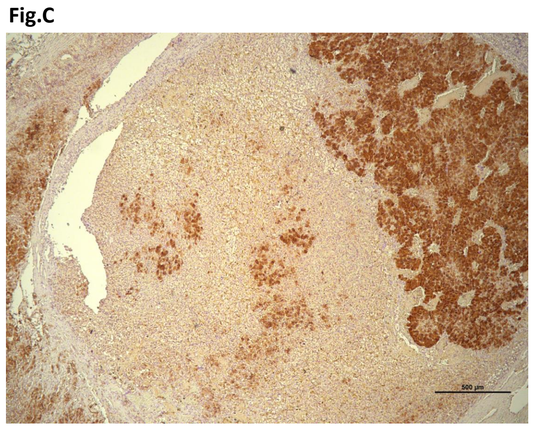


Fig.D

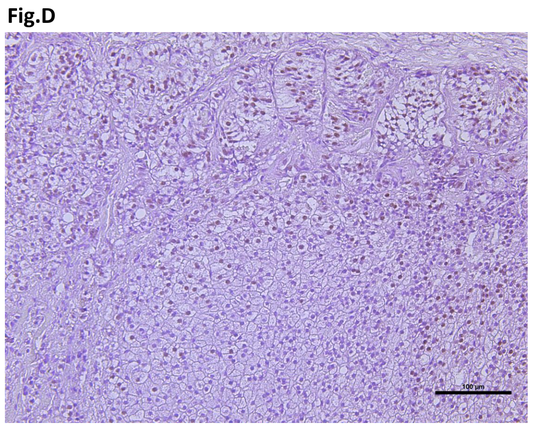


Fig.E

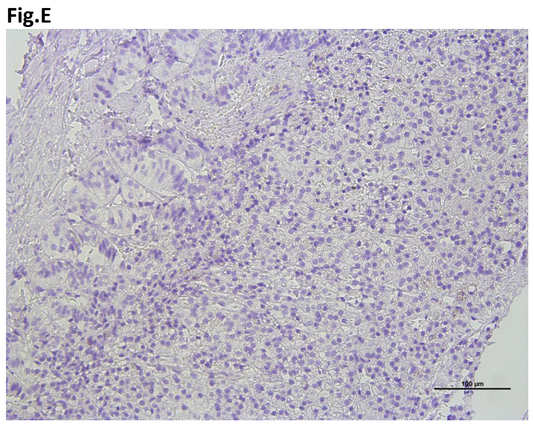
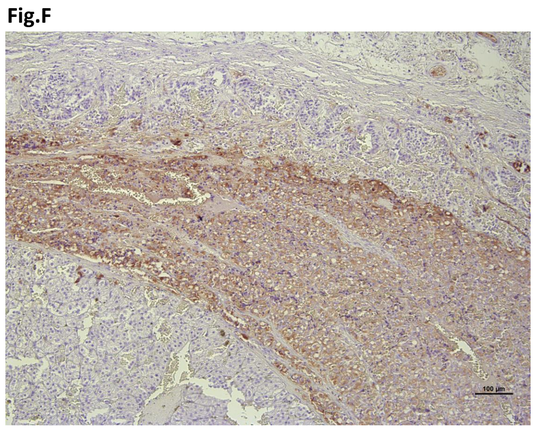


Fig.F



IHC staining for MART-1 (Fig.B), CYP17A1 (Fig.C), SF-1 (Fig.D), SYP (Fig.E) and CrA (Fig.F) of an ACT. Fig 1: hematoxylin and eosin (H&E).