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Biological variation and reference change values of feline plasma biochemistry analytes

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Abstract

This is the first report concerning biological variation and reference change values of feline plasma biochemistry components in peer reviewed literature. Biological variation refers to inherent physiological variation of analytes. The ratio of individual biological variation to group biological variation is referred to as an analyte's index of individuality; this index determines the suitability of an analyte to be assessed in relation to population-based or subject-based reference intervals.

A subject-based reference interval is referred to as a reference change value or critical difference and is calculated from individual biological variation. Fourteen cats were sampled for plasma biochemistry analysis once weekly for six weeks. Samples were stored and then tested at the same time. Results were assessed in duplicate and coefficients of variation for each analyte were isolated to distinguish variation within each subject, between all subjects and by the analyzer. From these results, an index of individuality and reference change values were determined for each analyte. Five plasma biochemistry analytes (ALP, ALT, cholesterol, creatinine, and globulin) had high individuality and therefore subject-based reference intervals are more appropriate; only one analyte (sodium) had low individuality indicating population-based reference intervals are appropriate. Most analytes had intermediate individuality so population-based reference intervals should be assessed in relation to subject-based reference intervals. The results of this study demonstrate high individuality for most analytes and therefore, that population-based reference intervals are of limited utility for most biochemical analytes in cats.

Introduction

Currently, veterinary clinical pathology results are assessed in relation to population-based reference intervals.[1] However, it is considered more appropriate to use 'subject-based' reference values to assess analytes that have a high degree of inter-individual variation because many unhealthy individuals may have values that significantly differ from their regular analyte determination, but fall within population-based reference intervals.[2-4]

Determination of 'subject-based' reference values requires knowledge of inherent physiological variation of analytes which is referred to as biological variation.[5]

Suitability of an analyte to be assessed in relation to population-based or subject-based reference intervals is determined by that analyte's index of individuality, a ratio of individual biological variation to group biological variation. A subject-based reference interval is referred to as a reference change value (RCV) or critical difference and is calculated from individual biological variation; it is used to determine if two consecutive results from an individual reach significance.[5-6]

Biological variation data are also vital to objectively assess imprecision,[7-8] accuracy and

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total error[9] of laboratory equipment[10] since it is not possible to recognize the acceptable degree of variation occurring within equipment without knowing how much normal variation occurs within an individual for that analyte.

Although biological variation data have been generated for several blood components in dogs,[11-13] cows[14] and budgerigars,[15] there are no biological variation data for any feline blood component published in peer-reviewed literature.

The objectives of this study were to determine biological variation data of feline plasma biochemistry analytes and calculate indices of individuality and RCVs as well as demonstrate the relevance of RCVs to feline medicine.

Materials and methods

Subjects

This prospective study was performed on client-owned cats maintained in their normal environment and being fed their regular diets. All cats were determined to be clinically healthy on the basis on physical examination and their owners' weekly reports. Physical examination included determination of body weight, hydration status, heart rate and nature, and abdominal palpation. The study was approved by The University of Sydney Animal Ethics Committee.

Sampling

Blood was collected from 14 cats weekly, for 6 weeks. Sampling occurred after an overnight fast of at least 10 hours, and all samples were collected between 8:30 to 10:30 am, with cats sampled in a consistent order so that sampling occurred at approximately the same time of day for each cat.

Samples were collected by a single operator (RMB) from each cat by jugular venipuncture. Samples were collected using 23-ga needles and 3mL syringes and immediately transferred to lithium heparin plasma collection tubes (BD Vacutainer, Becton, Dickinson and Company, NJ, USA). Each tube was centrifuged at 4000rpm (1790 x G) for 5 minutes within 10 minutes of collection before immediately decanting the plasma into plain (no additive) collection tubes (BD Microtainer, Becton, Dickinson and Company, NJ, USA). Plasma samples were stored at -20˚C for up to 6 weeks before thawing and analysis at a commercial veterinary laboratory (Gribbles, Bella Vista, NSW, Australia).

Laboratory methods

The following 20 biochemistry analytes were analyzed in duplicate: albumin, alkaline phosphatase (ALP), alanine aminotransferase (ALT), aspartate transaminase (AST), bicarbonate, calcium, chloride, cholesterol, creatinine, creatine kinase, gamma-glutamyl transferase (GGT), globulin, glucose, magnesium, phosphorus, potassium, sodium, total bilirubin, total protein and urea. All analytes were assessed using an Advia 1800 Clinical Chemistry System (Siemens Healthcare, Frankfurt am Main, Germany) on a single day by a single operator using single lots of reagents and calibrators for each analyte. The chemistry methods used by the Advia 1800 analyzer for these analytes are provided in Table 1.

Assessments and statistical analyses

Outliers were assessed for each analyte using Tukey's outlier identification method using Reference Value Advisor (Ecole Nationale Vétérinaire, Toulouse, France).[16] Outliers were

Table 1 Chemistry methods used by the Advia 1800 analyser for biochemical analytes

Analyte	Method
Albumin	Bromcresol green dye
Alkaline Phosphatase	Modified IFCC,no P5P, 25˚C
Alanine aminotransferase	Modified IFCC,no P5P, 25˚C
Aspartate aminotransferase	Modified IFCC,no P5P, 25˚C
Bicarbonate	Enzymatic
Calcium	Arsenazo III dye
Chloride	ISE- diluted
Cholesterol	Enzymatic
Creatinine	Enzymatic
Creatine Kinase	NAC- activated
Gamma- glutamyl transferase	Modified IFCC,no P5P, 25˚C
Globulin	Calculation (Total protein – albumin)
Glucose	Hexokinase- color
Magnesium	Xylidyl blue
Phosphorus	Phosphomolybdate- UV
Potassium	ISE-diluted
Sodium	ISE-diluted
Total bilirubin	Vanadate oxidation
Total protein	Biuret
Urea	Urease with GLDH, UV

IFFC: International Federation of Clinical Chemistry and Laboratory Medicine reference method, P5P: Pyridoxal-5'-phosphate , ISE: ion selective electrode, NAC: N-acetyl cystine, GLDH: Glutamate dehydrogenase, UV: ultra violet

assessed for each analyte on three levels: across the entire group of subjects, for each subject individually and for individual subjects with outlying variability compared to the other subjects in the group.

Most outliers detected appeared to result from analytical or pre-analytical variation since these samples demonstrated considerable variation between duplicate samples from the same patient from the same day. In these cases, both duplicates were excluded. Any outliers that had a similar result for both duplicates from that subject for that day were retained, as the variation was assumed to be normal physiological variation as all cats maintained good health through the study duration.

Restricted maximum likelihood (REML) was used to estimate variance components (including means and standard deviations) by specifying analytes as outcome variables, subject identification and day (nested within subject identification) as random effects using Genstat version 14 (VSN International, Hemel Hempstead, UK). Inter-individual or group coefficient of variation (CV_G), intra-individual coefficient of variation (CV_I) and the coefficient of variation occurring between duplicates, or analytical variation (CV_A) were then calculated from the variance components. Assumption of normality of residuals for REML was evaluated by visual inspection of histograms and normal plots of residuals.

For variables where the assumption of normality was valid, indices of individuality were calculated from coefficients of variation (CVs) by the 'reciprocal formula' with CV_G as the numerator (inverse index of individuality), i.e. $CV_G / \sqrt{(CV_A^2 + CV_I^2)}$ which results in higher values for increased intra-individual variation.[5-6, 13] With the traditional use of CV_G as the denominator, indices of individuality less than or equal to 0.6 indicate that subject-based reference values are more appropriate (and population-based reference intervals are of limited utility) and indices greater than or equal to 1.4 indicate population-based reference intervals

Table 2: Biological variation for each analyte expressed as coefficients of variation (CV) for between cats, or group variation (CV_G), and for within cats, or individual variation (CV_I), within run variation of the duplicate samples or analytical variation (CV_A) as well as the Index of Individuality (calculated as the more intuitive 'reciprocal formula'). Bolded analytes and indices of individuality indicate high individuality so subject based reference intervals are more appropriate.

Analyte	Mean (Range)	Between Cat CV (CV_G) (%)	Within Cat CV (CV_I) (%)	Analytical CV (CV_A) (%)	Inverse Index of individuality
Albumin (g/L)	37.22 (32-41)	3.88	3	1.97	1.08
Alkaline phosphatase(U/L)	28.17 (12-61)	33.7	12.5	4.64	**2.54**
Alanine aminotransferase(U/L)	37.86 (23-100)	23	13.2	3.81	**1.67**
Aspartate aminotransferase (U/L)	23.56 (16-46)	15	14.7	3.73	0.99
Bicarbonate (mmol/L)	16.01 (12-22)	9.57	5.4	5.19	1.28
Calcium (mmol/L)	2.38 (2.15-2.57)	2.51	2.34	1.43	0.92
Chloride (mmol/L)	120.68 (115-128)	1.16	1.17	0.97	0.76
Cholesterol (mmol/L)	4.31 (2.6-7.1)	22.4	10.7	3.37	**1.98**
Creatinine (umol/L)	115.72 (87-146)	11.4	5.97	1.92	**1.82**
Creatine Kinase (U/L)	141.25 (57-586)	34	31	3.14	1.09
Gamma-glutamyl transferase(U/L)	0.00 (0-0)	0	0	0	na
Globulin (g/L)	41.36 (31-55)	11.3	4.13	2.01	**2.46**
Glucose (mmol/L)	5.49 (4.5-8.0)	8.06	6.76	2.58	1.11
Magnesium (mmol/L)	0.94 (0.8-1.1)	4	3.71	4.03	0.73
Phosphorus (mmol/L)	1.31 (0.92-1.73)	11.8	8.49	1.64	1.36
Potassium (mmol/L)	3.96 (3.5-4.5)	4.91	3.63	1.77	1.22
Sodium (mmol/L)	153.38 (147-161)	0.57	0.86	0.92	0.45
Total bilirubin (mmol/L)	0.55 (0-1)	94.3	87.5	39.84	0.98
Total protein (g/L)	78.57 (67-90)	14	8.31	5.36	1.41
Urea (mmol/L)	8.98 (5.7-13.6)	15.5	10.4	1.9	1.47

are more appropriate. With the more intuitive inverse formula (CV_G as the numerator) indices of individuality greater than or equal to 1.67 indicate that subject-based reference values are more appropriate and indices less than or equal to 0.7 indicate population-based reference intervals are more appropriate.

RCVs were calculated to a 95% probability of significance in percentage terms according to: $RCV = Z. \sqrt{2} . \sqrt{(CV_A^2 + CV_I^2)}$. This means that any changes greater than this amount for two consecutive samples from an individual have a 95% probability of being significant. One-sided (Z = 1.65) or two-sided (Z = 1.96) results were calculated depending on whether

the analyte is likely to require interpretation when concentrations are either high, or low (one-sided) or for both high or low concentrations (two-sided).

For those analytes where results were not normally distributed, data were log transformed (to base e) and then assessed in the same way as for the raw data, using residual diagnostics. CVs were then back transformed by $CV = \sqrt{(\exp \sigma^2 - 1)}$ as previously described.[17-18] The RCV was calculated using the lognormal approach described by Fokkema *et al.*[17] Briefly, the 'lognormal' standard deviation was calculated from the untransformed CV such that $\sigma = \sqrt{\log (CV^2 + 1)}$ for each of CV_I and CV_A; then RCV was calculated as $\exp(+Z. \sqrt{[2. (\sigma_I^2 + \sigma_A^2)]}$ for increasing values and as $\exp(-Z. \sqrt{[2. (\sigma_I^2 + \sigma_A^2)]}$ for decreasing values; where Z=1.65 when 1-sided analysis is appropriate (interpretation of results only concerned with increased or decreased results) and Z=1.96 for 2-sided analysis (both increased and decreased results of significance) and accordingly these RCVs are not symmetrical.

The number of results with $CV_A < ½ CV_I$ were assessed as an indicator of adequate precision.[19] The number of results with $CV_A < ½. \sqrt{(CV_I^2 + CV_G^2)}$ were assessed as an indicator of whether analytical variation was sufficient to affect judgment of biological variation, individuality, and therefore, RCVs.[19-20]

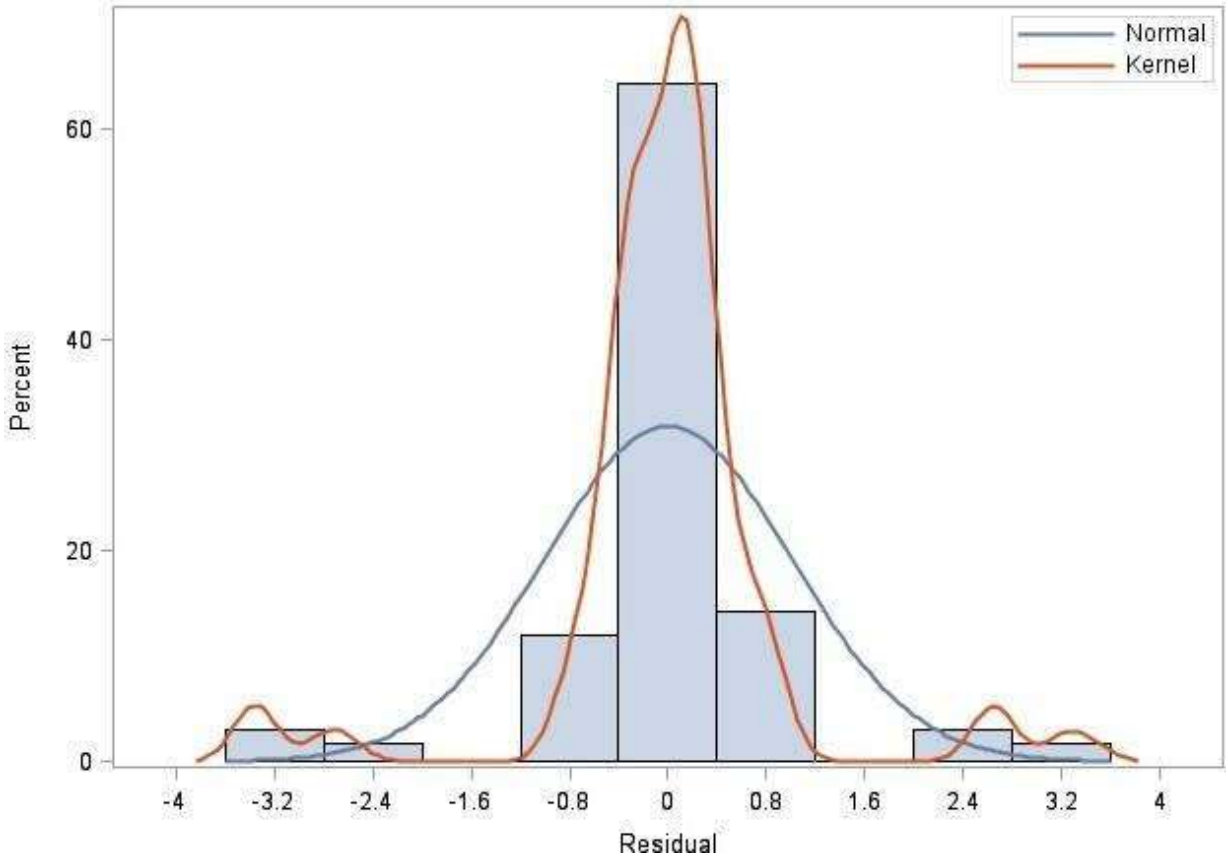

Figure 1 Residual plot for log transformed results (to base e) for total bilirubin. The y-axis represents the percentage of results, the x-axis represents the standardized residuals of the log transformed results. The normal trace represents the standard normal distribution curve for comparisons. The kernel trace represents a smoothed plot that approximates the probability density of the residuals. Although the curve appears to be normally bell shaped, note that over 60% of results are at the mean and this creates central peakedness (kurtosis) of the bell curve which indicates that this set of results approaches normality but is not entirely normal.

Results

Cats were aged 5 to 17 years (median: 10 years; mean: 10 years 5 months); three were neutered males, 11 were neutered females. All cats were domestic short-hairs.

Of the 84 sets of duplicate results, the following analytes had exclusions due to outliers: potassium (1 exclusion), urea (1), creatinine (2), albumin (3), globulin (3), total protein (3), phosphorus (7), calcium (9), chloride (9) and sodium (9). Three cats had exclusions from one week's testing, one cat had exclusions on two weeks, and one cat had exclusions on three weeks. Exclusions were from weeks 2 (affecting three cats), 3 and 4 (affecting two cats on each of these weeks) and weeks 1 and 5 (affecting one cat). A further 35 sets of results across 10 analytes were determined to be outliers (across all three levels assessed) but as the duplicate results were similar, these results were considered to be physiological variation and maintained.

Values for CV_G, CV_I and CV_A were determined for all analytes, and from these, indices of individuality were calculated (Table 2). All results for GGT (from all cats, all weeks and both replicates) were reported as <1 U/L and were tabulated as 0 U/L meaning that the index of individuality could not be calculated.

Analytical variation was less than half individual variation ($CV_A < ½ CV_I$) for twelve analytes (ALP, ALT, AST, cholesterol, creatinine, creatine kinase, globulin, glucose, phosphorus, potassium, total bilirubin and urea), indicating adequate precision for these analytes. Analytical variation (CV_A) was less than $½\sqrt{(CV_I^2 + CV_G^2)}$ for all analytes except chloride, magnesium, sodium. For these three analytes, analytical variation may have been great enough to affect judgment of biological variation, individuality, and therefore, RCV. For GGT all variation, including analytical variation, was zero.

Using the inverse index of individuality formula the highest individuality was recognized for ALP (2.54) and the lowest for sodium (0.45). Five analytes (ALP, ALT, cholesterol, creatinine and globulin) had an index of individuality ≥ 1.67, indicating that use of subject-based reference values (RCV) is appropriate, and only sodium had an index of individuality ≤ 0.7, indicating that the use of population-based reference intervals is appropriate.

Normal distributions of residuals were found for 15/19 analytes (GGT not applicable), the exceptions were ALT, creatinine kinase, glucose, total bilirubin. Three of these had normal distributions from log transformed values, the exception was total bilirubin. For total bilirubin, all raw results were either 0 or 1 U/L. In order to enable log transformation 0.1 U/L was added to all total bilirubin results and the distribution of the log transformed results approached normality but showed excess kurtosis (see Figure 1). For all of these analytes CVs were back transformed from the log transformed values and RCV calculated by the lognormal approach and therefore had differing values for increasing or decreasing values.[17]

RCVs for all analytes are presented in Table 3. RCVs varied from 0% (i.e. any change is significant) for GGT, to greater than 360% for increases of creatine kinase and total bilirubin (that is, serial samples must show a 3.6 fold increase to be significant). Important RCVs relevant for feline medicine include ALP with 31%, creatinine with 14.6%, and potassium with 11.2%.

Table 3 Reference change values for feline biochemistry analytes.

Analyte	Reference Change Value (%)		
	Decrease	Both	Increase
Albumin (g/L)		9.95	
Alkaline phosphatase(U/L)*		30.99	
Alanine aminotransferase(U/L)*	34.50		52.68
Aspartate aminotransferase (U/L)*		35.27	
Bicarbonate (mmol/L)		20.76	
Calcium (mmol/L)		7.59	
Chloride (mmol/L)		4.21	
Cholesterol (mmol/L)		31.21	
Creatinine (umol/L)		17.39	
Creatine Kinase (U/L)*	34.06		361.45
Gamma-glutamyl transferase (U/L)*		0.00	
Globulin (g/L)		12.74	
Glucose (mmol/L)	16.94		20.40
Magnesium (mmol/L)		15.17	
Phosphorus (mmol/L)		23.97	
Potassium (mmol/L)		11.20	
Sodium (mmol/L)		3.50	
Total bilirubin (mmol/L)*	78.43		363.63
Total protein (g/L)		27.41	
Urea (mmol/L)		29.39	

Those analytes with different values noted for increasing and decreasing concentrations had RCV calculated by the lognormal technique since the results did not have a normal distribution.

* One-sided values calculated (Z=1.65) since change at one of the end of the reference interval (high or low) is important clinically. All others are calculated as 2-sided (Z=1.96) since both ends of the reference interval are of clinical interest.

Discussion

This is the first report concerning biological variation of feline plasma biochemistry analytes in peer reviewed literature. The results demonstrate high individuality for most analytes and therefore, that population-based reference intervals are of limited utility for these analytes.

Using the criteria described by Fraser and Harris[6] and recently reviewed in the veterinary literature,[5] an index of individuality $\leq$0.6 indicates that subject-based reference values are more appropriate to use; when the index of individuality $\geq$1.4, population-based reference intervals are more appropriate and when between 0.6 and 1.4, population-based ranges should be used with caution. The semantic difficulty that low values indicate high analyte individuality can be overcome by using the inverse formula such that an inverse index of individuality $\geq$1.67 indicates that subject-based reference values are more appropriate to use; when inverse index of individuality $\leq$0.7, population-based reference intervals are more appropriate and when between 0.7 and 1.67, population-based ranges should be used with caution.[6, 13]

This study found five analytes with inverse index of individuality $\geq$1.67: ALP, ALT, cholesterol, creatinine and globulin; one analyte with inverse index of individuality $\leq$0.7 (sodium); and thirteen analytes with inverse index of individuality between 0.7 and 1.67. Both the indices of individuality for GGT could not be calculated as the CVs were zero (since

all results were the same) (see Table 2). This means that population-based reference intervals alone are only appropriate to use for sodium.

Interpreting clinical biochemistry results with RCVs requires a comparison to prior clinical biochemistry results from the same subject. For example, a cat may have previously had a creatinine concentration of a 120µmol/L; an increase to 145µmol/L (an increase of 21%) in a subsequent sample represents an increase greater than the reference change value of 17.4% (Table 3), above which, there is a 95% probability that the change is significant, there and thus is an indicator of azotaemia even if 145µmol/L is within a correctly determined population-based reference interval of 70-160µmol/L.

For those analytes with intermediate indices of individuality between 0.7 and 1.67, interpretations are made similarly, but with reference to the population-based reference interval as well. For example, a cat may have a urea concentration of 11.3mmol/L that is higher than the reference interval of 5.5-10.5mmol/L. If prior testing had noted a concentration of 9.4mmol/L, then 11.3mmol/L is only an increase of approximately 20%, within the reference change value of 29.4% and so may not indicate azotaemia (although it would be prudent to monitor further to see if the increase is part of a upwards trend).

Previous biological variation studies have used analysis of variance (ANOVA) techniques[11-14, 21-23] whereas REML was used to estimate variance components in this study. REML takes a similar approach as ANOVA, but yields variance components directly, whereas they have to be calculated from the output in ANOVA. The estimated variance components are identical for simple models with balanced designs but REML can also be used for non-balanced designs.[24] REML has become the standard method for estimating variance components.[25]

Biological variation data, including CVs and RCVs, as described by Cotlove[19] and Fraser and Harris[6] depend on normally distributed data and equality of variance since normal distributions provide predictability of percentage of results above and below set points. When a dataset is not normally distributed, routine calculations result in significant errors such as implausible decreases of 100% or more (which can occur when standard calculations are applied when CV_I exceeds 33.3%).[26] Due to non-normally distributed data for ALT, CK, glucose and total bilirubin lognormal approaches were used for these analytes in this study.[17-18, 27] Reasons for results not being normally distributed can vary but box and whiskers plots of the results from all cats for ALT (Figure 2) illustrate that cat 10 had results notably different from the other thirteen cats which resulted in the data not being normally distributed. Although this cat had ALT concentrations above the reference interval limits on two consecutive occasions, on neither occasion was the concentration greater than the reference change value of 52.68% (or 43.00% when calculated with this cat omitted) thus demonstrating that this increase above the reference interval likely did not indicate pathology.

The log transformed dataset for total bilirubin was not entirely normal, displaying excess kurtosis or central peakedness (Figure 1). This means that CVs and RCVs for this analyte must be assessed with caution, however, the results in Table 3 make intuitive clinical sense in that increases must be large (in the order of four-fold) to be clinically relevant. Future work will be required to refine these values. Along similar lines, conclusions can be made about GGT even though calculations for index of individuality could not be performed since all results were the same. Since no variation from 0 U/L (reported as <1 U/L) was found across

fourteen cats, tested weekly on six occasions and tested in duplicate (resulting in 168 analyses), any increase can be considered significant.

As first described by Cotlove for people,[19] the largest biological variability, both intra-individual (9 to 12%) and inter-individual (15 to 25%), were associated for serum constituents that are end-products of catabolism such as urea (CV_I=10.43% and CV_G=15.54% in this study) or are released from tissues such as the liver enzymes (CV_I's 12-15% and CV_G's =15-34% in this study). Physiological reasons may also explain the large variation for creatine kinase (CV_I=30.96% and CV_G=34.02%) as a cat may have sustained some tissue damage from minor trauma that resulted in no outward clinical signs. Outliers were recognized for creatine kinase (on two of the three levels assessed) but included in analyses as both duplicates were similar in each case.

Cotlove also reported [19] that the lowest variation occurred for analytes with strict homeostatic regulation of the stability of the composition and volume of extracellular and intravascular fluids as well as total calcium which has complex regulatory mechanisms. Accordingly these

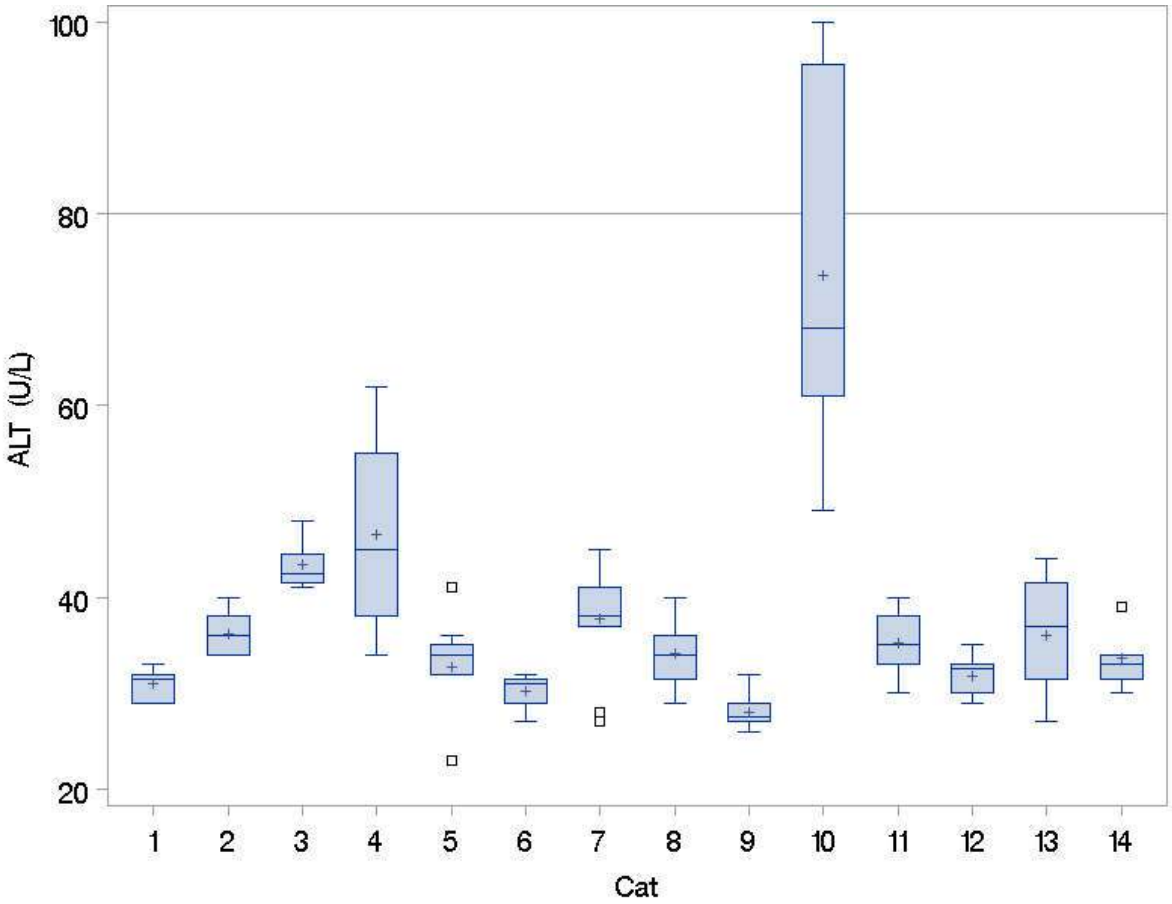

Figure 2 Box and whiskers plots of ALT concentrations (U/L) from all cats. The box is made by the first quartile (Q1), the median and the third quartile (Q3). The whiskers go up to the smallest and the largest observations within 1.5*IQR below and above the Q1 and Q3, respectively. Any observation outside these limits is displayed as an outlier. The + symbol indicates mean. Note that cat 10 has results notably higher than the other thirteen cats. This resulted in a deviation from normality when assessing results of all the cats. On two consecutive occasions this cat's ALT concentrations were above the laboratory reference interval of < 80 U/L but were less than the RCV of 52.68%, demonstrating the importance of subject based reference intervals for analytes with an appropriate index of individuality.

analytes had the smallest CV_I's: sodium (0.86%), chloride (1.17%), calcium (2.34%) and albumin (3.00%) and had little difference between CV_I and CV_G (see Table 2).

Analytes with low variation are faced with the problem that the 'noise' of analytical deviation may often mask their true biological variability, both within an individual and among individuals[19-20] as when CV_A approaches CV_I, there is no certainty whether any variation is intra-subject or due to the limitations of the analytical equipment.

Cotlove defined tolerable imprecision as $CV_A \leq 0.5.\ CV_I$[19] and this standard was not achieved for seven analytes in the current study (albumin, bicarbonate, calcium, chloride, magnesium, sodium and total protein). It is recognized that desirable performance standards are not attainable for all analytes with current technology and methodology, and in these circumstances, minimum quality imprecision is assessed as $CV_A \leq 0.75.\ CV_I$.[20, 28] Albumin, calcium and total protein reached this minimum standard but the tightly controlled electrolytes (bicarbonate, chloride, magnesium and sodium) did not. Artifactual increases of biological variation (CV_I) due to analytic variation are minimal when $CV_A < ½.\ \sqrt{(\ CV_I{}^2 + CV_G{}^2\ )}$[19] and this standard was achieved for all analytes except chloride, magnesium and sodium. A further difficulty in assessing true CV_A is made by the reporting of results as whole integers. For example, duplicate results for chloride reported as 115mmol/L and 116mmol/L represent analytical variation of 0.87% whereas if the true concentrations were 115.4mmol/L and 115.6mmol/L, the analytical variation would be 0.17%.

Fraser and Harris noted that: *"A single extraordinary observation, resulting from an analytical blunder in the assay, or a misidentification of the specimen, can exert a profound effect on summary statistics, especially variances. A distinction should be made between an aberrant observation, due to a mistake or accident in the analytical procedure, and an outlier. In some cases, the outlier is known to be aberrant, but more often no explanation can be found for an unusual value."*[6] The decision to exclude outliers from the final statistical analyses was not taken lightly and exclusions were made when recognized outliers were from duplicate samples (both results were excluded). For example, a duplicate for sodium with results of 145mmol/L and 170mmol/L was excluded. No reason was found for such dramatic variations that occurred in multiple cats, across multiple weeks. Possibilities such as inadequate centrifugation, aspiration of red blood cells when decanting plasma, effect of freezing and thawing samples or imprecision of analyzer all seem unlikely because the varied results were across multiple cats and multiple weeks and no other indicators of possible operating error, such as low glucose concentrations if red blood cells were aspirated, were detected.

Ideally, RCV should be calculated using the CV_A for the instrument upon which the specimens are analyzed. CV_A may differ amongst individual instruments of the same or different manufacturers and ages.[29] However, the calculations provided in the 'Materials and Methods' are easily applied and could be instituted for any analyzer, either at a commercial laboratory or in-house, using the information regarding biological variation from this study and the imprecision of the analyzer (CV_A) used for specimen analyses.

Conclusions

This study represents the first assessment of biological variation for feline blood components within the peer-reviewed literature. Results of this study should be interpreted cautiously for analytes with low CV_I and CV_G (particularly chloride, magnesium and sodium) since CV_A provides analytical 'noise' to the clinical 'signal'.[19-20] The high individuality of most analytes indicates that subject-based reference values should be used to assess feline plasma biochemistry samples. Biological variation studies such as this one and another recently published for dogs[13] provide strong evidence for serial sampling and reporting of RCVs values in small animal practice.

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Conflict of interest statement The authors declare that there is no conflict of interest.

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