IDENTIFYING HORSES WITH PPID – PART TWO: INTERPRETING RESULTS

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DAVID RENDLE concludes his article on equine Cushing's disease by examining the results that may be obtained by lab tests and assessing their value

Summary

Interest in the diagnosis of pituitary pars intermedia dysfunction (PPID or equine Cushing's) has never been higher and the licensing of pergolide mesylate has been accompanied by encouragement to test for the disease by measuring adrenocorticotropic hormone (ACTH). While ACTH is accepted as a reliable means of identifying horses with PPID, laboratories offer conflicting advice on how samples should be collected and processed – and even on how results should be interpreted. In part one (*VT*42.45) factors relating to sampling and processing were discussed. In this article, laboratory factors that may affect results are reviewed and advice is offered on interpreting results.

Key words

equine Cushing's, pituitary pars intermedia dysfunction, adrenocorticotropic hormone, laminitis

MEASUREMENT of ACTH in equine plasma has been validated by two methods; radioimmunoassay (RIA)[1] and chemiluminescent assay (CIA)[2].

There is a single CIA method available, which is more popular due to faster processing, better

integration into laboratory systems, avoidance of radioisotopes and (in general) less variation in results. The coefficient of variation for the CIA has been reported to be between 3.2 per cent and 12.9 per cent[2].

A number of RIA methods have been developed and while some are very good others have no published validation data and generate variable results[3]. To get around the high intra-assay variability of some RIA methods, it is standard practice to measure each sample in duplicate and report an average of the results. This is not considered necessary for the CIA method.

Agreement between CIA and RIA methods was variable in two investigations that compared different methods of assessing ACTH concentration[3],[4]. The CIA assay is the most widely used and most robustly validated method in the UK and US, and much of the published research relating to PPID has been performed using it, which is advantageous when research data is being applied to clinical results.

There is little published data to indicate what the commercially available assays that claim to measure ACTH actually do measure. This suggests some methods that return far higher results measure more ACTH than others. Horses with PPID have circulating “ACTH” that is not biologically active and may merely be a peptide, or mixture of peptides, that cross-react with ACTH[5]. Assay kits may react with incomplete ACTH fragments or other similar proopiomelanocortin (POMC)-derived peptides that are produced in horses with PPID.

Radioimmunoassays demonstrate lower sensitivity and specificity for the intact human ACTH molecule than CIA methods and consistently generate higher results, which may be a result of lower specificity for the intact ACTH molecule and greater cross-reactivity with other peptides[6]. As ACTH levels do not necessarily correlate with clinical signs (see discussion below), it may not be important which ACTH fragments are actually measured, so long as what is measured is a reliable and repeatable indicator of pituitary dysfunction.

Reference ranges – why do they vary?

Donaldson et al (2005) identified that plasma ACTH concentrations in healthy horses and ponies were significantly higher in late summer/autumn than at other times of year and other groups have -[15].

since repeated their findings; not only in the US, but also in Europe and Australia[7]

A peak in ACTH concentration can be expected to occur from August and diminish by the end of October in the northern hemisphere and it is most widely assumed that changes occur in response to changes in day length[10],[14].

The magnitude of seasonal changes has been identified to be greater in horses with PPID,

increasing the diagnostic accuracy of measurement of ACTH concentration at this time of year[12,15]. Therefore, establishing seasonal reference ranges has reversed the previously held belief that late summer and early autumn were inappropriate times to test due to the occurrence of false-positive results[4]. Seasonal fluctuations have been identified consistently in published studies, regardless of laboratory method, so the reliability of reference limits that do alter with season is open to question.

Multiple research studies that have used the CIA method have identified an upper reference limit around 35pg/ml from November to July[2,4,14,16-18]. In the UK, Copas and Durham (2012) used results from samples collected from 156 healthy horses and 941 horses diagnosed with PPID to calculate seasonal upper reference limits of 47pg/ml for August to October and 29pg/ml for November to July[19].

Another UK group using an RIA method suggested an upper limit of greater than 40-50pg/ml in March and June and greater than 80-100pg/ ml in September and December[12], which is consistent with reports from the US (and human reports) in which RIA methods consistently report slightly higher results than the CIA method and upper reference limits for various different RIAs are 45-50pg/ml out of the autumn period[9]. One UK laboratory uses an upper reference limit of 300pg/ml, although it is unclear how this reference limit was decided and why it is so disparate from all other published reports and from the findings of other groups that use RIA techniques[3]. The reference limit per se may not be important provided the method via which it was derived is robust.

What if I get a borderline result?

PPID is a progressive disease that is gradual in onset, and fluctuations in endogenous ACTH occur in some horses (part one *VT*42.45), so further testing is indicated when borderline results are obtained. One study, in which normal and PPID horses had ACTH concentrations repeatedly measured on 27 occasions over a twoweek period, found horses with ACTH concentrations below 20 and above 40 could be diagnosed confidently as negative and positive respectively, but concluded horses with concentrations between 20pg/ml and 40pg/ml should be subject to further testing with a thyrotropin releasing hormone (TRH) stimulation test[20], which has a slightly higher sensitivity and specificity than resting ACTH[16,17].

In a separate study comparing the results of resting ACTH concentrations against ACTH concentrations following administration of TRH, there was poor agreement over the same range of resting ACTH concentrations, leading to the same conclusion that further testing should be performed in horses that have resting ACTH concentrations between 20pg/ml and 40pg/ml[21].

Therefore, in horses with borderline ACTH concentrations, a TRH stimulation test is recommended (Table 1). Alternatively, retesting could be performed in the autumn when the disparity in ACTH concentration between normal horses and horses with PPID is increased.

ACTH concentration and disease severity

Anecdotally, it is not uncommon for horses with obvious clinical signs of PPID to have modest increases in ACTH concentration, while horses with few or no signs may demonstrate marked increases.

While it is a reliable marker of pituitary dysfunction, ACTH is probably not a reliable indicator of the magnitude of pituitary dysfunction and the severity of clinical disease.

There is no established link between ACTH levels and the risk of laminitis – indeed, the reverse may be true (Liphook Equine Hospital, unpublished data). ACTH is merely the marker of PPID we have available to us and, although it correlates with histopathological changes in the pars intermedia[22] and tends to increase with age[4], it does not necessarily predict the severity of clinical signs.

Although the links between PPID and laminitis (and other clinical signs) have not been established, it is likely laminitis occurs as a result of hyperinsulinaemia, the degree of which is associated with the risk of laminitis[23], and hence prognosis[24]. It is possible tests for other pituitary-derived peptides, which may correlate more closely with clinical signs, will be developed.

Should asymptomatic horses be treated?

Studies performed using necropsy as the gold standard indicate a high ACTH result has a high positive predictive value for PPID (false-positive results are uncommon[16],[17]).

Furthermore, evidence indicates plasma ACTH concentration (and other endocrine tests of pituitary dysfunction) only detect horses that have advanced histopathological changes within the pars intermedia and we are more likely to miss horses with early disease than falsely diagnose horses that do not have it (Dianne McFarlane, personal communication).

If the horse has had laminitis in the past then treatment is warranted. In the absence of current or previous clinical signs the main indication for the treatment of PPID is prevention of disease progression and reduction in the risk of future incidents of laminitis.

Published evidence is lacking on whether pergolide reduces the risk of laminitis in horses that have not suffered from it previously and whether pergolide slows the progression of pituitary pathology. However, dopamine agonists have been speculated to have a range of neuroprotective effects[25] and pergolide has been demonstrated to protect dopaminergic neurons and neuroblastoma cells from oxidative stress in vitro[26],[27].

Although the condition is not directly analogous, dopamine agonists are the treatment of choice for prolactin-producing microadenomas and macroadenomas; the most common pituitary tumours in

humans. In these tumours, pergolide and other dopamine agonists consistently result in dramatic tumour shrinkage in 75 per cent of patients and tumours may continue to shrink for months or even years[28-30].

Pergolide may similarly result in regression of adenomas of the pars intermedia in horses and, pending the results of equine clinical studies, one has to recommend treatment with pergolide following a diagnosis of PPID in order to reduce the risk of disease progression and onset of laminitis.

The alternative is to wait until laminitis occurs and then instigate treatment. As laminitis has been identified in 24 per cent to 82 per cent of horses with PPID[31-33] this seems a very risky alternative.

Is pergolide safe?

Pergolide is safe, with the only likely side effect being transient inappetence. If inappetence occurs it usually resolves if pergolide administration is stopped and then reintroduced gradually. There is no evidence that pergolide results in liver disease and nothing in the literature that gives any foundation to such a suggestion.

In a study of 24 horses treated with pergolide at one to two times the licensed dose for six months, one horse developed a mild increase in GGT that resolved spontaneously without treatment in the face of continued pergolide administration[34]. One of the eight control horses that did not receive pergolide developed a similar increase in GGT that also resolved without treatment. Subclinical liver disease is common, and if increases in liver enzymes are identified in horses treated with pergolide they are likely to be the result of unrelated toxic or infectious insults.

In research studies, doses far higher than those used clinically have been administered without long-term effects (author's unpublished data; Jill Beech personal communication).

How much pergolide should I administer?

The recommended starting dose of pergolide is 2µg/kg per os once daily. Individual responses are highly variable and do not appear to correlate closely with initial ACTH concentrations.

As there is no means to predict whether 2µg/ kg pergolide is sufficient based upon signalment or initial ACTH concentration, and as only one in three horses is likely to respond adequately to 2µg/kg, further endocrine testing should always be performed[35]. While testing does incur further expense this is outweighed by the costs of persisting with treatment at an ineffective dose and the development of further bouts of laminitis.

Conclusions

• When assessing ACTH concentration, use an assay that has been validated and has robust seasonally-adjusted reference intervals.

• Borderline ACTH results are inevitable with an insidious, progressive disease. If they occur, consider performing a TRH stimulation test.

• ACTH indicates the presence of PPID, not its severity. For an indication of prognosis and laminitis risk consider measuring insulin concentrations – either fasted or using a standardised dynamic test.

• Treatment should be considered in all horses with PPID to reduce the risk of laminitis.

• Pergolide is safe. The only adverse effect is reversible inappetence.

• Concerns that pergolide may result in liver disease are unfounded.

• Endocrine testing is required around a month into treatment to determine the appropriate dose of pergolide in horses with PPID.

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