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1. 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°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