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Long-term Intra-individual Variations and Critical Differences of Clinical Chemical Parameters in Dogs

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Abstract: The purpose of this study was to assess intra-individual variations of clinicochemical parameters and calculate critical differences in healthy dogs during long-term periods. To calculate the critical difference of clinicochemical parameters, blood samples from 20 apparently clinically healthy dogs were collected once weekly for eight consecutive weeks. The critical difference was calculated as 9.01 mg/dl for urea, 0.52 mg/dl for creatinine, 0.99 g/dl for total protein, 0.39 g/dl for albumin, and 20.64 mg/dl for glucose. If two consecutive results differ by less than the critical difference value, it can be concluded that the difference is probably due to physiological variation. However, when the difference is greater than the critical value, other factors, either related to progression of the disease or the presence of concurrent disease, are more likely to be involved.

Key words : critical difference, dogs, intra-individual variations, clinicochemical parameters.

Introduction

The scope of hematological and clinical chemical analysis is in most cases to observe whether the current measurement is part of a trend towards a pathophysiological state in a particular animal[3]. An analytical result outside the reference interval classifies the animal as abnormal indicating an unusual or pathological condition. However, other ways of assessment such as the critical difference (d_k) may be used, in which the patient serves as its own reference using a comparison of analytical results from samples obtained serially at appropriate intervals[4]. The critical difference allows consecutive results to be compared[1,6] and assists in determining whether the difference between two consecutive results can be safely ascribed to natural variation or whether it is caused by others factors such as disease, therapy or experimental procedures. The present study was designed to establish the critical difference values for clinical chemical parameters including blood urea nitrogen (BUN), serum creatinine, total protein, albumin, and glucose in canine serum samples in a week-to-week basis. Such long term intra-individual variations of clinical chemical parameters in dogs have not been reported previously.

Materials and Methods

Experimental animals

Breed and sex distributions were as follows; beagle (n=5, male: 3, female: 2), mongrel (n=5, male), Yorkshire terrier (n=3, male), schnauzer (n=3, male), maltese (n=2, male), shih-tzu (n=1, male), and poodle (n=1, male). The range of body weight was 3.2 to 6.9 kg and that of age was 1 to 6 years. The dogs were each housed in individual cages and maintained on commercial dry food and water provided *ad libitum*.

Sample collection and Analytical procedure

Each dog was sampled weekly for 8 consecutive weeks. Following a 15 hour fast, blood samples were obtained by jugular venepuncture from each dog between 10:00 a.m. and 11:00 a.m. hours. BUN, serum creatinine, total protein, albumin, and glucose were determined by Selectra 2 (Merck, The Netherlands).

Statistical analysis

First the set of results was evaluated statistically for approximate normality of distribution. Then, the d_k value was calculated as described by Costrongs GMPJ *et al.* (1985) from $d_k = z \times \sqrt{2\sigma^2}$. Usually, the critical difference is reported for the median value, with a probability of 95%, giving a z-value of 1.96. Theoretically, the standard deviation of the difference between two independent, random variables with the same expected mean value and the same standard deviation, σ, is $\sqrt{2\sigma^2}$. The probability that the difference between two variables is less than or equal to $1.96 \times \sqrt{2\sigma^2}$ is 95% and if the difference between two consecutive results is greater than $1.96 \times \sqrt{2\sigma^2}$, then from an analytical point of view the results can be regarded as being different (Stamm, 1982). The total variance for the results for the one animal concerned is dependent on intra-individual variance (σ^2_{Intra}), the analytical variance (σ^2_{Anal}), and the variance due to other factors such as, for example, specimen collection and preanalytical variation (σ^2_{others}). For the present study, the variation due to other factors can be neglected and, consequently, the critical difference can be calculated as $d_k = 1.96\sqrt{2(\sigma^2_{intra} + \sigma^2_{anal})}$. Analytical variance was established by 9 assays on three different levels of serum controls. Statistical analyses were performed using the Microsoft Excel 2003.

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Results

Mean values and absolute ranges for BUN, serum creatinine, total protein, albumin, and glucose are presented in Fig 1-5 respectively.

Table 1 summaries the intra-individual variance (σ^2_{Intra}), the analytical variance (σ^2_{Anal}), the overall mean value ($\bar{x}$), and the critical difference both in absolute values (d_k) for each of the 5 parameters examined in the present study. The critical difference was calculated as 9.01 mg/dl for BUN, 0.52 mg/dl for serum creatinine, 0.99 g/dl for total protein, 0.39 g/dl for albumin, and 20.64 mg/dl for glucose.

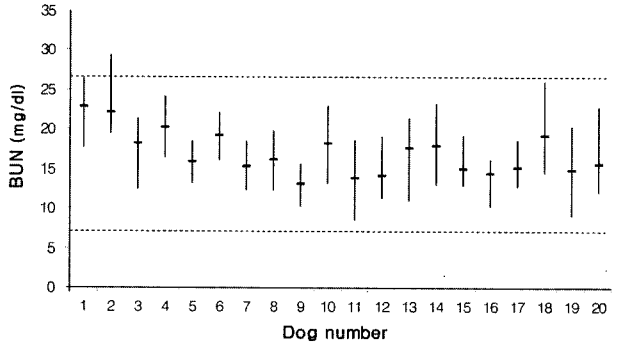

Fig 1. Mean and absolute range values for blood urea nitrogen (BUN) concentration in twenty clinically healthy dogs measured at weekly intervals over an eight-week period. The dotted lines represent the reference interval used in this laboratory.

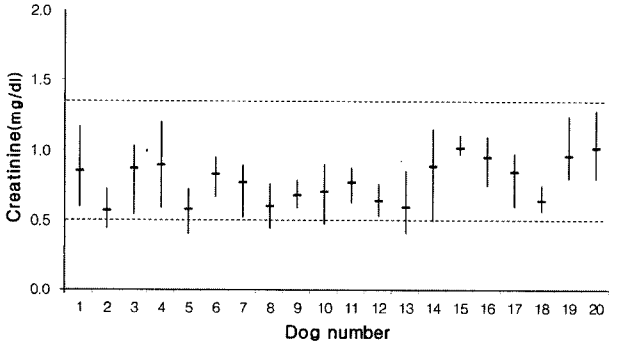

Fig 2. Mean and absolute range values for serum creatinine concentration in twenty clinically healthy dogs measured at weekly intervals over an eight-week period. The dotted lines represent the reference interval used in this laboratory.

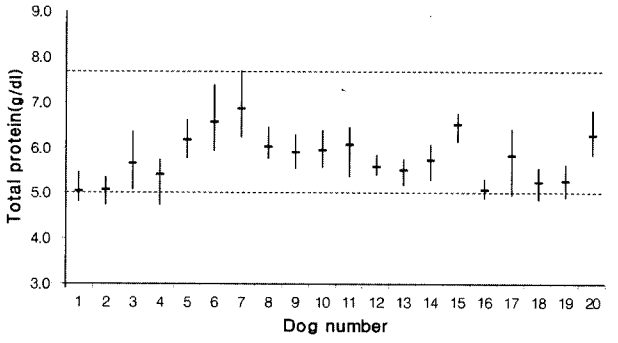

Fig 3. Mean and absolute range values for serum total protein concentration in twenty clinically healthy dogs measured at weekly intervals over an eight-week period. The dotted lines represent the reference interval used in this laboratory.

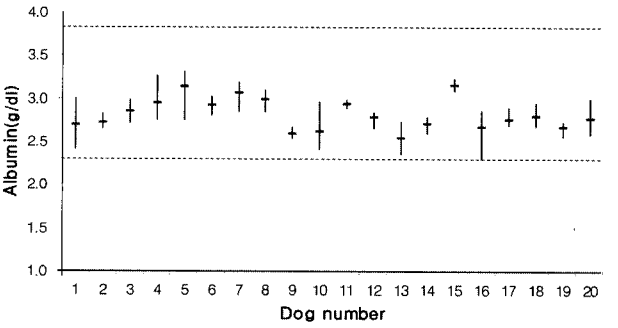

Fig 4. Mean and absolute range values for serum albumin concentration in twenty clinically healthy dogs measured at weekly intervals over an eight-week period. The dotted lines represent the reference interval used in this laboratory.

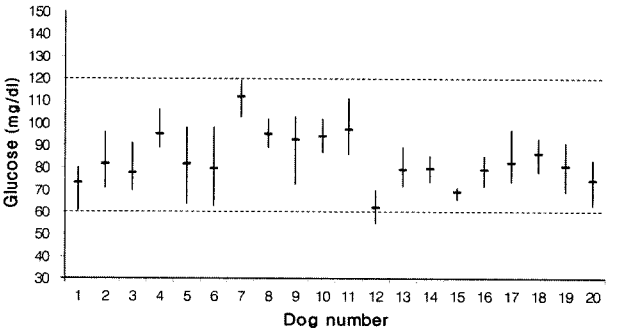

Fig 5. Mean and absolute range values for serum glucose concentration in twenty clinically healthy dogs measured at weekly intervals over an eight-week period. The dotted lines represent the reference interval used in this laboratory.

Table 1. The critical difference in absolute values (d_k), the component of variance for weeks within dogs (σ^2_{Intra}), and the component of variance for measurements (σ^2_{Anal}), and the overall mean value ($\bar{x}$) for five canine clinical parameters

Parameter	σ^2_{Intra}	σ^2_{Anal}	$\bar{x}$	d_k	
BUN	10.08	0.486	16.90	9.01 mg/dl	3.22 mmol/L
Creatinine	0.019	0.016	0.78	0.52 mg/dl	46.07 μmol/L
Total protein	0.099	0.028	5.78	0.99 g/dl	9.87 g/L
Albumin	0.009	0.011	2.81	0.39 g/dl	3.90 g/L
Glucose	50.625	4.794	83.87	20.64 mg/dl	1.14 mmol/L

Discussion

The present study is concerned with the critical difference for 5 clinicochemical parameters calculated from the intraindividual variance and the analytical variance of the parameters. This was to be expected, because only 95% of the values from the general population were included in determination of the reference interval. There is one report[3] on the d_k value for canine clinical-chemical parameters. Jensen and Aaes[3] collected blood samples from 20 apparently clinically healthy dogs once weekly for five consecutive weeks and measured concentrations by the Cobas Fara (Roche) centrifugal analyzer. The d_k values for canine BUN, serum creatinine, total protein, albumin, and glucose calculated in the Jensen and Aaes's report were 2.36 mmol/L, 35.0 μmol/L, 6.3 g/L, 2.8 g/L, and 1.49 mmol/L respectively and those of the present study were 3.22 mmol/L (9.01 mg/dl), 46.07 μmol/L (0.52 mg/dl), 9.87 g/L (0.99 g/dl), 3.90 g/L (0.39 g/dl) and 1.14 mmol/L (20.64 mg/dl) respectively. Except for glucose, estimates of average intra-individual variation were seemed to be a little bit larger than those of previously documented[3]; this is probably due to the elongation of experimental period. However, as illustrated in Fig 1-5, the variation around the mean value for each animal may be smaller than the dispersion of the reference interval. It is therefore possible that a single animal with a disease affecting a given parameter could have an analytical result outside its own reference interval, but within the corresponding population-based reference interval at the time of collecting the blood sample. If two consecutive results differ by less than the critical difference value, it can be concluded that the difference is probably due to physiological variation. However, when the difference is greater than the critical value, other factors, either related to progression of the disease or the presence of concurrent disease, are more likely to be involved.

Further, it has not yet been established whether the biological variation is of the same magnitude in health and disease. Therefore it could be argued that the critical differences calculated in this study are not directly applicable to diseased dogs as the calculations are based on apparently clinically healthy dogs. But, in human medicine, it has been reported that in non-acute pathological processes, where new homeostatic steady states are reached, biological variation around the new mean values are of the same magnitude as in healthy individuals[2,5]. Whether this also applies to animals has yet to be studied.

In conclusion, the critical differences calculated in the present study may be used as guidelines to evaluate the difference between two consecutive analytical results. However, analytical results should not be assessed by the critical differences alone, but should also be compared with the corresponding reference intervals. For practical purposes, decision-making criteria can be derived from the estimated biological component of intra-individual variation and the analytical variation established for each laboratory.

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개 혈청화학검사항목의 장기간의 개체 내 변이와 Critical Difference

최은화 · 신일섭 · 방동하 · 김유석 · 황철용 · 윤화영[1] · 이창우

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요 약: 이 연구의 목적은 BUN 및 혈청 creatinine, total protein, albumin과 glucose의 장기간에 걸친 개체 내 변이를 산정하여, 각각의 critical difference를 구하는 것이다. 임상적으로 건강한 20마리의 개에서 일주일에 한번씩 총 8주간 채혈하여, 장기간에 걸친 개체 내 변이를 구하고, critical difference를 산출하였다. BUN은 9.01 mg/dl, 혈청 creatinine은 0.52 mg/dl, total protein은 0.99 g/dl, albumin은 0.39 g/dl, glucose는 20.64 mg/dl로 critical difference가 산출되었다. 동일한 개에서 연속적으로 측정된 검사항목 치가 critical value보다 차이가 적으면, 이 차이는 생리적인 변화에 기인한다고 할 수 있으며, critical value보다 차이가 크면, 이는 다른 요인, 즉 질병의 진행이나 다른 동반된 질병의 존재를 의미한다고 할 수 있다.

주요어 : critical difference, 개, 개체 내 변이, 혈청화학검사 종목.