Determination of the calcaemic status of Oncology Patients at the Charlotte Maxeke Johannesburg Academic Hospital, using unadjusted serum total calcium.

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0401560A

Submitted in partial fulfilment of the requirements in respect of the Master's Degree (MMed) in the Department of Chemical Pathology at the Faculty of Health Sciences, the University of the Witwatersrand.

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Final submission:.....2019

DECLARATION

“I, Dr. Shaida Khan, declare that, my research report (submissible” format with protocol & extended literature review) that I hereby submit, towards the Master’s Degree qualification (MMed Chemical Pathology), at the University of the Witwatersrand, is my independent work, and that I have not previously submitted it for a qualification at any another institution of higher education.”

……..

……. Day of……2019

DEDICATION

I dedicate this MMed to my dear husband, Mahomed Shoaib Omar. Thank you for selflessly supporting me through my less than selfless pursuits and chocolate addiction during my studies.

With this, "Dobby is free!" Well, sort of, academics never do end.

ABSTRACT

Background:

When total serum calcium (TCa) is used to determine the calcaemic status of patients, it has to be adjusted, using one of several formulae to limit the effect of low or raised serum albumin levels. All the existing adjustment formulae, were not validated against ionised calcium, neither have they been adjusted for the presence of paraproteins. They have also not been validated for use in cancer patients complicated with chronic kidney disease and cachexia. This study therefore aimed to determine whether unadjusted serum TCa measurement, could be used successfully to determine the calcaemic status instead of the gold standard, ionised calcium (ICa), in two sub-groups of hospital patients with breast cancer (BCA) and multiple myeloma (MM).

Material and methods:

202 paired TCa/ICa data points for MM and 3 467 for BCA were reviewed retrospectively over a 1-year period for diagnostic concordance and discordance using ICa as the gold standard.

Results:

In the MM sub-group, the diagnostic concordance was 60.1% (18 - 59 years) and 78.8% (60 - 90 years) for hypocalcaemia, and 7.4% (18-59 years) and 42.9% (60 -90 years) for hypercalcaemia. Whilst in the BCA sub-group, the diagnostic concordance was 46.1% (18 - 59 years) and 44.5% (60 - 90 years) for hypocalcaemia, and 14.7% (18-59 years) and 21.5% (60 -90 years) for hypercalcaemia.

Conclusion:

In all cancer sub-groups, both hypocalcaemia and hypercalcaemia were underestimated to various degrees. This was similar to other studies published in

non-cancer- specific patients. The poor performance of unadjusted TCa in the study prompted a recommendation that ICa should be used preferentially in cancer patients.

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Finally, I would like to thank my family for their ongoing support and well wishes.

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LIST OF ABBREVIATIONS

ICa	Ionised calcium
ISE	Ion selective electrode
TCa	Total calcium
BCA	Breast cancer
MM	Multiple myeloma
BCG	Bromcresol green
CMJAH	Charlotte Maxeke Johannesburg Academic Hospital
NHLS	National Health Laboratory Service
LIS	Laboratory information system
CDW	Corporate data warehouse
CV	Coefficient of variation
CKD	Chronic kidney disease

Chapter 1: Publication ready manuscript

This manuscript meets the Clinical Chemistry Journal's requirements under "Information for Authors" (Appendix G)

1.1. Journal: Clinical Chemistry

1.2. Journal Category: Evidence-Based Laboratory Medicine and Test Utilization

1.3. Research Article Title: Determination of the calcaemic status of Oncology Patients at the Charlotte Maxeke Johannesburg Academic Hospital, using unadjusted serum total calcium.

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1.6. Keywords:

Ionised calcium, total serum calcium, calcaemic status, multiple myeloma, breast cancer, paraproteinaemia, hypoalbuminaemia, hypercalcaemia, hypocalcaemia, o-cresol-phthalein complexone.

This manuscript has not been presented previously

1.7. INTRODUCTION

The determination of the calcaemic status of any patient, is complicated by the fact that, intravascular calcium exists in three forms. Most of the calcium (50%) is ionised (free), 40% is protein- bound with mainly albumin, and 10% is complexed with phosphates, citrate, bicarbonate or sulphates (1).

In clinical practice, the gold standard measure of the calcaemic status of a patient, is the ionised calcium (ICa) level in whole blood. ICa is measured using ion selective electrodes (ISE) on a blood gas analyser (2). ICa is the physiological fraction that interacts with the calcium- sensing receptor on parathyroid gland cells (3, 4).

ICa, however, is not routinely used, because of challenging pre-analytical sample collection requirements that can affect the result. Instead, serum total calcium (TCa) is analysed, commonly by one of two methods, that is, the o-cresolphthalein and arsenazo III dye- binding methods.

Because TCa is a measure of all three calcium fractions, the concentrations of binding proteins such as albumin, may affect the TCa levels (5). For this reason, several albumin- adjustment formulae were derived to minimise inaccurate TCa interpretation, when albumin levels are either higher, or lower than the reference intervals. The most frequently used formula is that of Payne et al (6).

A number of concerns have been raised, regarding the validity of these adjustment formulae (7, 8), because they have to be derived by each laboratory, using a specific combination of albumin and calcium methods. This is of particular importance in

patients with multiple myeloma, who have high levels of circulating paraproteins that can bind calcium and therefore produce falsely high serum TCa levels (9, 10). Therefore, the use of both adjusted and unadjusted TCa is unreliable as a tool to assess the calcaemic status in these patients.

In addition, the dye- binding methods used for albumin measurement, such as bromcresol green (BCG), are not specific for albumin and may bind immunoglobulins as well (11). As a result, albumin concentrations may be overestimated in these patients, and invalidate the adjustment formulae.

Hypo- and hypercalcaemia are common metabolic complications that occur in patients with malignancies. Hypercalcaemia, which may be present in up to 30% of these patients, occurs via one of two mechanisms, namely, local bone osteolysis caused by tumour metastases, and humoral hypercalcaemia mediated by parathyroid hormone-related peptide secreted by tumour cells (12-14).

Hypocalcaemia is also a complication in this patient population group, often due to a combination of nutritional deficiencies, co-existing chronic kidney disease (CKD), as well as the use of cancer therapeutic agents (15).

In light of the above issues raised, the use of adjustment formulae to determine the calcaemic status in cancer patients is inappropriate, as none of them are able to adjust for the presence of paraproteins. For this reason, the study attempted to look at the performance of unadjusted TCa alone in the assessment of the calcaemic status of two subgroups of cancer patients, namely, breast cancer (BCA) and

multiple myeloma (MM). This has never been investigated before in previous published studies in cancer patients, although it is well known that paraproteins in the MM subgroup bind calcium and that these patients are complicated with chronic kidney disease and cachexia. The performance of unadjusted TCa was compared with the ICa gold standard method.

1.8. MATERIALS AND METHODS

A. Data Collection

TCa and ICa patient results with breast cancer and multiple myeloma, that were seen at the Charlotte Maxeke Johannesburg Academic Hospital (CMJAH) adult oncology ward, were reviewed retrospectively. These were biopsy-proven patient diagnoses, made over the period June 2015 to June 2016.

A total of 24 014 TCa results were retrieved from the National Health Laboratory Service (NHLS) electronic data base (Trakcare). The data mining of the laboratory information system (LIS) was performed by the NHLS Corporate Data Warehouse (CDW). As ICa results represent the gold standard, the TCa results were paired with ICa test results when sampled at the same time. Results that did not include these pairings, were excluded. As a result, only 3 674 paired ICa - TCa results were obtained, of which, 3 471 were breast cancer pairs, and 203 multiple myeloma pairs (Table 1).

B. Sample analyses

ICa samples were analysed at the CMJAH NHLS Auto laboratory, using the ion-selective electrode method on the Radiometer ABL 80/Flex blood gas analyser (Copenhagen, Denmark). TCa was measured with the spectrophotometric o-cresol-phthalein complexone (without deproteinisation) method on the Siemens Advia 1800 Chemistry platform (Siemens Healthcare Diagnostics Inc., Tarrytown, NY). The analytical imprecision (coefficient of variation %) of the methods used during the study period, was 2.6% and 1.04% for TCa and ICa respectively.

C. Reference intervals

Age- specific reference intervals were used for both TCa and ICa. TCa: 2.15- 2.50 mmol/L (18 - 59 years) and 2.2 - 2.55 (60 - 90 years); ICA: 1.15- 1.27 mmol/L (18-59 years) and 1.16- 1.29 mmol/L (60 - 90 years (16, 17).

D. Statistical analyses

The ICa results were used to determine the correct classification of the calcaemic status of the subjects. The sub-grouped, age-partitioned data, was analysed using the MedCalc Statistical software programme. The percentage calcaemic diagnostic concordance and discordance was calculated using the 95% confidence limits of the reference intervals for TCa and ICa in healthy populations. These confidence limits were represented as horizontal and vertical lines for TCa and ICa respectively on the Deming's regression scatter plots.

1.9. RESULTS

After the removal of outliers (1 for MM and 4 for BCA), the remaining 202 paired ICa/TCa data points were used for analyses in the MM group (both males and females), and 3 467 pairs used for the BCA group (females only).

A. Multiple myeloma subgroup concordance

In the 18 to 59-year age group, the diagnostic concordance is 60.1% for hypocalcaemia (bin vii), 72.7% for normocalcaemia (bin v) and 7.4% for hypercalcaemia (bin iii) (Figure 1a).

In the 60 to 90-year age group, the diagnostic concordance is 78.8% for hypocalcaemia (bin vii), 57.3% for normocalcaemia (bin v), and 42.9% for hypercalcaemia (bin iii) (Figure 1b).

B. Multiple myeloma subgroup discordance

The diagnostic discordance in the 18 to 59-year age group is 39.9% for hypocalcaemia (bin iv), 27.3% for normocalcaemia (bins ii, viii) and 92.6% for hypercalcaemia (bins vi, ix) (Figure 1a).

The diagnostic discordance is 21.2% for hypocalcaemia (bin iv), 42.7% for normocalcaemia (bin ii, viii) and 57.1% for hypercalcaemia (bin vi, ix) in the 60 to 90-year age group (Figure 1b).

C. Breast cancer subgroup concordance

The diagnostic concordance is 46.1% for hypocalcaemia (bin vii), 86.6% for normocalcaemia (bin v) and 14.7% for hypercalcaemia (bin iii) in the 18 to 59-year age group (Figure 2a).

The concordance is 44.5% for hypocalcaemia (bin vii), 78.8% for normocalcaemia (bin v) and 21.5% for hypercalcaemia (bin iii) in the 60 to 90-year age group (Figure 2b).

D. Breast cancer subgroup discordance

The diagnostic discordance is 53.9% for hypocalcaemia (bins i, iv), 13.4% for normocalcaemia (bins ii and viii) and 85.3% for hypercalcaemia (bins vi, ix) in the 18 to 59-year age group (Figure 2a).

The diagnostic discordance is 55.5% for hypocalcaemia (bins i, iv), 21.2% for normocalcaemia (bins ii and viii) and 78.5% for hypercalcaemia (bins vi, ix) in the 60 to 90-year age group (Figure 2b).

1.10. DISCUSSION

In the MM subgroup, unadjusted TCa underestimates hypocalcaemia in 8 out of 20 (39.9%) pairs in the 18 to 59-year age group, and 3 out of 14 (21.2%) pairs in the 60 to 90-year age group (Table 2). In this subgroup, unadjusted TCa also underestimates hypercalcaemia in 12 out of 13 (92%) pairs in the 18 to 59-year age group, and 8 out of 14 (57%) pairs in the 60 to 90- year age group (Table 2). Both hypercalcaemia and hypocalcaemia are more underestimated in the younger age

group. This difference could be related to the different age-determined reference intervals used.

In the BCA sub-group, unadjusted TCa underestimates hypocalcaemia in 149 out of 278 (53.9%) pairs in the 18 to 59-year age group, and 144 out of 259 (55%) pairs in the 60 to 90-year age group (Table 2). In this sub-group, unadjusted TCa also underestimates hypercalcaemia in 236 out of 277 (85.3%) pairs in the 18 to 59-year age group and 55 out of 70 (78.5%) pairs in the 60 to 90-year age group (Table 2). Hypocalcaemia and hypercalcaemia, is markedly underestimated in both age groups.

In both the MM and BCA subgroups, both hypocalcaemia and hypercalcaemia are underestimated to various degrees. Our finding of the underestimation of hypocalcaemia and hypercalcaemia is supported by findings in other previous studies (18-22). However, they did not divide their study groups into different age groups as was done in our study, and were not specifically directed to patients with cancer.

Pre- analytical issues like the incomplete filling of ICa heparinised collection devices that disturbs ICa levels, could not be factored into the study (23, 24). Further limitations of our study were that, the presence of CKD was not assessed, neither were the concentrations of albumin levels factored into the interpretation of the TCa results. This was due to the retrospective nature of the study. A follow up study in this specific patient population, can be prospectively investigated with greater sample numbers to include albumin levels and renal function parameters.

Our study, in patients with two malignancies, namely, MM and BCA, demonstrates poor performance of TCa in the majority of patients with hypocalcaemia and hypercalcaemia and at times were as good as guessing. This finding, together with those of Ijaz et al (25), supports our recommendation that ICa alone should be used to determine calcaemic status in patients with cancer.

1.11. Tables

Patient subgroup	Number/ Range/ %
Multiple myeloma (MM)	
Total number of TCa/ICa data pairs	203
Total number of patients	62
Mean number of data pairs per patient	3 (1- 10)
Sex	
Female	118 (58.1%)
Male	85 (41.8%)
Median Age (years)	58 (23- 86)
Breast cancer (BCA)	
Total number of TCa/ICa data pairs	3471
Total number of patients	827
Mean number of data pairs per patient	4 (1- 11)
Sex	
Female	3471 (100%)
Median Age (range)	52 (24- 87)

Table 1. Demographics of patients in the multiple myeloma (MM) and breast cancer (BCA) subgroups.

	Ionised calcium (ICa) (18- 59 years)			Ionised calcium (ICa) (60- 90 years)		
Total Calcium	Hypo	Normo	Hyper	Hypo	Normo	Hyper
Multiple myeloma						
Hypo	12	19	0	11	28	2
Normo	8	53	12	3	39	6
Hyper	0	1	1	0	1	6
Total	20	73	13	14	68	14
Breast cancer						
Hypo	129	215	8	115	153	4
Normo	147	1578	228	144	598	51
Hyper	2	31	41	0	8	15
Total	278	1824	277	259	759	70

Table 2. Paired results for both multiple myeloma (MM) and breast cancer (BCA) subgroups, displaying the numbers classified as hypocalcaemia (Hypo), normocalcaemia (Normo) and hypercalcaemia (Hyper).

1.12. Figures

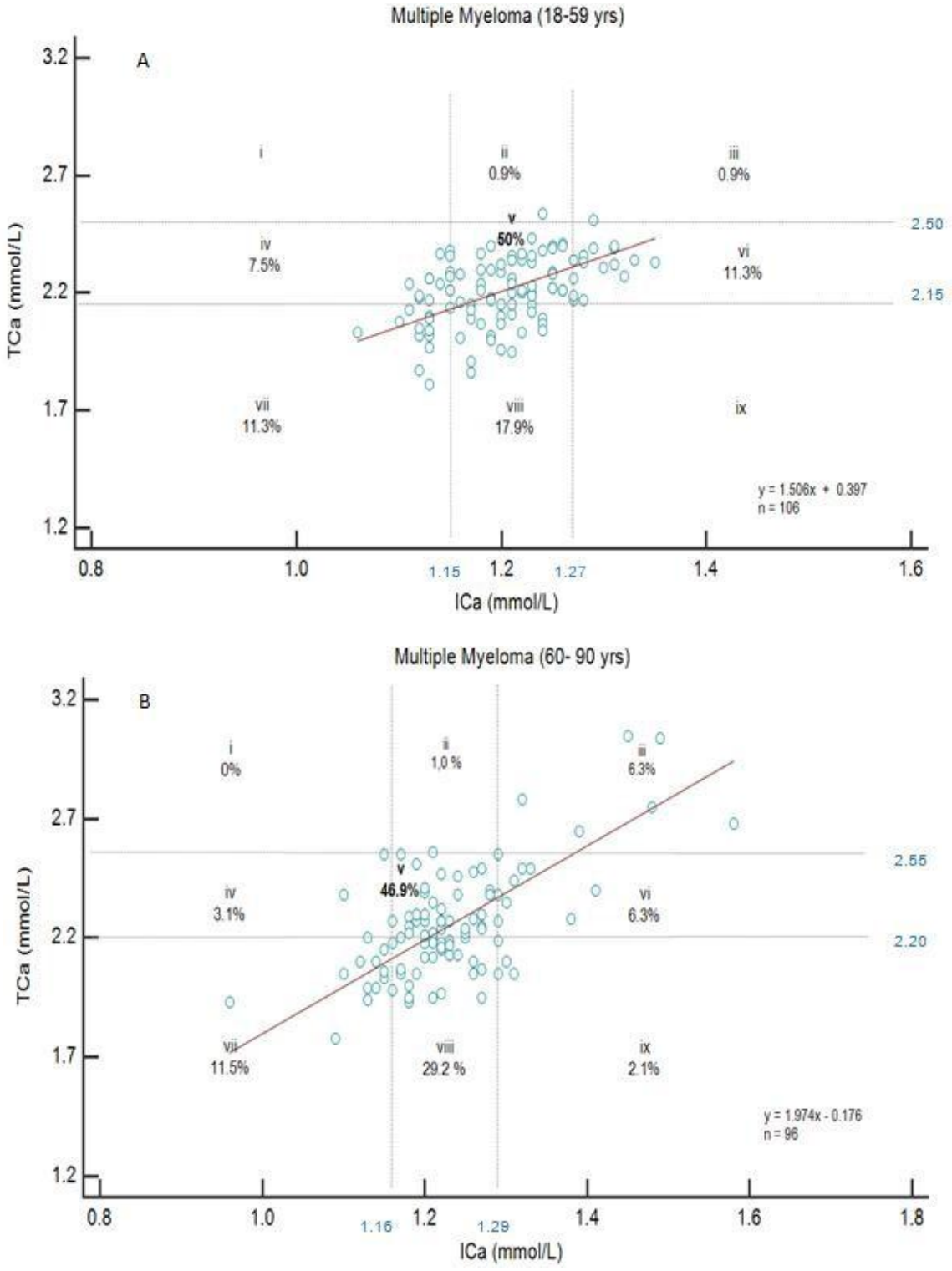

Figure 1. Concordance/ discordance plots of multiple myeloma (MM) according to age: 1a. 18 - 59 years and 1b. 60 - 90 years. The two horizontal lines represent the TCa reference interval, and the two vertical lines represent the ICa reference interval creating bins. Bins iii, v, and vii = areas of concordance. Bins i, ii, iv, vi, viii, ix = areas of discordance. Percentages per bin indicate number of paired results of the total

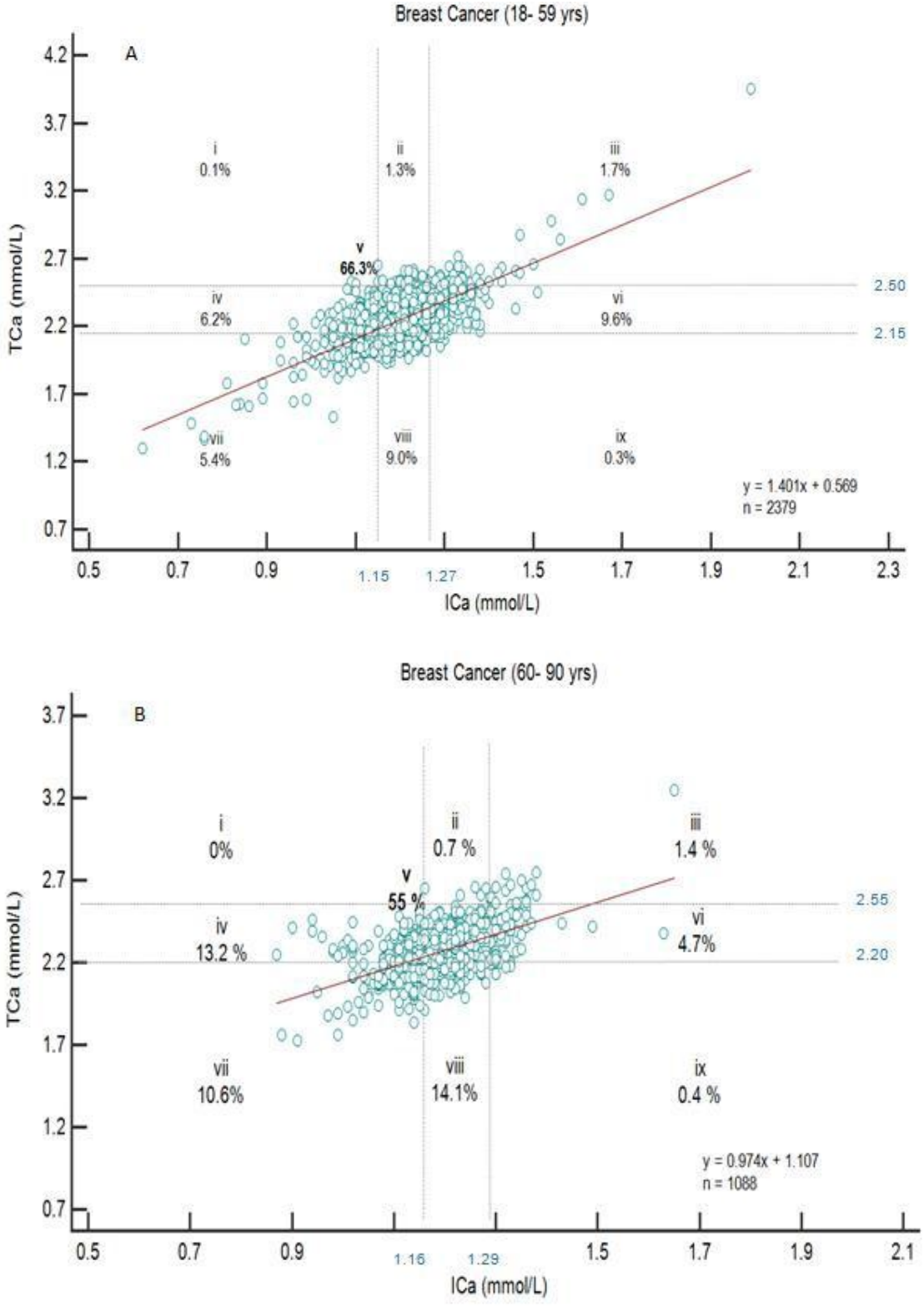

Figure 2. Concordance/ discordance plots of breast cancer (BCA) according to age: 2a. 18 - 59 years and 2b. 60 - 90 years. The two horizontal lines represent the TCa reference interval, and the two vertical lines represent the ICa reference interval creating bins. Bins iii, v, and vii = areas of concordance. Bins i, ii, iv, vi, viii, ix = areas of discordance. Percentages per bin indicate number of paired results of the total.

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Chapter 2: Approved protocol

RESEARCH PROTOCOL

TITLE:

Determination of the calcaemic status of Oncology Patients at the Charlotte Maxeke Johannesburg Academic Hospital using unadjusted serum total calcium.

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Background:

Calcium, an important cation in the human body has numerous intracellular and extracellular functions. These include bone mineralization, muscle contraction, cell signalling, coagulation and maintenance of the plasma membrane potential. (1)

The assessment of the calcaemic status of any patient is complicated by the fact that plasma calcium is separated into a protein-bound fraction (40%), complexed fraction (10%) and a free ionised fraction (ICa) (50%). The calcium found in all these fractions is measured in serum total calcium assays (TCa). Albumin is the major calcium binder (80%) followed by immunoglobulins (20%). (1)

ICa is the physiologically active form and is under tight hormonal control. A reduction in the ICa level is detected by the calcium- sensing receptor in the parathyroid glands. This results in the release of parathyroid hormone which acts on the bone and kidneys to return ICa levels to normal. The patients' calcaemic status can therefore be evaluated by measuring circulating ICa concentrations. (2)

ICa is often measured directly on a blood gas analyser employing ion-selective electrode (ISE) methodology. The measurement of ICa is regarded as the gold standard for the assessment of a patient's calcaemic status. (3) However, due to the manual nature of the test method, as well as the stringent sample collection and handling requirements, ICa measurements are not routinely used in all laboratories. (1) TCa measurement on the other hand, has less strict sampling requirements and is more routinely available on automated analysers. For these reasons, TCa has been the most frequently requested test when clinicians have wanted to assess the calcaemic status. (4)

Marked variations in the concentrations of serum albumin however, influence the TCa concentration. In cases with high or low albumin values, the ICa concentrations may

remain unaffected. The TCa levels on the other hand, may reflect this hypo/hyperalbuminaemic status (rather than the actual calcaemic status). (5) In order to limit the influence of these variations of serum albumin concentrations, which occur commonly in hospitalized patients, adjustment formulae have been mathematically derived for routine use. The aim of these adjustment formulae is to provide a serum TCa concentration that would have been achieved if albumin was within the normal levels. (3) Historically the Payne et al adjustment formula (see below) had been routinely used both internationally and locally at the Charlotte Maxeke Johannesburg Academic Hospital Laboratory (CMJAH). Payne adjustment formula is as follows (5):

Adjusted calcium (mmol/l) = Total calcium (mmol/l) + 0.02 (40- alb (g/l))

Other derived formulae include those from Orrell et al and Berry et al that use different coefficients to correct for albumin concentrations. (6,7) The Payne et al adjustment equation was derived from the slope of the linear regression relationship of serum TCa concentrations to albumin levels in patients who had no proven calcium disorders. (5) Although the practice of adjusting calcium for albumin has been widely adopted, issues have been raised regarding the diagnostic accuracy with regard to the hypo-/hypercalcaemic status of patients.

Several studies have demonstrated that the albumin adjustment formulae underestimate hypocalcaemia and overestimate hypercalcaemia in the general patient population. Possible reasons to explain this under- and overestimation relate to the albumin and calcium measuring methods. Commercially available dye-binding colorimetric assays for measuring albumin include bromocresol green (BCG), bromocresol blue (BCB) and

bromocresol purple (BCP) dyes. (8) All of the dyes, particularly BCG, can potentially bind other serum proteins apart from albumin, leading to an overestimation of albumin levels. This is particularly significant in patients who have marked hypergammaglobulinaemia that will bind the albumin-binding dyes.

Routine automated analyser TCa measurement methods include photometric methods which are based on the measurement of calcium-bound coloured complexes. Two common dyes used are o-cresolphthalein complexone and arzenazo lll dyes. (8)

Both calcium and albumin analytical methods are not standardized. The measurements and reference intervals are therefore, method and analyser specific. In addition, studies using adjustment formulae eg. Payne et al formula, were not validated against the ICa gold standard. The calcaemic status of patients using these formulae is therefore questionable. In addition, when a laboratory changes analyzers for calcium and albumin measurements, new adjustment formulae have to be developed. (4) The Clinical Practice Working Group of the Association of Clinical Biochemistry recommends that laboratories derive their own method specific adjustment formulae using their current analyzers. (4)

In this study, the calcaemic status of a specific group of oncology patients, with solid tumours (breast and colon) and haematological malignancies (paraproteinaemias) will be evaluated. Hypercalcaemia, is a common metabolic complication in patients with malignancies and complicates the disease course in about 10 - 30% of cases. (9) Humoral Hypercalcaemia of Malignancy (HHM) is mediated primarily through PTH related peptide (PTHrP). PTHrP has structural homology to PTH and can thus act similarly on the bones and kidneys to increase calcium concentrations. In addition, unlike PTH, PTHrP is not under regulatory control. Other causes for an abnormally

increased TCa can be due to localized osteolytic hypercalcaemia (LOH) particularly by solid tumours. The tumours (bone metastasis) cause local destruction and increased bone resorption by releasing osteoclast- activating factors (OAFs). Patients with malignancy-induced hypercalcaemia have higher rates of morbidity and mortality and in many cases; the hypercalcaemia is regarded as a poor prognostic sign. (9) In addition, patients with paraproteinaemias have high levels of circulating immunoglobulins that can also bind calcium. Adjustment equations do not correct for this enhanced immunoglobulin-calcium binding that occur in these patients and are therefore inappropriate to estimate these patients' calcaemic status and follow up their response to treatment. (10)

There are very few studies to determine what the preferred parameter would be to define these oncology patients' calcaemic status.

Aim:

This study aims to determine whether unadjusted TCA measurements alone can be used to define the calcaemic status of oncology patients.

Study objectives:

To assess the concordance of unadjusted TCA with ICA measurements in the determination of the calcaemic status of oncology patients.

Research hypothesis:

Unadjusted TCA alone can be used to determine the calcaemic status of Oncology patients.

Materials & Methods

Study design- retrospective cross sectional study.

Subjects- Inclusion criteria:

Patients will be selected according to the following selection criteria: All patients seen at the adult Oncology ward of CMJAH with biopsy proven solid tumours and haematological malignancies. (The two most common tumour types include breast cancer and multiple myeloma). All these patients should have at least one request for serum TCa and whole blood ICa on samples collected on the same occasion.

Data collection:

The study sample results will be obtained by electronic data mining (by the Corporate Data Warehouse - CDW) from the National Health Laboratory Service (NHLS) Trakcare (Laboratory Information System- LIS) database from June 2015 to June 2016. The number of participants is expected to exceed three hundred. The patient diagnoses will be obtained from patient records or from the Medical Records Department at CMJAH.

Due to the ICa samples being sent to the laboratory immediately for analysis (pre-analytical requirement), the samples for TCa may be requested under a different Trakcare episode number and will therefore be matched with the systems of CDW the use of the LIS audit trail and patient requisition forms.

Exclusion criteria:

Participants that don't have ICa measurements that were performed at the same time as TCa.

Possible limitations of this study

There may be loss of portions of data that cannot be retrieved by the CDW. The sample size might be reduced due to absence of Ica coupling with TCa results.

Data capture:

Data will be recorded on an Excel spread sheet with the following headings: anonymised patient number, age, sex, diagnosis as per biopsy result, ICa, TCa, Immunoglobulins and albumin blood results and patient classification in one of three groups; hypo, normo and hypercalcemia according to ICa results.

Sample handling & storage:

The laboratory uses standardized procedures for sample collection (as per Appendix A) to limit pre-analytical issues that may influence patient results. Patient dietary intake could not be controlled.

Sample analysis:

Samples will have been analysed at the CMJAH NHLS Auto laboratory. Analysis of ICa will have been performed on the Radiometer ABL 80/Flex blood gas analyser (Copenhagen, Denmark) using ion-selective electrode methodology. TCa measurements will have been performed on the Siemens Advia 1800 Chemistry platform (Siemens Healthcare Diagnostics Inc., Tarrytown, NY) using the spectrophotometric o-cresolphthalein complexone (without deproteinisation) method.

The laboratory analytical imprecision (analytical CV %) of both ICa and TCa will be obtained from the CMJAH laboratory quality control database. The sources of the reference intervals (95% confidence intervals) for healthy populations will also be recorded.

Statistical analysis

The data will be plotted using the EP evaluator statistical software program (Data Innovations LCC) using the CLSI EP9 method and Deming's simple regression analysis. The ICA results will be used to determine the correct classification of the calcaemic status of the subjects.

The percentage diagnostic concordance and discordance with calcaemic status using TCa will then be calculated using the 95% confidence limits of the reference intervals for healthy populations demarcated by horizontal & vertical lines (for ICa and TCa respectively)

Ethical Clearance

Application for ethical clearance will be made to the Health Sciences Ethics committee of the University of the Witwatersrand.

Research Timeline:

Task	Sep	Oct	Nov	Dec	Jan	Feb	Mar	Apr	May	Jun	Jul	Aug
Preparation of protocol												
Protocol Review by Department												
Ethics application												
Wits Research Committee												
Data collection												
Data analysis												
Write up												

Acknowledgements:

Professor P. Ruff for patient diagnosis and work up; the NHLS CMJAH laboratory for sample analysis and analytical performance information and the NHLS CDW for data collection using the NHLS- LIS database.

Funding

The diagnostic workup and monitoring will be part of the participants' routine investigation for complications related abnormal calcium status in malignancy.

REFERENCES

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APPENDIX A

WHOLE BLOOD IONISED CALCIUM SPECIMEN COLLECTION AND HANDLING

Pre-Phlebotomy recommendations:

- Rested patient - either sitting / lying for at least 5 minutes prior to procedure
- No food intake for at least 4 hrs.

Sampling requirements:

- A tourniquet can be used for < 3 min but exercising of the limb is not permitted.
- A heparinised sample in a lyophilised heparinised blood gas syringe or vacutainer blood collection tube is used. Heparin concentrations ≥ 30 U/ml can lower the ICa proportional to the heparin concentration. (The NHLS uses vacutainer collection tubes that have spray-dried Lithium Heparin in concentrations of 17 U/ml when tube is completely filled. Please check the heparin concentration of the pre-heparinised blood gas syringes that you are using in your Unit). Note: other anticoagulants bind Ca and their use is unacceptable. It is not advisable to use liquid heparin because of the dilution effect on the ICa concentration.
- Completely fill the vacutainer collection tube.
  Incompletely filled vacutainer tubes will have higher concentrations of heparin and therefore a proportional reduction in the ICa concentration.

- Mix the sample after collection to ensure even dispersion of the lyophilised heparin. This prevents the development of micro-clots that can affect the blood gas analyser.
- The collection tube should remain sealed or if using a blood gas syringe, seal immediately. Maintenance of anaerobic conditions until analysis minimises the loss of carbon dioxide from the sample. The proportion
  Ca bound to albumin is charge-related and therefore pH dependent. In vitro loss of CO_2 from the specimen will increase the pH and reduce the unbound Ca (ICa).

- Analysis within 30 min is preferred if the specimen is at room temperature. If a delay in analysis is anticipated, the specimen should be transported in an **ice - water slurry** (not dry ice as this will freeze samples) and analysed within 4 hr. Chilling minimises continued metabolism and a resultant change in pH.

Appendix A: Ethics approval

R14/49 Dr Shaidah Khan and Dr Taryn Pillay

HUMAN RESEARCH ETHICS COMMITTEE (MEDICAL)

CLEARANCE CERTIFICATE NO. M170104

NAME: (Principal Investigator)	Dr Shaidah Khan and Dr Taryn Pillay
DEPARTMENT:	Chemical Pathology National Health Laboratory Service Charlotte Maxeke Johannesburg Academic Hospital
PROJECT TITLE:	Determination of Calcaemic Status of Oncology Patients at the Charlotte Maxeke Johannesburg Academic Hospital using unadjusted Serum Total Calcium
DATE CONSIDERED:	27/01/2017
DECISION:	Approved unconditionally
CONDITIONS:	
SUPERVISOR:	Dr Jocelyn Naicker
APPROVED BY:	Prof P Cleaton-Jones, Chairpersn. HREC (Medical)
DATE OF APPROVAL:	03/02/2017

This clearance certificate is valid for 5 years from date of approval. Extension may be applied for.

DECLARATION OF INVESTIGATORS

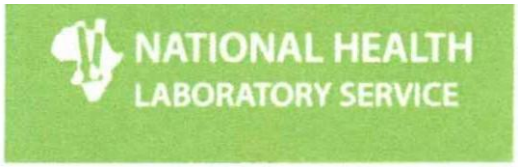
To be completed in duplicate and ONE COPY returned to the Research Office Secretary in Room 301, Third floor, Faculty of Health Sciences, Phillip Tobias Building, 29 Princess of Wales Terrace, Parktown. 2193, University of the Witwatersrand. I/we fully understand the conditions under which I am/we are authorized to carry out the above-mentioned research and I/we undertake to ensure compliance with these conditions. Should any departure be contemplated, from the research protocol as approved, I/we undertake to resubmit the application to the Committee. I agree to submit a yearly progress report. The date for annual re-certification will be one year after the date of convened meeting where the study was initially reviewed. In this case, the study was initially reviewed in January and will therefore be due in the month of January each year. Unreported changes to the application may invalidate the clearance given by the HREC (Medical).

Principal Investigator Signature

13/02/2017
Date

PLEASE QUOTE THE PROTOCOL NUMBER IN ALL ENQUIRIES

Appendix B: NHLS Permission



Modderfontem Road Sandnngham. 2031
Tel: +27 (0)11 386 6142
Fax +27 (0)11 386 6296
Email babatv1 k.gol..ong@nhs a1.. za
Web: vvWN ntl1s ac za

13March 2017

Applicant: Or Shaida Khan
Institution: Charlotte Maxeke Academic Hospital
Faculty: Chemistry
Tel: 011 489 8432
Email: sha.da khan@nhls.ac.za

Re: Approval to access National HeaHh Laboratory Service (NHLSl Data - Modification

Your application to undertake a research project "Determination of Calcaemic Status of Oncology Patients at the Charlote Maxeke Johannesburg Academic Hospital Using unadjusted Serum Total Calciumusing data from the NHLS database has been reviewed. This letter serves to advise that the application has been approved and the required data will be made available *without patient names* to you to conduct the proposed study as outlined in the submitted application.

Please note that the approval is granted on your compliance with the NHLS conditions of service and that the study can only be undertaken provided that the following conditions have been met.

- Ethics approvalis obtained from a recognised SA Health Research Ethics Committee.
- Processes are discussed with the relevant NHLS departments (i.e. Information Management Unit and Operations Office) and are agreed upon.
- Confidentiality is maintained at participant and institutional level and there is no disclosure of personal information or confidential information as described by the NHLS policy.
- A final report of the research study and any published paper resulting from this study are submitted and addressed to the NHLS Academic Affairs and Research office and the NHLS has been acknowledged appropriately.
- NHLS Data cannot be used to track patients as no pre-approval/consent is obtained from Patients.

Please note that this letter constitutes approval by the NHLS Academic Affairs and Research. Any data related queries may be directed to NHLS Corporate Data Warehouse, Tel: (011) 386 6074 email: Zar na sab ciln ac za

Yours sincerely,

Dr Babatyi Malope-Kgokong
National Manager: Academic Affairs and Research

Appendix C: CMJAH Permission

GAUTENG PROVINCE
EA
REPUBLIC OF SOUTH AFRICA

CHARLOTTE MAXEKE JOHANNESBURG ACADEMIC HOSPITAL

Enquiries: N. Mzila
Office of the Director: Clinical Services
Tell :(011)488-3365
Fax: (011)488-3753
16 February 2017

Dear Dr. Khan

RE: "Determination of the calcaemic status of oncology patients at the Charlotte Maxeke Johannesburg Academic Hospital using serum total calcium".

Permission is granted for you to conduct the above recruitment activities as described in your request provided:

1. Charlotte Maxeke Johannesburg Academic hospital will not in anyway incur or inherit costs as a result of the said study.
2. Your study shall not disrupt services at the study sites.
3. Strict confidentiality shall be observed at all times.
4. Informed consent shall be solicited from patients participating in your study.

Please liaise with the Head of Department and Unit Manager or Sister in Charge to agree on the dates and time that would suit all parties.

Kindly forward this office with the results of your study on completion of the research.

Supported / ~~not supported~~

Dr. M.I. Mofokeng
Director: Clinical Services
DATE: 27/02/2017

"?'pproNot approved

M shi
Chief Executive Officer
DATE: :J.13. O:J..d-£)1/

Appendix D: HOD Permission

1922 1997

UNIVERSITY OF THE WITWATERSRAND, JOHANNESBURG

7 York Road, Parktown, 2193 South Africa · Telephone (011) 488-3901 • fax (011) 488-4515
E-mail: Medicine@wits.ac.za

Department of Medical Oncology

5 April 2017

Postgraduate Committee
School of Clinical Medicine
Faculty of Health Sciences

RE: PERMISSION FOR MMED RESEARCH BY DR SHAIDA KHAN STUDENT NR. 04015608

I am happy that Dr Khan collects data for her MMed Research Report titled: "Determination of the calcaemic status of Oncology patients at the Charlotte Maxeke Johannesburg Academic Hospital using unadjusted serum total calcium" from patients treated at the Division of Medical Oncology, Charlotte Maxeke Johannesburg Academic Hospital and University of Witwatersrand, Faculty of Health Sciences.

Many thanks

PROFESSOR PAUL RUFF
PROFESSOR AND HEAD
DIVISION OF MEDICAL ONCOLOGY
DEPARTMENT OF MEDICINE
FACULTY OF HEALTH SCIENCES AND
CHARLOTTE MAXEKE JOHANNESBURG ACADEMIC HOSPITAL

Appendix E: Turnitin report

0401560a:17032019b - Final - MMed submissible format.docx

ORIGINALITY REPORT

5%	4%	3%	3%
SIMILARITY INDEX	INTERNET SOURCES	PUBLICATIONS	STUDENT PAPERS

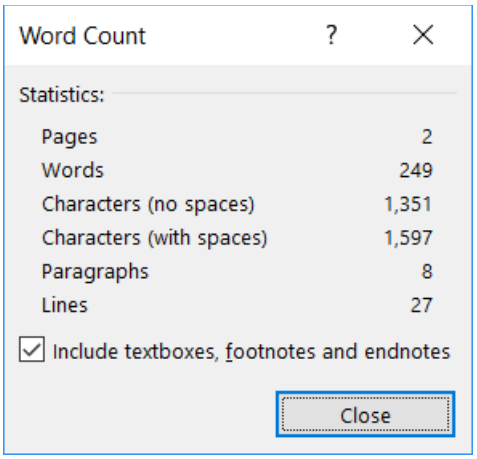
PRIMARY SOURCES

	www.ufs.ac.za Internet Source	1%
	Urolithiasis, 2012. Publication	1%
II	Submitted to University of the Free State Student Paper	1%
	www.crcsi.com.au.stage.gs Internet Source	1%
	www.isbnpa.org Internet Source	1%

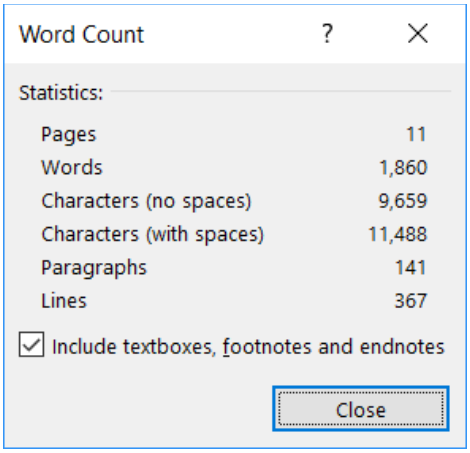
Exclude quotes	On	Exclude matches	
Exclude bibliography	On		

Appendix F: Proof of word count

Abstract



Manuscript: (includes tables and figures- not references)



Appendix G: Journal publication requirements

Clinical Chemistry

Information for Authors

Revised February 2019

Click to view an instructional video: Tips for Online Manuscript Submission. This video covers a range of topics to help authors navigate the electronic submission system, including registration procedures, how to upload files, and what to include in the author disclosure forms.

OVERVIEW

Clinical Chemistry, issued monthly, is published in print and electronically by the American Association for Clinical Chemistry. The journal welcomes contributions, either experimental or theoretical, in the field of laboratory medicine. It is the leading forum for peer-reviewed, original research on innovative practices in today's clinical laboratory. In addition to being the most cited journal in the field, *Clinical Chemistry* has the highest Impact Factor among journals of clinical chemistry, clinical (or anatomic) pathology, analytical chemistry, and the subspecialties, such as transfusion medicine and clinical microbiology.

Submissions of the following nature are welcomed:

- Basic materials or principles
- Analytical techniques
- Molecular diagnostics
- Test utilization or testing-related health or financial outcomes
- Instrumentation
- Data processing
- Statistical analyses of data
- Clinical investigations in which laboratory testing has played a major role

- Laboratory animal studies of chemically oriented problems of human disease

Contributions should consist of subject matter that is original and significantly advances the state of knowledge of clinical chemistry, and conclusions that are justified from the design of the experiments and the data presented. The information must be sufficiently detailed to permit replication of the work by a competent worker in the field. Lastly, the writing must be clear, concise, and grammatically correct.

Equal consideration is given to original manuscripts in English from any country, regardless of membership in the Association. It is, however, advised that all non-English speaking authors enlist the aid of a native-English speaking colleague to correct English language usage before submission. Submissions must adhere to the current International Committee of Medical Journal Editors (ICMJE) Recommendations for the Conduct, Reporting, Editing, and Publication of Scholarly Work in Medical Journals.

All *Clinical Chemistry* submissions, correspondence, and reviews must be in English. Manuscript content and writing responsibility remains with the author; however, several professional writing/editing services are available to assist authors. *Clinical Chemistry* does not endorse any editing service nor is *Clinical Chemistry* responsible for any services performed. These include but are not limited to:

www.bioedit.co.uk
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www.biosciencew riters.com
www.bluepenci!science.com
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www.editage.com
www.enago.com
www.journalexperts.com
www.prof-editing.com
www.sciencedocs.com
www.stallardediting.com

Contributions may be submitted via the manuscript tracking system at http://submit.clinchem.org. The "Information for Authors" will offer assistance with journal style and requirements. Please contact the Editorial Office via e-mail should you have any questions or need assistance: clinchemed@aacc.org.

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STANDARDS FOR REPORTING SCIENTIFIC DATA

- Description of Analytical Methods and Results
- Statistics
- Studies with Human Subjects
- Animal Studies
- NIH Funding/Open Access Requirements
- Checklist for the Description of Sequence Variants at the Human Genome Variation Society

Comparison-of-methods studies should compare results by the new or proposed method with those by a reference-quality method or other generally accepted analytical method for which assay performance is documented (4, 5).

It is desirable to test 100 to 200 different samples from patients who have been selected to include a wide variety of pathologic conditions and to present a range of values for the analyte that includes those likely to be encountered in routine application.

For a table of the required number of samples, see Linnet (6).

If regression analysis is used for statistical evaluation of the data, supply slopes and intercepts (and their standard deviations) and standard deviations of residuals (Sylx, often called standard errors of estimates). Unbiased (e.g., Deming) regression is typically required (7). A program to perform Deming regression is available online as a supplement from this journal (8).

The correlation coefficient has limited utility. Residuals plots [e.g., Bland-Altman (9, 1O)] are often useful. On the horizontal axis, plot the mean of results by the two studied methods, not the result of one method.

Analytical sensitivity and detection limit: These terms are commonly confused. The International Union of Pure and Applied Chemistry defines analytical sensitivity as the ability of an analytical procedure to produce a change 1n signal for a defined change of the quantity. This is often visualized as the slope of the calibration curve.

The limit of detection (LOO) is defined as the lowest concentration or amount of an analyte that can be reliably identified as being qualitatively present in the sample. The limit of quantification (LOO) is defined as the lowest concentration or amount of analyte that can be reproducibly quantified in a sample. The most acceptable criteria for ascertaining the LOO is the concentration of analyte that can be measured with an imprecision of less than 20% and a deviation from target of greater or less than 20% (1). The operational definition of the LOO and LOO must be supplied by the author. Additional considerations related to this topic are presented by Linnet (11).

Analytical quality: Results obtained for the performance characteristics should be compared objectively with well-documented quality specifications, e.g., published data on the state-of-the-art performance required by regulatory bodies such as CUA 88, or recommendations documented by expert professional groups (12).

Reference interval (normal range): Depending on the conclusions of the accuracy studies, modification of an accepted reference interval may be indicated. Description of the reference interval study should include details about sampling; selection of subjects, including their number, age, and sex distribution; the statistical method for summarizing the results (13); and other factors that would influence the values obtained.

Mass spectrometric assays must be evaluated for matrix effects (ion suppression or enhancement) (14, 15).

Chromatograms: Chromatograms from gas-liquid and liquid chromatography should usually be presented so that readers can see the efficiency of the separation and observe the resolution from interferents in the matrix. Similar images are often needed for electrophoretic separations.

Enzyme activities: Enzyme activities may be expressed in international units (U) or katals. Temperature and other key assay features must be described in the text or by reference to a published method.

When first mentioned in the text, enzymes (whether measured by activity or mass assays) must be numbered (EC no.) in accordance with the recommendations of the Nomenclature Committee of the International Union of Biochemistry and Molecular Biology on the Nomenclature and Classification of Enzymes {16).

and a total of 6 tables and/or figures. Supplemental data are permitted for Review articles.

Reviews should list no more than 15 authors, with any additional contributors listed in the Acknowledgments. Although exceptions are rare, you may email clinchemed@aacc.org detailing each author's contribution to your submission, which will be forwarded to the editor.

SPECIAL REPORT

Special Reports may be submitted directly by authors or invited by the journal. The types of papers that would be considered include consensus reports, guideline development, position statements, or evidence-based recommendations on test utilization or quality specifications. The editors may also decide to classify other miscellaneous submissions under this heading.

A Special Report should consist of a structured or unstructured abstract limited to 250 words. The main text should be no more than 5,000 words. The manuscript should have no more than 40 references and a total of 4 tables and/or figures. Supplemental data are permitted for Special Reports.

Special Reports should list no more than 20 authors, with any additional contributors listed in the Acknowledgments. Although exceptions are rare, you may email clinchemed@aacc.org detailing each author's contribution to your submission, which will be forwarded to the editor.

UNVEILING THE RIGHT SIDE

Submissions should highlight the creative side of someone in the field of chemistry. This can be poetry, a short story, photographs, or other creative artwork. Submissions are limited to 400 words and/or one image, photograph, or poem. All submissions are subject to review. Cover letter should state interest in contributing to Unveiling the Right Side and must be submitted under the category of Clinical Chemist.

WHAT IS YOUR GUESS?

Submissions for this 1-page quiz should consist of an image or lab values, a case description (no more than 75 words), 3 questions, case discussion (no more than 75 words), and no more than 5 references. Cover letter should state interest in contributing to What Is Your Guess? and must be submitted under the category of Clinical Chemist.

What Is Your Guess? submissions should list no more than 5 authors. Although exceptions are rare, you may email clinchemed@aacc.org detailing each author's contribution to your submission, which will be forwarded to the editor.

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MANUSCRIPT PREPARATION

- Author Contribution Requirements
- Manuscript Guidelines
- Submission/Publication Fees

- Journal Categories
- Title Page
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- Text
- Disclosures/Conflict of Interest
- Acknowledgments
- References
- Tables
- Figure Captions
- Figures
- Color
- Supplemental Data

AUTHOR CONTRIBUTION REQUIREMENTS

General Guidelines

Clinical Chemistry follows the recommendations for authorship set out by The International Committee of Medical Journal Editors (ICMJE). In accordance with these recommendations, manuscripts are considered for publication with the understanding that each listed author must meet the following criteria:

- 1. Substantial contributions to the conception or design of the work; or the acquisition, analysis, or interpretation of data for the work; AND
  2. Drafting the work or revising it critically for important intellectual content; AND
  3. Final approval of the version to be published; AND
  4. Agreement to be accountable for all aspects of the work in ensuring that questions related to the accuracy or integrity of any part of the work are appropriately investigated and resolved.

Author limits may be imposed for certain submission types. Please review the specific requirements for your submission type. Please list only the allowed number of authors in the author list, with the remaining contributors listed in an Acknowledgment. Exceptions may be made at the discretion of the editor.

If more than one author should be considered as the first authors, or as the corresponding authors, please indicate this clearly in a footnote on the first page of the manuscript.

Any alterations made to the manuscript after submission must be approved by the editor. Authors may upload the request letter to the online submission system as a supplemental file or send the letter via e-mail to the *Clinical Chemistry* editorial office at clinchemed@aacc.org. The editor may contact any of the authors and/or contributors to ascertain whether they have agreed to any alteration.

1. The ICMJE Recommendations for the Conduct, Reporting, Editing, and Publication of Scholarly Work in Medical Journals state that contributors who do not meet all 4 criteria for authorship should be named in the acknowledgments.
2. Important contributions to an article should be recognized and appropriately attributed in that article. Good medical writers and editors can make valuable contributions to the publication process, often improving the clarity of the communication, broadening the scope of literature review, providing an extra level of data review, adding balance and objectivity, and shortening the time needed for manuscript development.

The American Medical Writers Association (AMWA) believes that these important contributions deserve recognition.

3. Readers benefit from knowing about the involvement of professional writers and editors. Disclosing the editorial contribution and the source of funding of the writer and editor allows the reader to make informed judgments about the objectivity of the article.

Note that the AMWA position statement recommends acknowledgment of pertinent professional or financial relationships as well as acknowledgment of the contributions of writers and editors.
ft also recommends that the person being acknowledged be given the opportunity to grant or refuse permission for the acknowledgment.

Changing the Author List

Any change in authors and/or contributors after initial submission must be approved by all authors. This applies to additions, deletions, change of order to the authors, or contributions being attributed differently. If a change is made to the author fist after submission or during any revision, the submitting author must send an e-mail to clinchemed@aacc.org explaining the reason for the change and copying all authors for their approval. Any additions or deletions must be made in the electronic submission record as well as on the manuscript itself. At revision, be sure to select "Yes, the authorship has changed" when prompted.

Group Authorship

If you are including the name of a group or consortium in your author list, please clearly identify the group members who can take credit and responsibility for the work as authors by fisting them in a footnote or at the end of the manuscript. Please also provide email addresses for each listed group member, either in the manuscript or by emailing clinchemed@aacc.org. All members of the group named as authors should meet all four criteria for authorship, including approval of the final manuscript, and they should be able to take public responsibility for the work and should have full confidence in the accuracy and integrity of the work of other group authors. They will also be required as individuals to complete the author disclosure and contribution forms. This is in accordance with the recommendations of ICMJE.

MANUSCRIPT GUIDELINES

- MS Word document (.doc or .docx) is required for all submissions.
- All figures must be uploaded separately as Image Fifes in Tagged Image File Format (.tiff), Encapsulated Postscript (.eps) or PowerPoint (.ppt) with embedded fonts.
- All submissions must be double-spaced, 1 inch margin, twelve-point font size in Arial, Helvetica, Times New Roman and Symbol font (for non-text characters).
- All submissions must be page numbered.
- All submissions must have fine numbers included in the text.
- Do not use headers or footers.
- Use standard abbreviations and define all nonstandard abbreviations.
- All submissions require a title page.
- Reporting of Concentration Units:

1. Analyte concentrations will be expressed in the text in the traditional mass unit (mg/dl, ng/ml, and so forth) followed by the SI unit in parentheses. Exceptions would include those analytes in which SI units are used globally, such as electrolytes (use mmol/L for sodium, potassium, chloride, and $C0_2$ values), or cases in which the traditional unit and the SI unit differ by only a factor of 1000 in both the numerator and denominator (e.g., ng/ml vs µg/L). In such cases, the unit of measure consistent with common practice will be used.
2. The unit of measure mg/L should be used only when referring to SI units or when national or international guidelines require or recommend that the concentration of an analyte be expressed in that unit of measure, such as for high-sensitivity C-reactive protein. The unit of measure U/L will be used for most enzyme activites.
3. Only traditional units will be used for tables and figures in the printed version of a report; SI conversion factors will be provided in legends. All tables and figures will also be presented in SI units. These tables and figures will be made available in online supplements to published articles and letters. Authors will provide both versions before final acceptance of a manuscript. SI units are available at Bureau International des Poids et Mesures.

- Supplemental Data are accepted for online publication only and are limited by submission types (See Types of Submissions for details).
- Follow the guidelines for length restrictions, abstract, reference, table and figure, and supplemental data limits as outlined in the chart below:

Type of Submission	Word Limit*	Structured...(S) or Unstructured (U) Abstract: Word Limit	Maximum Number of References	Total Number of Tables/Figures	Supplemental Data Permitted
Article	3,500	S 250	40	6	Yes
Citation Classics	600	Nonapplicable	6	Nonapphcable	No
Clinical Case Studies (Case description) w/ 3-5 questions and up to 5 points to remember	1,000 (500)	Nonapplicable	10	2	No
Commentary	300	Nonapplicable	2	Nonapplicable	No
Editorial	1,500	Nonapplicable	15	Nonapplicable	No
Letter to the Editor / Reply	750	Nonapplicable	5		No
Mini-Review	3,500	S. 250	40	4	Yes
Obituary	600	Nonapphcable	Nonapplicable		No
Opinion	1,500	Nonapplicable	15		No
Perspective	1,500	Nonapplicable	5		No
Point/Counterpoint	1,500	Nonapplicable	15		No
Reflection	2,000	Nonapplicable	20		No
Review	5,000	S. 250	75	6	Yes
Special Report	5,000	S or U. 250	40	4	Yes

*Word limit consists of the body of the manuscript only; it does not encompass the title page, abstract, acknowledgments, references, tables, figure legends, figures, or Clinical Case descriptions, questions, and points to remember.

**Structured abstracts contain the headings (1) BACKGROUND, (2) METHODS, (3) RESULTS, (4) CONCLUSIONS for all applicable article types except for Reviews and Mini-Reviews. Abstracts for Reviews and Mini-Reviews contain the headings (1) BACKGROUND, (2) CONTENT, (3) SUMMARY.

***If a figure accompanies the paper, the image should not be multipart (i.e., Fig. 1A, 1B, 1C, Part 1, Part 2).

SUBMISS/ ON/PUBLICAT/ON FEES

Authors are not required to pay submission fees in order to submit their work to *Clinical Chemistry.* If color figures are present in the submission, authors of accepted papers may be required to pay a fee for color printing at the time of publication.

Authors have the option to request that their papers be made open access immediately at the time of online publication, for a fee of $3500. For more information or to request open access publication, contact the Editorial Office at clinchemed@aacc.org. All papers are made open access one year after publication.

JOURNAL CATEGORIES

Articles are grouped in the journal according to subject. Upon submission, authors are required to select the journal category that best describes their manuscript from the list indicated below:

- Molecular Diagnostics and Genetics (MDG)
- Evidence-Based Laboratory Medicine and Test Utilization (TUO)
- Hemostasis and Thrombosis (HAT)
- Proteomics and Protein Markers (PPM)
- Cancer Diagnostics
- Lipids, Lipoproteins, and Cardiovascular Risk Factors (LLP)
- Drug Monitoring and Toxicology (DMT)
- Hematology (HEM)
- Endocrinology and Metabolism (END)
- Point-of-Care Testing
- Automation and Analytical Techniques (MT)
- Informatics and Statistics
- Laboratory Management (LMA)
- General Clinical Chemistry (GCC)
- Animal Clinical Chemistry (ANI)
- Clinical Immunology (CLI)
- Pediatric Clinical Chemistry (PED)
- Nutrition (NUT)
- Infectious Disease

- Other Areas of Clinical Chemistry (OTH)

TITLE PAGE

The first page of the manuscript should include the following information:

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