Canine snake envenomation in New South Wales – development of adjunctive diagnostic tests

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Abstract

This study was undertaken to identify and evaluate clinicopathological data for use in the diagnosis of Red-bellied Black snake (*Pseudechis porphyriacus*) (RBBS) envenomation in dogs in the Camden region of Australia. Serum biochemical variables were compared between dogs with and without RBBS envenomation. Five variables were identified as significantly different and sensitivity, specificity, predictive values and likelihood ratios were obtained for these analytes used alone or in combination (with consideration of conditional covariance) for the diagnosis of RBBS envenomation in the dog. Manipulation of these analytes by varying cut-off values and combining tests in series or parallel, allowed optimisation of sensitivity and/or specificity appropriately for use in different clinical scenarios. Three analytes (AST, CK and albumin) were found, used in different combinations, to be the most accurate.

Introduction

Accurate diagnosis of snake envenomation in dogs may be challenging to the clinician. Reasons for difficulty include an over-reliance on non-specific clinical signs, lack of published definitions of the clinical syndromes resulting from envenomation by less common snake species, and both inter- and intra-species variation in toxin composition (Hudelson and Hudelson 1995). The use of diagnostic tests such as snake venom detection kits (CSL Ltd, Parkville, Australia) for diagnosis of species-specific snake envenomation has been shown to be rare in a population of NSW veterinarians, and the need for identification of cheaper adjunctive diagnostic tests has been acknowledged (Heller *et al* 2005).

Objective

The aim of the current study was to identify and evaluate the accuracy of serum biochemical analytes that could be developed either independently or in combination as adjunctive diagnostic tests for RBBS envenomation in the dog.

Materials and Methods

Serum biochemical results were obtained prospectively for cases of snake envenomation in dogs in the Camden region of NSW (RBBS and other snake species). Strict criteria were required to be met prior to inclusion in the study. Retrospective data collection was also undertaken over a similar two year period to obtain data for non-snake envenomed cases. Mann-Whitney U tests were used to compare results between snake species and also between cases of RBBS envenomation and all other diseases.

Percentile distributions were obtained for analytes significantly altered in envenomed dogs using pooled data. Sensitivity, specificity, positive and negative predictive values, positive and negative likelihood ratios were calculated for each of the analytes, using varying percentile cut-off points to dichotomise the test results. Receiver Operator Characteristic (ROC) curves were also produced for each analyte, allowing optimal cut-off values to be obtained for maximisation of both sensitivity and specificity, and the area under each curve to be calculated.

The conditional dependence between analytes was assessed by calculating the conditional covariance using a technique described by Gardner and colleagues (2000). Parameters were obtained for all analytes when used in multiples of two in serial and parallel combination and using each of 75th, 90th, 99th and optimal percentile cut-off values.

Results

Five analytes (creatine kinase (CK), aspartate aminotransferase (AST), total bilirubin (Tbil), albumin and phosphate) were found to have a) significant differences between RBBS envenomed cases (N=18) and Brown snake envenomed cases (N=9), b) significant differences between RBBS envenomed cases and all other diseases (N=367) and c) medians that fell outside the 10th percentile for all cases (making them immediately identifiable as abnormal).

Sensitivity and specificity were similar for each of the five analytes at varying percentile cut-off values. Not surprisingly, specificity increased with an increase in cut-off value for most analytes and sensitivity decreased. In general, the analytes showed low sensitivity at the higher percentile cut-offs (sensitivity less than 50%) which increased to moderate (60-89%) as the cut-offs were lowered. In contrast, the specificity of all of the analytes were high at the higher percentile cut-offs (specificity greater than 95%) and reduced slightly (lowest value of 77.5%) as the cut-offs were lowered. Low positive predictive values (maximum 38%) and high negative predictive values (97% - 99.6%) were found for all five analytes

Results of ROC curve analyses revealed the area under the curves to be 0.920 (95% Confidence interval; 0.853–0.986) for AST, 0.905 (0.857–0.953) for albumin, 0.904 (0.817-0.992) for CK, 0.795 (0.661-0.929) for Tbil and 0.792 (0.667-0.917) for Phosphate.

Combining the diagnostic tests in series or parallel, using optimal cut-off points as identified by ROC analysis, and accounting for conditional dependence between analytes resulted in high values for sensitivity and positive likelihood ratios when certain biochemical analytes were used in parallel (CK OR albumin, AST OR albumin or albumin OR phosphate resulted in maximal sensitivity), and high specificity (maximal level of 97.9%) and negative likelihood ratios when used in series (AST AND albumin resulted in highest specificity).

Discussion

The study presented here is the first quantitatively to identify and characterise laboratory changes that may be used to aid the diagnosis of RBBS envenomation in the dog. A percentile analysis technique was used for the interpretation of test results and provides a greater objectivity than the more common reference range approach.

The current study found that, in the Camden region, AST, CK and albumin were the most useful serum biochemical analytes for the diagnosis of RBBS envenomation in the dog (area under curve greater than 0.90 for all tests). These analytes could be used alone, resulting in a high specificity and negative predictive value, or in combination to enhance either the sensitivity (e.g. AST OR albumin) or specificity (e.g. AST AND albumin). Varying cut-off values could also be used further to optimise certain test properties, depending on the clinical scenario and desired use of the test.

An assessment of post-test probability or likelihood of disease can be made after the appropriate diagnostic test/s have been chosen, with the use of calculated likelihood ratios and with an informed estimate of pre-test probability (or prevalence) of disease in the animal being tested. An intuitive way to do this is with the use of Fagan's nomogram (Fagan 1975).

Diagnosis of RBBS envenomation, as with other snake species, is notoriously difficult, and is susceptible to many potential sources of bias. Although every attempt has been made to control these biases in the current study, the ultimate effect has been to limit the applicability of the findings to the population and region in which the study was conducted.

References

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