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Indirect derivation of biological variation data and analytical performance specifications for therapeutic drug monitoring activities

Jia Hui Chai[1], Robert Flatman[2], Benjamin Teis[2], Sunil Kumar Sethi[1], Tony Badrick[3], Tze Ping Loh[1,4]

[1]*Department of Laboratory Medicine, National University Hospital, Singapore;* [2]*Sullivan Nicolaides Pathology, Brisbane, Qld, Australia;* [3]*Royal College of Pathologists of Australasia Quality Assurance Programs, Sydney, NSW, Australia;* [4]*Biomedical Institution for Global Health and Technology, National University of Singapore, Singapore*

Summary

We applied the indirect approach using anonymised data from an Australian and a Singapore laboratory to derive biological variation data for a group of 10 therapeutic drugs routinely monitored. A series of inclusion and exclusion criteria were applied on the data. The within- (CVi) and between-individual (CVg) biological variation data were then derived as previously described. The corresponding index of individuality and analytical performance specifications were also calculated.

The biological variation data were overall very similar between the two study sites. Moreover, the biological variation data were also comparable between males and females, as well as whether the data originated from patients who only had two episodes of measurement during the study period or from the last two results from patients who had more than two episodes of measurement during the study period.

The results presented in this study contribute towards the biological variation data for therapeutic drugs, which can be used to inform discussions about the setting of harmonised analytical performance specifications for these measurands.

Key words: Therapeutic drug monitoring; biological variation; analytical performance specification; data mining; indirect approach.

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INTRODUCTION

After administration, a medication (an exogenous substance) needs to stay within a range of plasma concentrations to be efficacious.[1] This is known as the therapeutic range. When the plasma concentration of a medication exceeds the therapeutic range and reaches a toxic range, the patient is at risk of harm from the adverse side effects. Below the therapeutic range, a medication may not be effective at treating the illness. Therapeutic drug monitoring is the measurement of plasma concentration of medication and comparing it to the therapeutic range to guide dose titration decisions. This activity is particularly important for medications with narrow therapeutic windows and those with severe adverse side effects.

Biological variation data is important in several aspects of laboratory medicine practice. It can be used to determine the analytical performance specification for bias and imprecision for a measurand according to the latest Milan consensus on analytical quality.[2] It can also be used together with analytical imprecision to define the reference change value, which is the smallest difference between sequential laboratory results that is considered significant.

However, the derivation of biological variation data for therapeutic drugs in healthy individuals is inherently difficult. It requires administration of medication in volunteers until it reaches steady state concentration within the therapeutic range, before sampling the subject multiple times over several days. This poses significant ethical and operation challenges. Moreover, the assumption that biological variation data obtained from health subjects can be applied to disease state is challenged by (1) health subjects do not consume these medications, and (2) subjects with disease may have comorbidities (e.g., renal and liver diseases), which may affect pharmacokinetics. By contrast, biological variation data can be derived indirectly from existing clinical laboratory data.[3–5] These data optimise the use of existing laboratory databases and produce values that are more representative of patients found in routine practice. Here, we applied the indirect approach using data from an Australian laboratory and a Singapore laboratory to derive biological variation data and analytical quality specifications for a group of 10 therapeutic drugs routinely monitored.

MATERIALS AND METHODS

The National University Hospital, Singapore is a tertiary-care institution with 1100-bed capacity. It encompasses all clinical disciplines, catering from neonates to geriatrics and palliative care patients. On the other hand, Sullivan Nicolaides Pathology is a medical testing laboratory based in Bowen Hills, Brisbane, Queensland. It serves a mixture of community and hospitalised patients and analyses over 25,000 episodes a day. It serves all disciplines of pathology.

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Table 1 Percentage analytical coefficient of variation (%CVa) of the assays derived from at least 12 months of running internal quality control results at mean concentration closest to the study cohort means

Measurand	Unit	SNP			NUH		
		Assay	Mean concentration	%CVa	Assay	Mean concentration	%CVa
Carbamazepine	mg/L	Abbott Architect PETINA	2.89	2.51	Beckman Coulter AU5800	5.5	5.88
Cyclosporine	µg/L	Beckman AU480 Immunoassay	60.39	12.78	Abbott Architect i1000	88.86	12.39
Digoxin	µg/L	Abbott Architect PETINA	1.12	4.1	Beckman Coulter AU5800	1.65	6.18
Gentamicin	mg/L	Abbott Architect PETINA	1.51	6.27	Beckman Coulter AU5800	1.60	7.55
Lithium	mmol/L	Roche Integra 400 ISE	0.66	2.15	Beckman Coulter AU5800	0.80	3.89
Methotrexate	µmol/L	Abbott Architect Immunoassay	0.07	10.75	Abbott Architect i1000	0.07	6.50
Phenytoin	mg/L	Abbott Architect PETINA	11.76	3.03	Beckman Coulter AU5800	15.0	5.29
Tacrolimus	µg/L	Abbott Architect Immunoassay	4.42	5.9	Abbott Architect i1000	5.87	4.79
Valproate	mg/L	Abbott Architect PETINA	31.7	2.03	Beckman Coulter AU5800	28.0	3.34
Vancomycin	mg/L	Abbott Architect PETINA	7.51	2.82	Beckman Coulter AU5800	6.2	5.50

CVa, analytical precision; NUH, National University Hospital, Singapore; SNP, Sullivan Nicolaides Pathology, Brisbane, Australia.

The laboratory methods for the measurands are summarised in Table 1. For this study, an individual 18 years old and above was considered an adult.

Anonymised data for therapeutic drug monitoring were extracted from the laboratory information system of the respective laboratories, and analysed separately (Table 1) for the period between 8 March 2015 and 8 March 2017. Invalid results (i.e., unacceptable runs, insufficient samples, measurements outside of analytical measurement range) were first removed from the extracted laboratory data. Following this, several exclusion criteria were applied such that the remaining laboratory data are likely to represent steady state concentrations, as previously described.[3] These exclusion criteria were (1) laboratory results that fell outside of the therapeutic range, (2) patients with only a single episode of the measurand, and (3) differences of >75% between two consecutive measurand measurements.

After applying the above initial exclusion criteria, the remaining laboratory data were further subjected to several outlier exclusion criteria, as previously described.[4,5] Tukey's criteria, defined as any value lying below the 1st quartile value minus 3 times the interquartile range, or above the 3rd quartile value plus 3 times the inter-quartile range, were applied to individual laboratory results to identify outlier results, and then to the averaged laboratory results of an individual patient to identify outlying individuals. Finally, Cochran's criteria were used to identify abnormally large variance between consecutive laboratory results of the same patient. All outlying results, individuals and variance were excluded from further analysis.

The remaining laboratory data were then divided into those with only two laboratory results per patient, and those with more than two laboratory results per patient and analysed separately. For the latter group, only the last two consecutive laboratory results were included in the final analysis. The analytical precision (CVa) for the measurands was derived from at least 12 consecutive months of running internal quality control results (Table 1).

The within- (CVi) and between-individual (CVg) biological variation data were then derived as previously described.[4,5] The CVi is calculated by subtracting the total within-subject variation of the paired results with the CVa. On the other hand, CVg is calculated by $[(kr - 1)/k(r - 1)]\{CVT^2 - CVa^2 - [(N - 2)/(N - 1)]CVi^2\}]^{0.5}$, where k = number of specimens, r = number of subjects, N = total number of measurements, CVT^2 = the total variance of all N measurements. The corresponding index of individuality [calculated as the ratio of CV(i+a)/CVg] and analytical performance specifications [calculated as: desirable imprecision = 0.50 × CVi, desirable bias = $0.25 \times (CVi^2 + CVg^2)^{0.5}$, total allowable error = (1.65 × imprecision) + bias] were also derived. The statistical analysis was performed using Microsoft Excel 2010 (Microsoft, USA).

This study was exempted from our institutional ethics review board.

RESULTS

The total number of laboratory results extracted and those that were included in the final analysis after application of the exclusion criteria are summarised in Supplementary Tables 1 and 2 (Appendix A). The biological variation data that were derived for the male and female patients are summarised in Tables 2 and 3, respectively. Using the biological variation data from this study, the desirable analytical performance specifications were derived and are summarised in Table 4.

DISCUSSION

The setting of analytical performance specifications is an important aspect of the total quality management of a laboratory. It is used to determine the acceptance criteria for analytical performance. The latest Milan consensus proposes a hierarchy of such analytical performance specifications for the laboratory. They include those derived from outcome-based clinical studies, biological variation data and state of the art.

The use of biological variation data to derive analytical performance specifications provides a direct link between analytical performance and clinical observation. The desirable analytical specifications are set as a fraction of the biological variation to limit the amount of analytical variation (noise) contributing to the observed biological variation in clinical results. These specifications also limit the amount of shift in a population result distribution that may lead to significant misclassification of patients.[6]

Moreover, biological variation data are important for determining the critical difference, which is the minimum difference between sequential laboratory results before they are considered significantly different.[6,7] However, biological variation data are very challenging to obtain by direct patient sampling, as they require significant operational and financial resources and stringent ethics approval.

The biological variation in plasma concentration of therapeutic drugs depends on the dose and the pharmacokinetics (absorption, distribution, metabolism and excretion) of the drug administered to an individual. Changes in the drug dosage and pharmacokinetics produce variations in plasma drug concentration that constitute the biological variation of these exogenous substances. In the steady state, they reflect the biological variation of these exogeneous compounds.

Because of the above reasons, biological variation data for therapeutic drugs have traditionally been hard to derive. In the absence of such data, expert opinion and state of the art have been the pragmatic way used to set the analytical

Table 2 Within- (CVi) and between-individual biological variation data (CVg) and index of individuality (II) derived from male subjects with only two episodes (N=2) of measurement or the last two results from males with more than two episodes of measurements (last 2), who attended the Sullivan Nicolaides Pathology (SNP) and National University Hospital, Singapore (NUH)

Measurand, unit	Male, N=2										Male, last 2 subjects									
	Subjects		Mean concentration		CVi, %		CVg, %		II		Subjects		Mean concentration		CVi, %		CVg, %		II	
	SNP	NUH	SNP	NUH	SNP	NUH	SNP	NUH	SNP	NUH	SNP	NUH	SNP	NUH	SNP	NUH	SNP	NUH	SNP	NUH
Carbamazepine, mg/L	170	17	7.7	8.3	13.3	8.7	4.9	20.1	2.8	0.5	280	28	7.9	8.0	12.4	12.0	6.7	18.3	1.9	0.7
Cyclosporine, µg/L	32	9	135.1	142.9	12.4	10.1	16.6	18.3	1.1	0.9	126	39	136.1	140.7	11.5	11.7	16.0	17.3	1.1	1.0
Digoxin, µg/L	586	28	0.7	0.7	17.3	16.5	14.4	13.3	1.2	1.3	973	34	0.7	0.7	17.3	15.3	13.6	14.7	1.3	1.1
Gentamicin, mg/L	NA	37	NA	1.1	NA	25.1	NA	38.7	NA	0.7	NA	84	NA	1.2	NA	26.0	NA	32.5	NA	0.8
Lithium, mmol/L	363	10	0.6	0.6	12.8	4.7	9.9	12.8	1.3	0.5	969	21	0.6	0.6	13.1	15.3	10.0	8.5	1.3	1.9
Methotrexate, µmol/L	14	3	0.07	0.1	38.7	27.7	18.2	13.7	2.2	2.1	68	21	0.08	0.1	39.8	37.5	26.3	3.8	1.6	10.0
Phenytoin, mg/L	191	31	14.3	14.7	13.4	15.4	13.0	10.8	1.1	1.5	345	54	14.3	14.5	13.8	17.6	12.6	10.6	1.1	1.7
Tacrolimus, µg/L	30	111	5.6	5.5	17.7	27.6	30.9	24.5	0.6	1.1	349	520	5.6	5.4	19.6	21.3	27.3	25.2	0.8	0.9
Valproate, mg/L	472	77	67.7	72.8	15.2	14.3	11.9	9.7	1.3	1.7	857	126	68.3	73.2	14.8	13.8	12.2	11.5	1.2	1.4
Valproate, children, mg/L	25	28	68.8	73.7	17.6	12.7	11.8	9.5	1.5	1.4	33	55	69.8	73.1	16.3	14.9	11.4	8.9	1.4	1.8
Vancomycin, mg/L	39	104	15.4	19.1	9.0	12.5	5.2	4.5	1.8	3.0	68	380	15.5	19.0	10.0	11.1	4.4	5.6	2.4	2.2

CVg, between-individual biological variation data; CVi, within-individual biological variation data; NA, not available.

Table 3 Within- (CVi) and between-individual biological variation data (CVg) and index of individuality (II) derived from male subjects with only two episodes (N=2) of measurement or the last two results from males with more than two episodes of measurements (last 2), who attended the Sullivan Nicolaides Pathology (SNP) and National University Hospital, Singapore (NUH)

Measurand, unit	Female, N=2										Female, last 2 subjects									
	Subjects		Mean concentration		CVi, %		CVg, %		II		Subjects		Mean concentration		CVi, %		CVg, %		II	
	SNP	NUH	SNP	NUH	SNP	NUH	SNP	NUH	SNP	NUH	SNP	NUH	SNP	NUH	SNP	NUH	SNP	NUH	SNP	NUH
Carbamazepine, mg/L	180	13	7.7	8.3	11.9	12.9	6.8	21.6	1.8	0.7	278	25	7.9	8.9	11.9	13.1	7.0	14.8	1.7	1.0
Cyclosporine, µg/L	22	4	143.3	128.1	6.9	14.3	16.6	NA	0.9	NA	93	28	138.6	143.8	9.6	15.4	19.3	11.9	0.8	1.7
Digoxin, µg/L	805	27	0.7	0.7	17.4	18.2	13.5	13.5	1.3	1.4	1387	33	0.7	0.7	17.3	19.5	13.2	10.9	1.3	1.9
Gentamicin, mg/L	NA	20	NA	1.1	NA	16.7	NA	46.8	NA	0.4	NA	50	NA	1.2	NA	25.2	NA	33.1	NA	0.8
Lithium, mmol/L	543	22	0.6	0.6	14.3	14.5	9.2	8.5	1.6	1.8	1441	33	0.6	0.6	13.8	13.7	9.2	9.1	1.5	1.6
Methotrexate, µmol/L	7	1	0.06	NA	40.1	NA	20.2	NA	2.1	NA	32	7	0.08	0.1	41.8	37.5	34.9	36.1	1.2	1.1
Phenytoin, mg/L	106	19	14.0	14.5	15.9	15.3	10.2	16.1	1.6	1.0	195	36	14.1	14.5	14.6	17.3	11.7	11.6	1.3	1.6
Tacrolimus, µg/L	53	73	5.8	5.4	17.0	24.3	32.7	30.7	0.6	0.8	256	420	5.7	5.5	19.3	20.7	29.6	23.2	0.7	0.9
Valproate, mg/L	469	58	67.2	73.7	14.5	11.4	13.1	10.5	1.1	1.3	788	96	67.6	74.0	14.3	12.5	12.6	9.8	1.1	1.5
Valproate, children, mg/L	25	20	73.7	71.5	14.6	19.4	8.2	NA	1.8	NA	33	50	73.5	71.9	13.9	16.2	8.9	8.5	1.6	2.0
Vancomycin, mg/L	40	56	15.0	19.2	9.4	12.5	5.9	4.5	1.7	3.0	72	217	15.1	19.2	8.7	11.8	6.2	5.0	1.5	2.6

CVg, between-individual biological variation data; CVi, within-individual biological variation data; NA, not available.

Table 4 Desirable analytical specifications derived from biological variation data from subjects with only two episodes (N=2) of measurement or the last two results from males with more than two episodes of measurements (last 2), who attended the Sullivan Nicolaides Pathology (SNP) and National University Hospital, Singapore (NUH)

Measurand, unit	Male, N=2						Male, last 2 subjects					
	Desirable precision, CV%		Desirable bias, %		Desirable total error, %		Desirable precision, CV%		Desirable bias, %		Desirable total error, %	
	SNP	NUH	SNP	NUH	SNP	NUH	SNP	NUH	SNP	NUH	SNP	NUH
Carbamazepine, mg/L	6.6	4.3	3.5	5.5	14.5	12.7	6.2	6.0	3.5	5.5	13.8	15.4
Cyclosporine, µg/L	6.2	5.1	5.2	5.2	15.4	13.6	5.7	5.8	4.9	5.2	14.4	14.9
Digoxin, µg/L	8.7	8.2	5.6	5.3	19.9	18.9	8.6	7.7	5.5	5.3	19.8	17.9
Gentamicin, mg/L	NA	12.6	NA	11.5	NA	32.3	NA	13.0	NA	10.4	NA	31.8
Lithium, mmol/L	6.4	2.4	4.0	3.4	14.6	7.3	6.6	7.6	4.1	4.4	14.9	17.0
Methotrexate, µmol/L	19.3	13.9	10.7	7.7	42.6	30.6	19.9	18.7	11.9	9.4	44.8	40.3
Phenytoin, mg/L	6.7	7.7	4.7	4.7	15.7	17.4	6.9	8.8	4.7	5.1	16.0	19.7
Tacrolimus, µg/L	8.8	13.8	8.9	9.2	23.5	32.0	9.8	10.7	8.4	8.2	24.6	25.8
Valproate, mg/L	7.6	7.1	4.8	4.3	17.4	16.1	7.4	6.9	4.8	4.5	17.0	15.9
Valproate, children, mg/L	8.8	6.4	5.3	4.0	19.8	14.5	8.1	7.5	5.0	4.3	18.4	16.7
Vancomycin, mg/L	4.5	6.2	2.6	3.3	10.1	13.6	5.0	5.5	2.7	3.1	11.0	12.2

Measurand, unit	Female, N=2					Female, last 2 subjects					
Carbamazepine, mg/L	6.0	6.4	3.4	6.3	13.3	16.9	5.9	6.5	3.4	4.9	13.2	15.7
Cyclosporine, µg/L	3.5	7.1	4.5	3.6	10.2	15.4	4.8	7.7	5.4	4.9	13.3	17.5
Digoxin, µg/L	8.7	9.1	5.5	5.7	19.8	20.7	8.6	9.7	5.4	5.6	19.7	21.6
Gentamicin, mg/L	NA	8.3	NA	12.4	NA	26.2	NA	12.6	NA	10.4	NA	31.2
Lithium, mmol/L	7.2	7.2	4.3	4.2	16.1	16.1	6.9	6.9	4.2	4.1	15.6	15.4
Methotrexate, µmol/L	20.0	NA	11.2	NA	44.3	NA	20.9	18.8	13.6	13.0	48.1	44.0
Phenytoin, mg/L	7.9	7.7	4.7	5.6	17.8	18.2	7.3	8.6	4.7	5.2	16.7	19.5
Tacrolimus, µg/L	8.5	12.2	9.2	9.8	23.3	29.8	9.7	10.4	8.8	7.8	24.8	24.9
Valproate, mg/L	7.3	5.7	4.9	3.9	16.9	13.2	7.1	6.3	4.8	4.0	16.6	14.3
Valproate, children, mg/L	7.3	9.7	4.2	NA	16.3	NA	6.9	8.1	4.1	4.6	15.6	18.0
Vancomycin, mg/L	4.7	6.2	2.8	3.3	10.5	13.6	4.4	5.9	2.7	3.2	9.9	13.0

NA, not available.

performance specifications for these tests. More recently, the indirect approach has been applied to some of these tests, generating useful information for laboratory practice.[3–5] Another method of obtaining analytical performance specifications for therapeutic drug monitoring measurands directly relates to the pharmacokinetic profiles of the drug of interest.[8]

In this study, biological variation data were derived from two laboratories serving different populations. Yet, the biological variation data were overall very similar between the two study sites. Moreover, the biological variation data were also comparable between males and females, as well as whether the data originated from patients who only had two episodes of measurement during the study period or from the last two results from patients who had more than two episodes of measurement during the study period. When compared to the biological variation data published previously,[3] the CVi and CVg were generally smaller in our study.

The results presented in this study contribute towards the biological variation data for therapeutic drugs. It is hoped that more laboratories can produce and publish local biological variation data, perhaps using alternate indirect approaches.[9] Biological variation studies are prone to confounding factors, particularly when using the indirect approach. The availability of data from other laboratories will allow systematic curation[10] to derive consensus values that approximate the intended population and identify sources of variation. This will in turn provide evidence and data for informed discussion for setting of harmonised analytical performance specifications for these measurands.

A major limitation of this study is the use of historical drug measurements performed in routine laboratories. Using this approach, variations in practice of drug dosing, timing of specimen collection for drug measurement, drug-drug interactions, pharmacokinetics/pharmacodynamics and other patient-related confounding factors (e.g., liver or renal function for drug metabolism and elimination) are not routinely captured by the laboratory information system and cannot be controlled or adjusted for in this study. These factors may affect the estimation of biological variation data.[8] In this study, we had sought to reduce the potential impact of these factors by using results that fall within the therapeutic range and excluding gross outlier results. Another limitation of this study is the assumption that CVa is constant throughout the therapeutic range in derivation of the biological variation data, which may not hold true for all assays. Nevertheless, the difference in CVa across the therapeutic range is likely to be smaller than the biological variation. Finally, for some measurands where the number of results included is small, the biological variation data estimated may be less reliable and should be interpreted with caution, since this indirect approach does not permit estimation of confidence intervals.[11]

In conclusion, this manuscript builds on the previous work by Pauwels and colleagues and provides biological variation and analytical performance specification data for additional therapeutic drugs, obtained from two laboratories serving different populations. It also provides early evidence suggesting comparability in the biological variation data derived from individuals with only two episodes of measurement and the last two results from patients with more than two episodes of measurements.

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APPENDIX A. SUPPLEMENTARY DATA

Supplementary data to this article can be found online at https://doi.org/10.1016/j.pathol.2018.12.418.

Address for correspondence: Tze Ping Loh, 5 Lower Kent Ridge Road, 119074, Singapore. E-mail: tploh@hotmail.com

References

1. Sayers J, Friedman M. How clinicians use therapeutic drug monitoring. *Lab Med* 1997; 28: 524–8.
2. Sandberg S, Fraser CG, Horvath AR, *et al*. Defining analytical performance specifications: consensus statement from the 1st strategic conference of the european federation of clinical chemistry and laboratory medicine. *Clin Chem Lab Med* 2015; 53: 833–5.
3. Pauwels S, Vermeersch P, Desmet K, *et al*. Practice-oriented quality specifications for therapeutic drug monitoring. *Clin Chem* 2014; 60: 559–60.
4. Loh TP, Metz MP. Indirect estimation of pediatric between-individual biological variation data for 22 common serum biochemistries. *Am J Clin Pathol* 2015; 143: 683–93.
5. Loh TP, Ranieri E, Metz MP. Derivation of pediatric within-individual biological variation by indirect sampling method: an LMS approach. *Am J Clin Pathol* 2014; 142: 657–63.
6. Fraser CG, Harris EK. Generation and application of data on biological variation in clinical chemistry. *Crit Rev Clin Lab Sci* 1989; 27: 409–37.
7. Ricós C, Perich C, Minchinela J, *et al*. Application of biological variation – a review. *Biochem Med* 2009; 19: 250–9.
8. Fraser CG. Desirable standards of performance for therapeutic drug monitoring. *Clin Chem* 1987; 33: 387–9.
9. Cembrowski G, Topping K, Versluys K, *et al*. The use of serial outpatient complete blood count (CBC) results to derive biologic variation: a new tool to gauge the acceptability of hematology testing. *Int J Lab Hematol* 2016; 38: 111–8.
10. Bartlett WA, Braga F, Carobene A, *et al*. A checklist for critical appraisal of studies of biological variation. *Clin Chem Lab Med* 2015; 53: 879–85.
11. Røraas T, Petersen PH, Sandberg S. Confidence intervals and power calculations for within-person biological variation: effect of analytical imprecision, number of replicates, number of samples, and number of individuals. *Clin Chem* 2012; 58: 1306–13.