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Use of Tandem Mass Spectrometry (LC-MS/MS) for the Measurement of Thyroid Hormones in Dogs with Spontaneous Hypothyroidism  
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In human medicine, liquid chromatography tandem mass spectrometry (LC-MS/MS) is actually considered the "gold standard" for measurement of many hormone concentrations, and it is widely used in clinical practice; its diagnostic performance has never been investigated in dogs with hypothyroidism (DWH).

The aim of this study was to determine whether serum concentrations of  $fT_4$ ,  $fT_3$ ,  $rT_3$ ,  $3.3-T_2$ ,  $3.5-T_2$ , measured with LC-MS/MS, were able to differentiate DWH (n=13) from dogs with non-thyroidal illness (DNTI) (n=12), septic dogs (SD) (n=12), and healthy dogs (HD) (n=12).

Hypothyroidism was diagnosed based on consistent clinical signs, laboratory findings, total  $T_4$  ( $TT_4$ ) and cTSH concentrations below and above the reference interval (RI), respectively; in dogs with normal cTSH, a rhTSH stimulation test was performed to confirm the diagnosis. In DNTI, hypothyroidism was excluded upon a negative result of a rhTSH stimulation test. SD were diagnosed based on alteration of temperature, cardiac and respiratory frequency, differential leukocyte count and C-reactive protein concentration above RI. HD were considered healthy upon history and physical examination. Hormone evaluations were performed with LC-MS/MS on surplus serum stored at  $-80^\circ\text{C}$ .  $TT_4$  and cTSH were measured using a validated immunoassay (Immulite®).

Non-significant differences considering signalment, age and body weight were found between groups. Median  $TT_4$  and  $fT_4$  serum concentrations were significantly higher ( $p < 0.001$ ) in HD compared to DNTI, DWH and SD. Median  $fT_3$  serum concentration was significantly lower in DWH and DNTI compared to SD ( $p < 0.001$  and  $p = 0.0091$ , respectively) and HD ( $p < 0.001$  and  $p = 0.0024$ , respectively). Median  $rT_3$  serum concentration was significantly lower in DWH compared to SD ( $p = 0.0141$ ) and HD ( $p = 0.0128$ ). Median  $3.3-T_2$  serum concentration was significantly higher in DWH compared to DNTI ( $p = 0.0038$ ) and HD ( $p = 0.0447$ ). There were non-significant differences regarding median  $3.5-T_2$  serum concentrations among the dogs of the four groups.

Using the ROC curve analysis to differentiate DWH from DNTI+SD, an AUC of 0.86 ( $p = 0.003$ ), 0.76 ( $p = 0.009$ ) and 0.75 ( $p = 0.012$ ) was obtained for  $fT_3$ ,  $fT_4$  and  $TT_4$ , respectively. Values of  $fT_3 < 0.61$  pmol/L better discriminated hypothyroidism with 69% sensitivity (95%CI: 39–91%), 83% specificity (95%CI: 63–95%) and accuracy of 0.86 (95%CI: 0.74–0.98).

Although serum  $fT_3$  and  $fT_4$  (LC-MS/MS) have shown better performances than the serum  $TT_4$  (Immulite®) in identifying DWH, the overlap between DWH and DNTI+SD was unfortunately relevant also for the thyroid hormone measurements with LC-MS/MS. Despite the introduction of new analytical methods, the use of dynamic tests (e.g., rhTSH stimulation test) remains the better method to discriminate DWH from DNTI.

#### DISCLOSURES

No disclosures to report