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Full Paper

Journal of Hong Kong Institute of Medical Laboratory Sciences 2019-2020 Volume 16 No 1 & 2

A Common Sense Approach for Establishing QC Frequency

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Abstract

Background: There is a wide variation in laboratory practices with regard to implementation and review of internal quality control (IQC), particularly against requirements of laboratory accreditation organizations or bodies. A poor approach can lead to a spectrum of scenarios from validation of incorrect patient results to over investigation of falsely rejected analytical runs. Major problems in IQC are the number and levels of control materials, the frequency of QC and the use of patient samples as QC. And yet every laboratory worker knows that the frequency of running QC is often problematic.
Methods: Simple rules and models are established for the purpose of adopting quality standards to ensure that the results of the laboratory service that are provided meet the specific requirements and characteristics of the clinical users. We make use of a common sense approach, based on risk management and patient safety to determine the frequency of QC to control the analytical process individualizely without the application of complex rules and/or models.
Results: Ideally patient samples should be run in batches, perhaps every 50 or 100 patient samples, starting and ending with a QC evaluation. Most analyzer systems presumably, run 60 tests per hour. Additional QC should be run every 60, 50, 40, and 30 patient samples for Sigma values of 6.0, 5.0, 4.0 and 3.0, respectively. Other factors may cause the laboratory to decide to test controls more frequently, which include stability of the analyte and the method system, number of patient tests that are routinely performed, Sigma Value (frequency, levels and rules), Clinical Alert (0.1, 0.2, 0.3 ; low, moderate, high), Calibration Stability (0.3, 0.2, 0.1 ; low, moderate, high), Reagent Stability (0.3, 0.2, 0.1 ; low, moderate, high), and Error Rates (0.1, 0.2, 0.3; low, moderate, high).
Conclusions: The minimum frequency for QC testing is the frequency defined by the manufacturer or the frequency defined by the regulatory agency that inspects or assesses your laboratory, whichever is more stringent. However, adverse effects to the Sigma Metric may lead to an increase in the frequency of QC, based on a calculated Risk Score, and the number of patient samples for individual tests.

Key words: *quality control, patient safety, accreditation, Risk management*

Introduction

Laboratories across the Asia Pacific region are facing significant challenges in meeting demands for quality of their services despite more and more laboratories are in progress of application or have successfully completed their accreditation programs. ISO 15189 is a globally recognized standard developed specifically for medical laboratories. It provides a mechanism for regulating authorities and accreditation bodies to confirm the technical competence of a laboratory in meeting specific requirements consistent with delivery of quality test results. The latest revision of ISO 15189: 2012, in one of the technical requirements 5.6.2.1states that: "*The laboratory shall design quality control procedures that verify the attainment of the intended quality of results."(Note: In several countries, quality control, as referred to in this subclause, is also named "internal quality control.")*. [1]

There is a wide variation in laboratory practices with regard to implementation and review of internal quality control (IQC), particularly against requirements of laboratory accreditation organizations or bodies. Such IQC policies and procedures are designed primarily to detect those deficiencies in the workflow and control analytical process. A poor approach can lead to a spectrum of scenarios from validation of incorrect patient results to over investigation of falsely rejected analytical runs. However, there is no specific guidance in this clause. It has been recommended that guidance on QC issues should be sought from publications of the relevant professional societies. Supplementary criteria are published elsewhere. [2, 3] Major problems in IQC are the number and levels of control materials, the frequency of QC and the use of patient samples as QC. And yet every laboratory worker knows that the frequency of running QC is often problematic.

There is also an increasing need to determine whether traditional QA/QC mechanisms are truly adequate for risk management (i.e. in terms of reduction of the probability of error reporting and minimizing patient harm). Recent experience has shown that mistakes are the most common cause of problems in health care as well as in other industrial environment. [4] Excessive product and process complexity in the medical laboratory contributes to both excessive variation and unnecessary mistakes. Strategies for mistake proofing have to be established for preventive measures. Using these mistake proofing techniques, nonconformities can be controlled at an affordable cost of the traditional QC methods. The traditional QC procedures based on statistical process control (SPC) could effectively cut down the probability of false rejection (Pfr) and increase the probability of error detection (Ped).

Methods

Simple rules and models are established for the purpose of adopting quality standards to ensure that the results of the laboratory service that are provided meet the specific

requirements and characteristics of the clinical users. We make use of a common sense approach, based on risk management and patient safety, to determine the frequency of QC to control the analytical process individually without the application of complex rules and/or models.

First of all, the common sense approach is based on the following strategic planning and strategic thinking of the QC experts in the field.

(I) Practical QC Design Process as proposed by James Westgard, PhD[5]

1. Define TEa for test of interest

2. Estimate SD or CV from routine QC data

3. Estimate Bias from comparison of methods, PT survey, Peer Comparison

4. Calculate Sigma =(%TEa-%Bias)/%CV

5. Use QC software or paper charts to translate Sigma into Statistical QC (control rules, N)

(II) Ideas for QC Strategy that Don't Require Math Calculations as suggested by Curtis Parvin, PhD[6]

1. Always end patient testing with a QC.

2. Make the time between QCs shorter than the time needed to correct results.

3. Compare CVs to your peers, if you're not in the top 20% -get there.

4. Set the QC Target to the group mean for multiple instruments.

5. Know your number of patient tests between QCs.

6. Estimate the magnitude of the Out of-Control condition before correcting it.

7. Use a realistic TEa: What magnitude will cause patient harm?

8. If running a 1:2s rule and you have a failure, repeat it. Just ONCE.

9. Divide analytes into High/Low Sigma groups

a. High Sigma rules goal: reduce false rejection rate

b. Low Sigma rules goal: increase frequency of QC

The minimum frequency for QC testing is the frequency defined by the manufacturer or the frequency defined by the regulatory agency that inspects or assesses your laboratory, whichever is more stringent. Other factors may cause the laboratory to decide to test controls more frequently. These factors include:

1. Stability of the analyte and the method system

2. Number of patient tests that are routinely performed

3. Change of instrument operators at change of work shift

4. Change of reagent lots

5. Recalibration

A good common sense approach to begin is to consider which assays are more stable compared to others? Some assays naturally perform better than others, giving

consistently better results. On the other hand, some assays perform inconsistently, having a higher rate of error and much lower stability. It's important that laboratories can recognize which assays are more stable and consistent in comparison to others and ensure that they are running QC at an appropriate frequency i.e. higher rate of error and much lower stability. Secondly, which tests are at higher risk and have a higher impact for an erroneous result? It's important that you run QC more frequently for higher risk tests. With higher risk tests there is a greater risk of harm to the patient; therefore it's of utmost importance that the results released are both accurate and reliable.

There is no straightforward answer to how frequently that the laboratory should run QC. Ideally patient samples should be run in batches, perhaps every 50 or 100 patient samples, starting and ending with a QC evaluation (Table 1). This will ultimately save time and repeats, in case of an out of control condition, and most importantly will reduce the risk of harm to the patient. Therefore, it is vital to consider the time between QC evaluations in correlation to the number of patient samples being tested. There is a good rule of thumb to ensure that the laboratory has selected an appropriate QC frequency i.e. keep the time between QC evaluations shorter than the time needed to undertake any corrective action in the case of an erroneous result, at least every 8 hours of operation or every shift duty (Table 2). Finally QC samples should also be tested before and after any event that has the potential to adversely affect the testing process e.g., change of reagent batch, instrument maintenance and calibration (Table 3). Testing prior to the event provides confidence that patient results since the last successful QC check are reliable. Testing QC samples immediately after the event ensures the test system is in control prior to running more patient samples.

Table 1. Basic QC Requirements

- Start up QC
- End-of-Run QC
  - end of batch
  - end of shift
  - end of day
- Event (adverse effect) changes
  - change of reagent batch
  - instrument maintenance and calibration

Table 2. QC Frequency Common Sense Approach

Every *8 hours* of operation

Table 3. Adverse Factors that would affect QC frequency

- Sigma Value (actual number)
- Clinical Alert (0.1, 0.2, 0.3 ; low, moderate, high)
- Calibration Stability (0.3, 0.2, 0.1 ; low, moderate, high)
- Reagent Stability (0.3, 0.2, 0.1 ; low, moderate, high)
- Error Rates (0.1, 0.2, 0.3; low, moderate, high)
- Risk Scores

Results

Ideally patient samples should be run in batches, perhaps every 50 or 100 patient samples, starting and ending with a QC evaluation. Most analyzer systems presumably, run 60 tests per hour. Additional QC should be run every 60, 50, 40, and 30 patient samples for Sigma values of 6.0, 5.0, 4.0 and 3.0, respectively (Table 4). Other factors may cause the laboratory to decide to test controls more frequently, which include stability of the analyte and the method system, number of patient tests that are routinely performed, Sigma Value (Table 5-frequency, levels and rules), Clinical Alert (Table 6-0.1, 0.2, 0.3 ; low, moderate, high), Calibration Stability (Table 7-0.3, 0.2, 0.1 ; low, moderate, high), Reagent Stability (Table 8-0.3, 0.2, 0.1 ; low, moderate, high), and Error Rates (Table 9-0.1, 0.2, 0.3; low, moderate, high).An example of the frequencies of QC for a few chemistry tests with different Sigma values for their respective QC levels is illustrated in Table 10.

Table 4. Assumption(s)

- Most autoanalyzers presumably, run 60 tests per hour
- If Sigma Value:
  - =6 run 1 additional QC every 60 samples
  - =5 .....every 50 samples
  - =4 .....every 40 samples
  - =3 .....every 30 samples
  - =2 .....every 20 samples

Table 5. QC Frequency (Collective Opinion Paper)

- >6σ (excellent performance) – evaluate with one QC per day (alternating levels between days) and a 1-3.5s rule.
- 4σ–6σ (suited for purpose) – evaluate with two levels of QC per day and the 1-2.5s rule.
- 3σ–4σ (poor performance) – use a combination of rules with two levels of QC twice per day.
- <3σ (problematic) – maximum QC, three levels, three times a day. Consider testing specimens in duplicate.

Clin Chem Lab Med 2011; 49: 793-802.

Table 6. Clinical Alert Level

- For an outpatient service, one can estimate this cycle time to be as long as 2-3 days or even one month.
- For a non-acute hospital setting, the test repeat cycle may be perhaps 4 hours.
- For an intensive care setting, the cycle time may be as short as 1 hour for chemistry tests and 30 minutes for blood gases.

Table 7. Calibration Stability

- Manufacturer's recommendations
- Calibration intervals
  - >1 month
  - 1 week to 1 month
  - <1 week

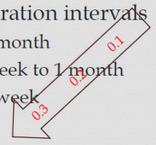

Table 8. Reagent Stability

- Manufacturer's recommendations
- Once opened
  - >1 month
  - 1 week to 1 month
  - <1 week

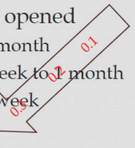

Table 9. Error Rates

- Error Rate Categories
  - Low = method that experiences <3% QC flags/year
  - Moderate = method that experiences 3-10% QC flags/year
  - High = method that experiences >10% QC flags/year

Per year or Per month

Table 10. Risk Assessment Metrics

Adverse effects to Sigma Metric

Test	QC Level	Sigma Value	Clinical Alert Level	Calibration Stability	Reagent Stability	Error Rates	Risk Score	QC Frequency No. of Patient Samples per QC	Samples per Day	QC per Day
Serum K	1	5	0.3	0.1	0.1	0.1	4.4	44	500	11
ALT	2	6	0.2	0.1	0.1	0.1	5.5	55	500	9
Ca	1	4	0.3	0.3	0.3	0.3	2.8	28	300	11
Glu	3	6	0.3	0.1	0.1	0.1	5.4	51	200	4
FT4	2	3	0.2	0.3	0.2	0.2	2.2	21	100	5
AFP	3	3	0.1	0.1	0.2	0.2	2.4	24	50	2
TnI	1	4	0.3	0.2	0.2	0.1	3.2	32	20	-

Discussion

Excessive product and process complexity contributes to both excessive variation and unnecessary mistakes. The whole purpose of QC is to give you confidence in the quality of the results that you are reporting. Those metrics should include measurements to determine if control systems are actually in control. CLSI EP23 says; "*Medical judgment is used to estimate the overall probability of harm due to receiving an incorrect result…*" and hence, ***Patient Safety*** concerns. [7]

Currently manufacturers of devices do not give much, if any information about device risk. Complex and ever-changing national or international regulations controlling the marketing of medical *in-vitro* diagnostic (IVD) devices are forcing manufacturers to provide risk assessment (analysis and evaluation) of their IVD products including their QC procedures but there have been no global consensus or harmonization yet. CLSI (EP23) also recommends the use of risk management for customizing QC in the laboratory. In this approach for "alternate QC," IVD manufacturers would make a risk assessment, including analysis and evaluation of their products, eliminate or reduce risks of failure as far as possible, mitigation those risks, and report the "residual risks" to the laboratory, that would then help the laboratory identify those potential risks before customize its QC plan. In case the risk(s) cannot be eliminated, inform the laboratory of the residual risks. This customization should include mechanisms for prevention measures, a primary concern being the frequency of QC to monitor the residual risks and implications for patient safety. Current approaches to Patient Safety Risk Analysis and Management are based on the Failure Mode and Effects Analysis (FMEA), which is a semi-quantitative, proactive approach with engineering origins in industry. ISO 14971:2007 specifies a process for a manufacturer to identify the hazards associated with medical devices, including *in vitro* diagnostic (IVD) medical devices, to estimate and evaluate the associated risks, to control these risks, and to monitor the effectiveness of the controls. [8] Quality costs are offset by quality payoffs. In an era of financial constraint, nowadays, laboratories are always being asked to do more with less.

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But every time work is redone e.g. corrective actions needed due to QC failures, the cost and the potential adverse effects on patient safety continue to challenge the laboratories, and therefore, the cost of quality -increases. [9]

Conclusions

The minimum frequency for QC testing is the frequency defined by the manufacturer or the frequency defined by the regulatory agency that inspects or assesses your laboratory, whichever is more stringent. However, adverse effects to the Sigma Metric may lead to an increase in the frequency of QC, based on a calculated Risk Score, and the number of patient samples for individual tests. The laboratories are recommended to run QC more frequently for high risk and unstable tests; start and end patient testing with a QC evaluation; and make the time between QC evaluations shorter than the time needed to take corrective action in the case of an erroneous result.

In summary, many of the requirements of ISO 15189 are all principles and not practical. Although total laboratory QC management systems are in place in all accredited medical laboratories, patient harm does occur due to laboratory errors in routine practices, and there is still room for improvement. A common sense approach integrating risk management and patient safety into ISO 15189 IQC compliance, despite lack of theoretically statistical support, will act as a continuous improvement process, potentially reduce the probability and severity of incidents and create action plans to improve coordination of patient safety and more reliable laboratory service performance over time.

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Competency of Medical Laboratory Technologists in Hong Kong

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Competency has been defined as "the ability to perform the activities within an occupation or function to the standard expected in employment". The term "competency" embodies attributes such as knowledge, skills, abilities, attributes and attitudes required in professional practice. Competency may be core, general or task-specific. During any one procedure it is expected practitioners will demonstrate elements of practice across a number of broadly-defined domains of competence. This recognises that competent professional practice is more than a sum of each discrete part. It requires an ability to draw on and integrate the breadth of competencies to support overall performance.

Competencies can be used in a variety of ways. Competencies are a way to address both the technical skills of a job and the more difficult-to-define behavioral expectations of a job – sometimes referred to as the "soft skills." , which they are well-defined set of competencies can help an organization better evaluate and measure performance. Competency models focus on accountability of the practitioners. Organizations are required to not only determine the level of competency, but to document how it was verified. They can be integrated into performance appraisals, hiring practices, succession planning, as well as on-boarding orientations and other forms of employee communication.

Background

At the Supplementary Medical Professions ("SMP") Council's meeting held in April 2019, the Council Chairman instructed at the meeting that all 5 SMP Boards should work out a document on core competences and skills expected of the respective SMP graduates. One of the purposes of the competency document is to provide the academic institution with a benchmark for designing and delivering a curriculum in meeting the registration requirements and standard. With the competency document, the re-accreditation exercise will be conducted on competency-based approach, instead of curriculum based one, as it is a global trend to make outcome-based assessment in accreditation.

The Medical Laboratory Technologists Board is established under the Supplementary Medical Professions Ordinance, Cap. 359. The Board's main function is to maintain a register of persons practising medical laboratory science and to promote adequate standards of professional practice and professional conduct amongst these persons. The Medical Laboratory Technologist ("MLT") Board has agreed to task the Registration Committee of the Board ("BRC") to develop the document on core competences and skills expected for registered MLT in Hong Kong.

During the preparation of this article, the "Competency Document for Medical Laboratory Technologists in Hong Kong" is undergoing internal consultation and discussion within MLT Board, therefore as the Chairman of the BRC and who has been writing up the first draft, here would like to have a very general overview of the document, so that as an introduction and engage more professionals to understand the process and the importance of developing this document. Providers of pre-registration education programmes are expected to use the competence document to inform the development of graduate curricula. This will assist new registrants in understanding the professional competencies required of them once registered.

This document specifies competency statements for registered MLT in Hong Kong in a diagnostic pathology setting. These standards have been developed to reflect the contribution normally expected from an entry level of a scientist to this profession with a combination of qualifications, skills and the assumption of personal responsibilities and accountability. This document concentrates on the general competencies for the Medical Laboratory Technologists.

The competency standards described here will cover both generic and functional competencies as well as from initial to ongoing competence which would be required over additional time and are expected of from working medical laboratory technologists performing supervisory roles. A MLT shall be competent in a wide range of skills, including investigative, analytical, problem solving, planning, communication, presentation, team building and leadership skills and has to demonstrate a contemporary knowledge and understanding of the broader issues of clinical laboratory practice.

Given the wide range of professional groups encompassed by "Medical Laboratory Science", it is intended that a registered MLT may be measured against these competencies in relation to a specific discipline or across several disciplines.

In contrast, professional and discipline-specific competencies refer to individual disciplines (e.g. haematology, microbiology, clinical chemistry, anatomical pathology, etc.) will not be elaborated and included in the competency document.

Purpose of the "Competency Document for Medical Laboratory Technologists in Hong Kong"

This document contains sets of competence statements which serve purpose to provide the undergraduate teaching institution with a benchmark with which to establish and apply outcome measures to assess the effectiveness of the undergraduate programme. Competency assessment is required to determine the effectiveness of initial training and readiness to function in work environment. It has to be noted in this document, only major competences are outlined. It offers the flexibility and diversity for any teaching institutions to define related supporting competences in order to achieve all major competences and these standards may change in the future.

While this document on competence statements can be useful in accreditation of any medical laboratory science teaching institution which provide undergraduate programme, it is understood that other standards on institutional effectiveness, faculty and staff, educational support services, patient care services and research programme should also be identified for the purpose.

Reference information used during the development of the competency document including the competency documents for biomedical scientists adopted by the UK and for medical scientists by Australia, core competencies at workplace in Hong Kong, and the competency documents for the Hong Kong registered healthcare professionals, all serve to be the reference information during the process. Furthermore, The Code of Practice for Registered MLT issued by the MLT Board outlines the basic ethical principles that a registered MLT shall follow, is also being included as a reference.

The scope of competency domains

The present document is structured from the general to the more specific competency domains. Five domains have been identified that represent the broad categories of professional activity and concerns that occur in the general practice of registered MLT. The details will be accessible during the consultation process after internal discussion and upon advice from the MLT Board. The following domains are subject to further review.

1: Professional Ethics, Attitude and Behavior

2: Functional Competency
2.1 Provision of diagnostic services
2.2 Ability to provide timely, accurate and reliable laboratory analyses to enable clinical diagnosis
2.3 Operation Management of a medical laboratory

2.4 Management of occupational health & safety and Risks

3: Personal Competency
3.1 Effective communication
3.2 Effective decision making
3.3 Integrity

4: People and Team Competency
4.1 Teamwork
4.2 Leadership
4.3 People development

5: Organizational Effectiveness and Quality Management

Competency standard and descriptions

Within each domain, one or more "competency standard" is identified as relating to that domain's activity. Under each competency standard, descriptions are laid out to assist the interpretation of the standard and for providing guidance in formulation of assessment checklist in future. Its complexity suggests that multiple and more specific abilities are required to support the performance of any competence standard

Consultation and review

A formal consultation during the development of the process is required. We will continue to listen to our stakeholders and will keep our competency statements or standards under continual review. Therefore, we may make further changes in the future to take into account changes in practice. We will always publicize any changes to the standards that we make by, for instance, publishing notices on our website and informing professional bodies, as required.

Conclusion

A Competence document with specified standards are a description of the ability of a medical laboratory science practitioner to practise safely and effectively in a variety of contexts and environments. The competence standards have been articulated so as to be sufficiently broad-based to allow for universal applicability across a variety of practice settings, while at the same time

being sufficiently focused to articulate the particular competencies specific to medical laboratory science practice.

Competence is influenced by many factors including, but not limited to, the practitioner's qualifications, clinical experience, professional development and his/her ability to integrate knowledge, skills, attitudes, values and judgements within a practice setting. A critical value of competence standards is the capacity to support and facilitate professional practice and growth. Developing a competency framework can take considerable effort. To make sure the framework is actually used as needed, it's important to make it relevant to the people who'll be using it – and so they can take ownership of it. This framework serves the purpose to provide the undergraduate teaching institution with a benchmark with which to establish and apply outcome measures to assess the effectiveness of the undergraduate programme. Proper engagement of stakeholders is required to gain continued support and collecting feedback. Board Registration Committee of MLT Board has been reviewing the competence document which intended to be sufficiently flexible and versatile to be relevant to a variety of stakeholders. We hope the MLT Board can use the competence document to serve purpose to provide the undergraduate teaching institution with a benchmark with which to establish and apply outcome measures to assess the effectiveness of the undergraduate programme. In addition to, it could serve as a reference point of professional competence when exercising its statutory functions.

The utilitarian perspective - an ethical issue of predictive genetic testing on ovarian cancer

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Abstract

With the development of predictive genetic testing, the woman at high risks for breast cancer and ovarian cancer can be identified by the BRCA 2 gene. Certain arguments were raised including relatives of someone with a positive predictive genetic test should be informed of the results, the concern of patient confidentiality and the threat of discrimination. The moral theory of utilitarian supports predictive genetic tests, the principles of maximizing benefits, minimizing harm, and justice were employed to explain the ethical standpoints on these issues. The recommendations and precautions of the predictive genetic test were illustrated by the American College of Obstetricians and Gynecologists (ACOG). In conclusion, the healthcare practitioner should recognize the ethical, psychological and social impact behind predictive genetic testing, so that to minimize the negative impact on the patient.

Key words: predictive genetic testing, ovarian cancer, utilitarian, moral, ethical

Background of ovarian cancer

Ovarian cancer ranks sixth among the top 10 female cancers in Hong Kong. There are about 400 new cases every year with most of the patients over 50 years of age.[1] The diagnosis and treatment are often delayed, the reasons are the ovaries located deeply inside the pelvic cavity and early symptoms are not obvious to recognize. Besides, abdominal discomfort is the major complaint during the early stage of the disease, but many patients recognize it as stomachache or indigestion.[2]

Investigation and diagnosis of ovarian cancer

If ovarian cancer is suspected after health history taking, the doctor may request radiology examinations or laboratory tests to confirm the diagnosis. For example, vaginal examination, ultrasound, blood tests on CA125 index, computerized tomography scan (CT scan) and magnetic resonance imaging (MRI) to detect the location of the tumor. Besides, the laparoscopy to visually inspect the inside of the abdomen cavity may use to inspect the tissues inside the abdominal cavity and a sample of the tumor will be taken for pathological diagnosis when found. Moreover, a biopsy will be taken to classify the cell type of the abnormal cells. If ovarian cancer is highly suspected, other examinations may be needed to determine the area affected by the tumor such as chest X-ray, CT scan or MRI.[1]

Risk factors of ovarian cancer

There are several risk factors for ovarian cancer, including obesity; high-fat diet; spontaneous abortion or infertility; family history of breast cancer, colorectal cancer and/ or ovarian cancer. Research studies have shown that a woman with a single first-degree relative (e.g. mother, sisters or daughter) with ovarian cancer, the relative risk will be approximately 3.6 times for developing ovarian cancer compared with the general population. If the woman has three or more first-degree relatives who have ovarian or ovarian and breast cancer, a genetic mutation on BRCA1 gene and/ or BRCA2 gene will be predicted for that family and transmitted in an autosomal-dominant inheritance.[1,2]

Predictive genetic test on ovarian cancer

Presently, predictive genetic testing on BRCA genes is available aimed at identifying woman at high risks for breast cancer and ovarian cancer,[3] however, the negative result could not exclude the person will get the disease or not. There are ethical issues towards the use of this predictive genetic test for patients, her family, and relatives, physician, and society.[4] For example, the information of test and informed consent matter; the right to be tested or not; the rights of others, confidentiality and privacy concerns, together with the threat of discrimination.

Predictive BRCA genetic test

Arguments

In a general view, the suspected woman should take the test as she has her right to know about her health. On the contrary, there are some arguments against her to take the test. Firstly, a positive test does not necessarily mean that she will get ovarian cancer. Secondly, a negative test result does not mean that she will not get ovarian cancer. Thirdly, some of her family members may not want to know this genetic risk or know her test result. Lastly, there are neither lifestyle changes nor medical interventions that are effective at preventing ovarian cancer, even though defensive surgical removal of both ovaries. While in utilitarianism theories, how ethical principles and theories can be adopted on a predictive genetic test?

An explanation of the Utilitarianism

Utilitarianism defines the right action as the action that maximizes the total well-being. When we assess a person's well-being, we are assessing how well or badly that person's life is going.[5] According to the utilitarian perspective, it generally supports genetic testing. The following paragraphs have explained and illustrated some key concepts of this theory:

Maximize benefits to the patient and her relative, doctor, and society

It is described as individuals should try to directly help others, acting in other's best interests. From a utilitarian's view, it is morally acceptable to promote overall well-being to other persons, even if sacrifice a person's interest.[5] The woman should take the test and her test result should be informed to her family members by herself or her doctor. No matter the result is positive or negative, her test result can be maximizing to more persons, especially her family members or relatives that are at risk.[6] Besides, the moral decisions should be decided by calculating a burden over benefit ratio from a societal viewpoint. It promotes the good of society over that of the individual. For example, the utilitarian regards if having the test, both the woman and her doctor will be surer about whether the BRCA gene was presented and the risk of getting ovarian cancer. As the BRCA gene may cause a severe and lethal ovarian cancer in a vertical inheritance, her family members should be noticed of her results. The goods are the family members would be more certain to make their decision for taking the genetic test themselves, then their test results may allow them to take medical intervention for decrease the intensity of pain for them; reduce the duration of symptoms; delay the onset of the possible propinquity symptoms. Also, they would have more adequate planning for themselves and have the lifestyle changes to lessening the certainty of physical pain and mental stress that may occur; increase their quality of life as well as prevent the passing on the BRCA gene to their next generation.

If the test result is negative, she and all her family members will be happier by feeling more relieved. Even if her test result is positive, it does not mean that she must develop ovarian cancer, but she can be proactive about her health conditions. By taking the test, she may have more chances for alternative treatment for curing herself and helping her family members in the future. It allows all of her family members to make earlier arrangements for their living, all of them would have more time for psychological preparation on health care planning, and ready to support anyone who may get ovarian cancer. For the doctor and patient relationship, her test result would let both patient and doctor with real-life experience gain. For the societal aspect, the test results may contribute to medical research on ovarian cancer detection and treatment on account of the genetic composition of each family may express differently. The findings may further assist the development of a more effective protocol for a particular patient.

Minimize the harms of patient and family members

It is meant an obligation not to inflict harm intentionally. When knowing about test results regardless of positive or negative, the result may lessen the long-term stress of the woman being unknown of conceivable risk of getting ovarian cancer. By informing family members of her test results, it may allow them to protect future generations of getting potential harm if they chose not to have a child.

Justice to the society

On the whole, justice is interpreted as individuals who are equals should qualify for equal treatment such as the risks, resources, and costs should be distributed equally.[7] As the test results have a strong risk factor for ovarian cancer, she should tell her family members who are at risk, especially those who want to know as it is fair to them. Then, they may receive equal opportunities to perform BRCA tests and various kinds of examination, preventive measures, medical intervention, genetic counseling, and suitable treatment if they find to be required. Besides, the woman who is earlier being noted of the possibility of cancer, the society may spend lesser resources and lower costs on treatment.

Recommendation and precaution on predictive genetic test

American College of Obstetricians and Gynecologists (ACOG) guidelines

In general, when physicians recommend patients for the predictive test, they should aware of the inclusion and exclusion, the pre-test genetic counseling on patients to be prepared for tests, informing and disclosure about the results of pre-test and post-test counseling. At present, ACOG has four guidance for Obstetricians and Gynecologists (O&G) to follow:

- "Clinicians should be able to identify patients within their practices who are candidates for genetic testing & should maintain competence in the face of increasing genetic knowledge."
- "Clinicians should discuss with patients about the importance of genetic information for their kindred, as well as

a recommendation that information is shared with potentially affected family members as appropriate, should be a standard part of genetic counseling."

- "O&G physician should recognize that geneticists & genetic counselors are an important part of the health care team & should consult with them and refer patients as needed."
- "O&G physician should be aware that genetic information has the potential to lead to discrimination in the workplace and to affect an individual's insurability adversely. Steps that physicians can take to fulfill this obligation could include, among others, advocacy for legislation to ban genetic discrimination."

Genetic counseling on pre-test and post-test

Adequate genetic counseling is important to both patient, family and relatives. The counseling should assist individuals to cope with the continuing uncertainty regarding the potentially inherited nature of the gene in the offspring, and regarding their genetic status. Meanwhile, counseling can prevent false reassurance, after all, a 'negative' test result in a given family does not exclude the inherited nature of cancer in this family. Because of the future development of a more informative genetic test, the counselor should also discuss the possibility of re-contacting the patient.[3]

The post-test counseling correspondingly serves a variety of purposes. At first, psychosocial counseling can help individuals to cope with either a positive or negative test result. Some individuals have profound difficulty in accepting 'negative BRCA1 results.[9] Second, the post-test counseling concerns medical management. After all, the major motivation for suggesting predictive testing is the hope for medical management following a 'positive' test result, which can help reduce patient morbidity and mortality. The counselor should provide adequate and balanced information about various options to the patients, and guide them in deciding with measured patient's values and preferences.[3]

Disclosure of genetic information by healthcare professionals

Genetic information is private and is directly related to an individual identity. Social stigmatism is attached to genetic disorders. Individuals having genetic defects are often discriminated against and harassed by other members of society or even family and friends. Therefore, healthcare professionals should be aware of the negative issues that will arise when disclosure of genetic test results. For example, discrimination in health insurance, the insurance company may not accept the person who noticed the pre-disposition of cancer risk.[7] The discrimination may occur in the workplace as an employer predicted the employee carries the defective gene will potentially get more sick leave than a usual employee.

Conclusion

To summarize, genetic information is necessarily imprecise for it deals with probabilities and risks, it can be easily manipulated, especially under social pressure. For a healthcare practitioner, they should be competent to provide ongoing accurate information to the patients; understand the ethical, psychological and social ramifications behind predictive genetic testing. Considering the patient aspect, they should respect individual beliefs and priorities, together with sensitivity to individual differences in perception of their genetic risk. For societal aspect, they should participate in educational programs not only on technical and medical context, but also promote to the general public for mutual understanding of the genetic test, and hope to reduce the probable misconception or discriminations.

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Serum ferritin concentration in a local Hong Kong population from private sector

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Abstract

Background: Ferritin is an essential protein stores of iron in a non-toxic form and transports it when the human body needs it for haemoglobin synthesis. Thus, measurement of serum ferritin level is a reliable indicator for assessing iron storage status in the body. Iron deficiency in blood has led to growing number of deferred blood donors in Hong Kong.

Objective: This study assessed the iron status in both male and female subjects in Hong Kong, and further compared the derived reference intervals and cut-off values recommended by World Health Organization (WHO) and manufacturers, in order to aid the establishment of serum ferritin reference intervals in a Hong Kong Chinese population. The correlation between CBC parameters and serum ferritin were investigated. This study also assessed the use of Combined Cell Index (CCI) for the assessment of iron storage.

Methods: A retrospective study was undertaken which was based on data derived from a private clinical laboratory in Hong Kong, in which patient raw data was obtained from January 2011 to December 2017. Statistical analysis of serum ferritin, haematological parameters [haemoglobin (Hgb), haematocrit (HCT), red blood cell (RBC), mean cell volume (MCV), mean cell haemoglobin (MCH), mean cell haemoglobin concentration (MCHC), red cell distribution width (RDW)], sex and age were performed. After applying inclusion and exclusion criteria (subjects having haemoglobin concentrations within the adult sex specific haemoglobin reference intervals), 1435 (85.2%) female and 249(14.8%) male subjects were selected for data analysis.

Results: The derived serum ferritin reference intervals in this study were 4.7-202.7 μg/L (female) and 23.6-273.45 μg/L (male) respectively. 331 female (19.7 %) and 8 male (0.5%) subjects had serum ferritin levels lower than 15 μg/L (WHO's cut-off). The highest AUC value was recorded for CCI (AUC=0.758) in female subjects, RDW (AUC=0.945) in male subjects and Hgb (0.775) in the total study population.

Conclusion: WHO's serum ferritin cut off may not be suitable in Hong Kong, which is the main reason why there is an increased of deferred blood donations. Results illustrated a potential diagnostic value of CCI in assessing iron stores, suggesting the potential use in daily routine.

Key words: serum *ferritin, haemoglobin, iron status*

Introduction

There is a constant need for regular blood donation in Hong Kong medical service, as the number of blood donors has been increasing in recent years. However, there is also a growing number of deferred blood donors due to their low haemoglobin level- a protein in blood cells which carries an iron molecule and mainly aids the transport of oxygen in the body. Thus, the main reason of deferred donors is iron deficiency in their blood.[1]

The function of iron is vital to human, transporting oxygen from lungs to other organs or tissues. The total iron content of an adult body is approximately 4 grams, in which two-thirds comes from haemoglobin. Iron storage in spleen, liver and bone marrow contain about a quarter of the body iron, while the remainder of iron locates in myoglobin. Only 0.1% of the total body iron exists in the plasma, where it is nearly all bound to a transport protein-called transferrin. Iron also contributes to the production of hormones, tendons, and ligaments.[2]

Iron status assessment is required when iron deficiency or overload is suspected or when there are signs of abnormal metabolism or distribution of iron. [3] Conventional haematological tests include haemoglobin and red cell indices (MCV, MCH, MCHC, RDW). Iron is stored mainly in the form of ferritin, which a ferritin molecule may consist of over 20% iron by weight when it is fully saturated.[3]

Ferritin is an essential protein that stores iron in a non-toxic form and transports it when the human body needs it for haemoglobin synthesis. Therefore, measurement of serum ferritin level is a reliable indicator for assessing total iron storage, thus, some blood tests are ordered routinely as a diagnostic testing for iron deficiency anemia.[4]

The accessibility of sensitive methods for measuring serum ferritin levels has significantly advanced the capability to detect iron deficiency and overload. Since iron deficiency is present before the onset of anemia, detection of iron depletion is important for the management of nutritional anemia. Clinical assessment of iron storage status usually depends on determination of serum iron, total iron-binding capacity (TIBC) and transferrin saturation (ratio of serum iron and TIBC) or direct examination of bone marrow after staining for iron.

A low haemoglobin concentration is the most readily observable sign of anaemia, but a significant decline in haemoglobin

level cannot be detected until late onset of iron deficiency anemia. Recent studies suggest that serum ferritin level provides a better sensitivity, specificity and reliability in determining iron deficiency at an early phase. [5] In patients prescribed with oral iron supplement, measurements of serum ferritin have been shown to be useful for monitoring the recovery of iron storage and determining whether therapy can be suspended.[5]

Serum ferritin level has been considered as an important biomarker associated with chronic inflammatory and rheumatologic diseases.[6] Serum ferritin concentration is increased in iron overload (e.g. haemochromatosis), hepatocellular carcinoma. Lung cancer may also lead to a high concentration of serum ferritin as a result of liver cell damage and inflammatory responses. Furthermore, recent study suggests increased serum ferritin level is associated with higher risk of cardiovascular risk factors and the occurrence of insulin resistance in type 2 diabetes patients with familial history.[7] A low serum ferritin level as a result of iron store depletion is highly specific for iron deficiency anaemia, but not necessarily how severe is the depletion as it progresses.[8]

As the concentration of serum ferritin can be altered by various factors, hence correct interpretation of serum ferritin results for apparently healthy subjects relies on the availability of relevant information of the underlying diseases with studies on the correlation of serum ferritin level with other parameters such as complete blood count (CBC) has not been reported in Hong Kong. In addition, reference intervals of serum ferritin levels used by majority of private or government laboratories are usually advised by manufacturers and World Health Organization (WHO), where their data are usually collected from Western countries, and may not be suitable and applicable to our Hong Kong Chinese population with various factors affecting serum ferritin level.[9]

Therefore, the current study aims at investigating the factors which may affect serum ferritin level in aiding clinicians for better diagnosis and treatments and also to aid the establishment of serum ferritin reference intervals in local population that is applicable to the Hong Kong Chinese population. This study also assessed the application of using combined cell index (adapted from *Vuk et al*, 2016) [10] in the assessment of serum ferritin.

Materials and Methods

Subject data

A retrospective study was undertaken which was based on data derived from the Laboratory Information System (LIS) of a private clinical laboratory in Hong Kong, in the period of January 3, 2011 to December 30, 2017. Data on gender, age and results of complete blood count (CBC), serum ferritin

concentration were analysed, correlation between different CBC parameters, combined cell index (CCI) and serum ferritin were observed. The CCI was calculated by the formula: RDW x 10^4 x MCV^{-1} x MCH^{-1}.

Inclusive criteria for non-anaemic subjects included subjects with haemoglobin concentrations within the adult sex-specific haemoglobin reference intervals. The haemoglobin reference intervals for female and male adults from private laboratory were 11.5 – 15.5 g/dL (female); 13.0 – 17.0 g/dL (male) respectively. The haemoglobin reference intervals as recommended by the World Health Organization (WHO) are ≥ 12.0 g/dL for non-pregnant women and ≥ 13.0 g/dL for men at 15 years of age and above.[11] All datasets must pass the exclusion criteria before subjected to statistical analysis. The exclusion criteria were (i) Subjects with haemoglobin concentrations beyond the upper or lower cut-off values of the reference intervals; and (ii) subjects with serum ferritin concentrations outside the upper or lower cut-off values of the reference intervals. The serum ferritin reference intervals for female and male adults from the private laboratory (provided by the manufacturer) are [female (4.6 – 204.0 µg/L) and male (21.8 – 274.9 µg/L) adults]. Other patient personal information was not disclosed, while results of this study had no influence on the clinical management and outcome of all subjects.

Measurement of serum ferritin concentrations

Serum ferritin concentrations were measured by the automated immunology analyser (Abbott Architect i2000, Abbott Laboratory, USA.). The ARCHITECT Ferritin assay is a two-step chemiluminescent microparticle Immunoassay (CMIA) for the quantitative determination of ferritin in human serum and plasma.[12]

Measurement of haemoglobin concentrations

CBC parameters were measured by the automated haematology analyser on EDTA blood samples from the subjects (Sysmex XT-1800i, SYSMEX Corporation, Japan). The following haematological parameters in a CBC test were assessed: haemoglobin (Hgb), haematocrit (HCT), RBC, MCV, MCH, MCHC and RDW.[12]

Data Analysis

Statistical analysis of serum ferritin, CBC parameters: haemoglobin (Hgb), mean cell volume (MCV), mean cell haemogoblin (MCH), red cell distribution width (RDW), and combined cell index (CCI) were performed by IBM SPSS statistics version 23 (IBM, New York, U.S.) and MedCalc (MedCalc Software, Ostend, Belgium) and Excel 2016 (Microsoft, Washington, U.S.). All data were categorized into three groups: Combined group (female + male subjects), female group and male group.

Receiver operating characteristic (ROC) analysis with area under the curve (AUC) calculation was adopted to access the diagnostic performance of the haematological parameters in detecting iron depletion- serum ferritin level <12 µg/L.[10] The ROC curve plots 'true positivity rate'

(sensitivity) against 'false positive rate' (1-specificity) has a ROC curve that passes through the upper left corner (100% sensitivity & 100% specificity), thus, the shifting of the curve to the top left indicates better diagnostic efficiency of the parameter. The higher the AUC (closer to 1.0) is considered as a better parameter.[13] The Youden index was employed to identify optimal cut-off values of certain parameters in order to look at their maximum sensitivity and specificity and to determine its potential effectiveness.[14] A p value of less than 0.05 was considered as statistical significant.

Results

Cumulative results of laboratory testing are shown in Table 1. A total of 1837 subjects (age >5) with measured haemoglobin concentrations within the reference intervals, serum ferritin levels and other CBC parameters were selected from the retrieved datasets of the private laboratory in Hong Kong. The mean age (range) with 95% confidence interval for the female, male and combined subjects were 39.4 (6-88), 41.1 (6-83), 40.3 (6-88) respectively. After applying the exclusion criteria mentioned above, 1684 subjects were included and analyzed in the study, in which 1435 (85.2%) were female subjects and 249 (14.8%) were male subjects. Table 2 shows a comparison between serum ferritin level of female and male non-anaemic subject with different serum ferritin cut-off adopted by WHO. Among all non-anaemic subjects, 331 female (19.7 %) and 8 male (0.5%) subjects had serum ferritin levels lower than 15 µg/L (WHO's cut-off), 242 female (14.4%) and 3 male (0.2%) subjects had serum ferritin levels lower than 12 µg/L (cut-off adopted by Vuk *et al,* 2016)[10], 176 female (10.5%) and 3 male (0.2%) subjects lower than 10 µg/L (manufacturer's cut-off) respectively.

Figure 1 showed the distribution of 1684 non-anaemic subjects after applying exclusion criteria. Serum ferritin concentrations were more dispersed in male subjects, while most female subjects had serum ferritin levels lower than 50 µg/L and haemoglobin concentration lower than 14 g/dL.

Figure 2 showed the distribution of serum ferritin concentrations according to age and sex, the median serum ferritin concentration in male subjects was the highest (median: 207.8 µg/L) at age of 19-24, while there were no significant differences in other age ranges. In female subjects, the median serum ferritin concentrations across different age ranges were lower than that in male in general, mostly below 40 µg/L except for the age range between 55-74.

The AUC values of the parameters were compared and were used to predict iron depletion (serum ferritin <12 µg/L)[10] as shown in Table 3. CCI had the highest AUC value (AUC=0.758; p<0.001) in female subjects, while RDW was the highest (AUC=0.945, p<0.001) in male subjects and Hgb was the highest (AUC=0.775, p<0.001)

in total population. The optimal cut-off value of certain parameters was calculated by the Youden index, the maximum sensitivity and specificity at these optimal cut-off values are presented in Table 4, only top 6 parameters with the highest AUC were included. It was seen that CCI >52.75, RDW >14.9% and Hgb ≤12.9 g/dL can predict iron depletion (serum ferritin <12 µg/L) with highest sensitivity and specificity in female, male and total combined subjects respectively. ROC curves of these parameters were drawn for the total study population and in male and female subjects which are shown in Figure 3a-c.

	Female subjects (n=1435)	***Male subjects (n=249)***	***Combined (n=1684)***
Parameters	***Mean (±SD)***		
Ferritin (ug/L)	46.54(±45.03)	136.43(±78.35)	59.849(±60.42)
Hgb (g/dL)	12.87(±0.84)	14.49(±0.99)	13.11(±1.04)
MCV (fL)	86.71(±6.77)	87.04(±6.99)	86.75(±6.80)
MCH (pg)	28.97(±2.61)	29.34(±2.87)	29.03(±2.65)
MCHC (g/dL)	33.27(±1.33)	33.67(±1.22)	33.33(±1.32)
RDW (%)	13.99(±2.33)	13.46(±1.51)	13.92(±2.23)
HCT (%)	37.88(±4.67)	43.06(±2.87)	38.64(±4.82)
RBC(x10^12/L)	4.46(±0.41)	4.98(±0.57)	4.54(±0.48)
CCI	58.14(±21.61)	55.13(±19.40)	57.69(±21.32)

Table 1. Cumulative results of laboratory tests.

Study	*WHO*	*Vuk et al. (2016)*	*Private Laboratory*
Ferritin Cut-off values	15 µg/L	12 µg/L	10 µg/L
No. of iron deficient subjects			
Female	331/1684	242/1684	176/1684
Male	8/1684	5/1684	3/1684
Total % of iron deficient subjects	20.1%	14.8%	10.6%

Table 2. Comparisons between serum ferritin concentration of 1684 subject data with different serum ferritin cut-off values.

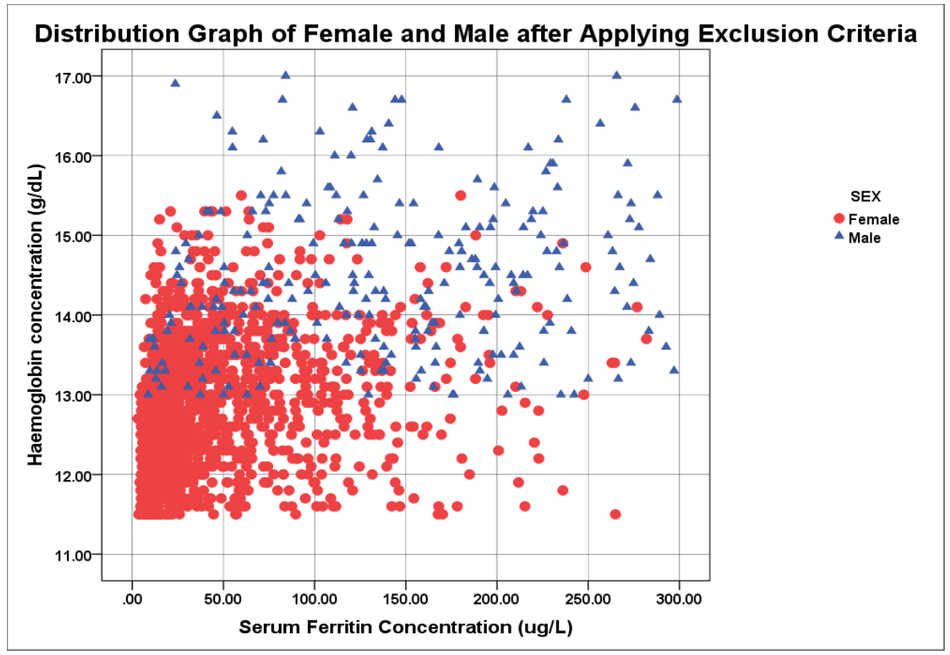

Figure.1 Distribution graph of female and male subjects after applying exclusion criteria.

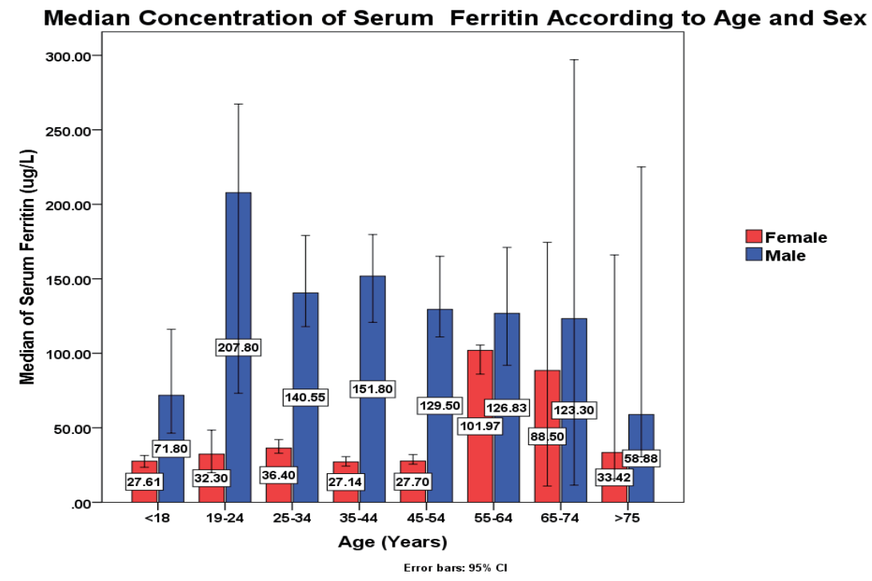

Figure 2. Median concentration of serum ferritin according to age and sex.

Parameter	*Combined subjects (AUC with 95% CI)*	*Female subjects (AUC with 95% CI)*	*Male subjects (AUC with 95% CI)*
CCI	0.766 (0.745 - 0.786)	**0.758** (0.735 - 0.780)	0.909 (0.866 - 0.942)
RDW	0.757 (0.736 - 0.777)	0.747 (0.723 - 0.769)	**0.945** (0.910 - 0.970)
Hgb	**0.775** (0.754 - 0.795)	0.743 (0.719 - 0.765)	0.832 (0.779 - 0.876)
MCV	0.720 (0.698 - 0.742)	0.716 (0.692 - 0.740)	0.900 (0.856 - 0.934)
MCH	0.757 (0.736 - 0.777)	0.752 (0.729 - 0.774)	0.878 (0.831 - 0.916)
HCT	0.694 (0.671 - 0.716)	0.650 (0.624 - 0.675)	0.761 (0.703 - 0.812)
MCHC	0.691 (0.668 - 0.713)	0.687 (0.597 - 0.718)	0.575 (0.511 - 0.637)
RBC	0.510 (0.486 - 0.534)	0.557 (0.531 - 0.583)	0.660 (0.597 - 0.718)

The highest AUC values indicated in bold

Table 3. AUC values of parameters used to predict iron depletion (ferritin <12 µg/L)

Female subjects						
Parameter	Cut-off value	Youden index	Sensitivity	95% CI	Specificity	95% CI
CCI	>52.75	0.436	80.58	75.0 - 85.4	63.03	60.2 - 65.8
RDW (%)	>13.6	0.439	76.03	70.1 - 81.3	67.9	65.2 - 70.5
Hgb (g/dL)	≤12.7	0.346	76.45	70.6 - 81.6	58.51	55.7 - 61.3
MCV (fL)	≤86	0.36	68.6	62.3 - 74.4	67.39	64.7 - 70.0
MCH (pg)	≤27.8	0.414	61.57	55.1 - 67.7	79.8	77.4 - 82.0
HCT (%)	≤38.7	0.227	75.62	69.7 - 80.9	47.11	44.2 - 50.0

Male subjects						
Parameter	Cut-off value	Youden index	Sensitivity	95% CI	Specificity	95% CI
CCI	>70.4	0.898	100	47.8 - 100.0	89.75	85.2 - 93.3
RDW (%)	>14.9	0.906	100	47.8 - 100.0	90.57	86.2 - 93.9
Hgb (g/dL)	≤13.7	0.746	100	47.8 - 100.0	74.59	68.6 - 79.9
MCV (fL)	≤81.4	0.877	100	47.8 - 100.0	87.7	82.9 - 91.5
MCH (pg)	≤27.6	0.836	100	47.8 - 100.0	83.61	78.4 - 88.0
HCT (%)	≤42.3	0.557	100	47.8 - 100.0	55.74	49.3 - 62.1

Combined subjects						
Parameter	Cut-off value	Youden index	Sensitivity	95% CI	Specificity	95% CI
CCI	>52.75	0.4499	80.97	75.5 - 85.7	64.02	61.5 - 66.5
RDW (%)	>13.6	0.4577	76.52	70.7 - 81.7	69.25	66.8 - 71.6
Hgb (g/dL)	≤12.9	0.4145	83	77.7 - 87.5	58.46	55.9 - 61.0
MCV (fL)	≤86	0.3708	69.23	63.1 - 74.9	67.85	65.4 - 70.3
MCH (pg)	≤28	0.427	65.18	58.9 - 71.1	77.52	75.3 - 79.7
HCT (%)	≤39.3	0.3005	82.59	77.3 - 87.1	47.46	44.9 - 50.1

Table 4. Sensitivity and specificity at optimal cut-off values.

Figure 3a

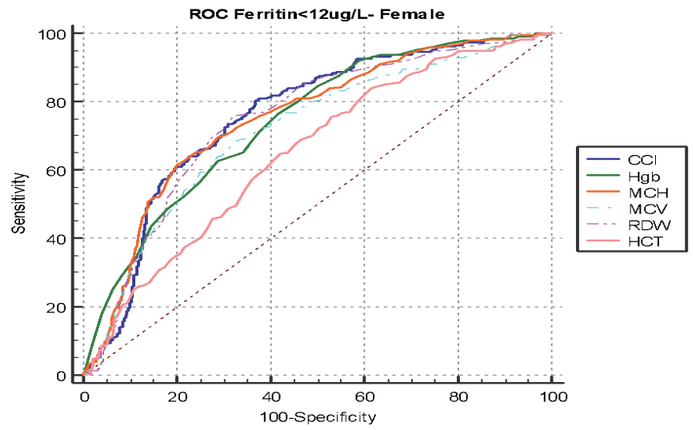

Figure 3b

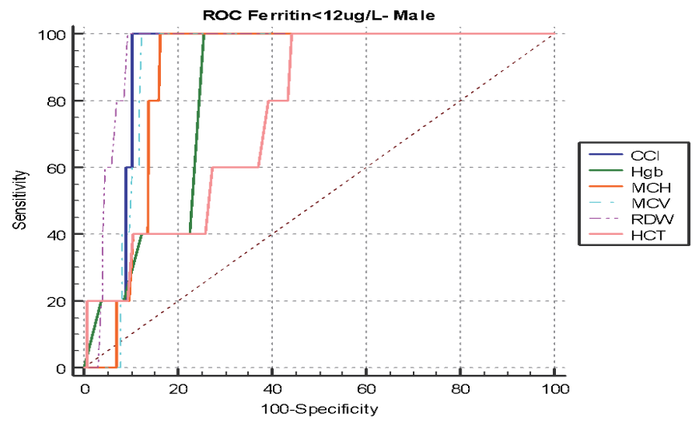

Figure 3c

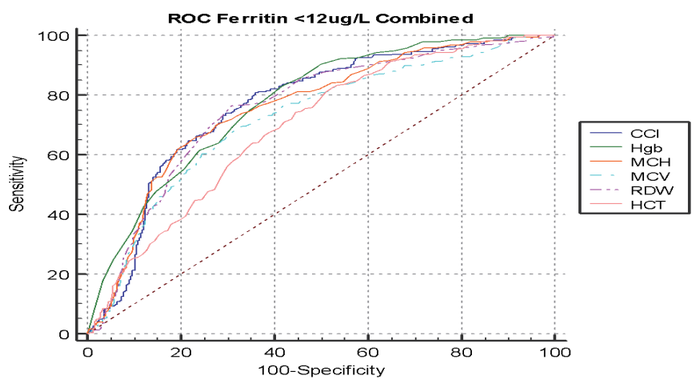

Figure 3a-3c. ROC Curve for haematological parameters predicting low ferritin level.

Discussions

The serum ferritin reference intervals derived in this study were 4.7-202.7 µg/L, 23.6-273.45 µg/L, 4.7-273.45 µg/L in female, male and total populations respectively. The body iron status and serum ferritin could vary with age, and a number of studies have shown a high prevalence of iron depletion in teen's donors compared to adult donors.[16] Hence, the reference intervals vary in adults and child. The derived serum ferritin reference interval in female subjects at age >18 and age <18 were 6.8-195.6 µg/L and 4.7-202.7 µg/L, and 25.9-200.7 µg/L and 27.9-273.45 µg/L in male subjects at age >18 and age <18 respectively. The range of serum ferritin levels in female subjects were much lower than the male subjects. A low serum ferritin level is significantly associated with low iron storage in the body, thus, factors leading to a low iron level may affect serum ferritin concentration, reflecting that female's lower iron stores are possibly caused by loss of significant amount of blood which are iron-rich during regular menstruation. Due to the fact that the loss of iron cannot be readily replenished before next menstrual cycle, low body iron level will be prolonged. Heavy blood loss during childbirth may also cause iron deficiency anaemia directly. Therefore, females at childbearing age (age 18-40) may have lower serum ferritin concentrations than females at age >40 as shown in Figure 2. Iron supplement is necessary for women suffered from severe iron deficiency or having symptoms of anaemia. Iron malabsorption caused by blood loss in gastrointestinal tract disorders such as celiac disease and Crohn's disease may also be one reason accounting for low body iron level.[17]

The cut-off for serum ferritin concentrations recommended by WHO that reflects the status of iron depletion is set at 15 µg/L for female and male subjects who are 5 years old or older. A 12 µg/L cut-off was adopted from Vuk *et al*, 2016[10], and a 10 µg/L cut-off was adopted from the current manufacturer used by the private laboratory. Hong Kong Red Cross Blood Transfusion Service[18] reported that one in eight (12.5%) blood donors were deferred for blood donations by using the current WHO ferritin cut-off at 15 µg/L. According to this study (shown in Figure 2), there were 339 (20.1%), about one in five, non-anaemic subjects had serum ferritin concentrations lower than 15 µg/L, which would be classified as deferred blood donors if the WHO standard is adopted. This would significantly reduce the supply of donated blood to hospitals for emergency uses. In addition, the number of deferred blood donations would be 247 (14.8% about one in seven) and 179 (10.6% about one in ten) if adopting the cut-offs <12 µg/L, <10 µg/L from Vuk *et al* and the current manufacturer respectively. These findings were similar to the results of the previous study by Yeung *et al*.[9] in which they found that 28 (20.3%), 22 (15.9%) and 15 (10.9%) female subjects had serum ferritin concentrations of <15 µg/L, <12 µg/L and <10 µg/L respectively. Since the reference intervals suggested by WHO and the manufacturer are often based on data from Western countries, Europeans or Americans who are Caucasians usually have a larger body mass than Chinese which may

be associated with different lifestyle, diet and heredity. Therefore, it is better to adopt a lower serum ferritin cut-off in Hong Kong population, so more non-anaemic subjects would be qualified blood donors. In order to maintain serum ferritin level and be qualified as blood donors, it is recommended to have a constant intake of iron-rich food such as red meat, seafood, poultry, iron-fortified cereals, whole grains, beans, peas, and dark green vegetables, Vitamin C is also useful for iron absorption.[19] Avoiding tea or coffee after meal is useful to prevent interference of iron absorption. Dietary monitoring would be helpful for women to maintain haemoglobin level.[19]

Another objective of this study was to test the appropriateness of CCI (adopted from Vuk *et al*) on assessing iron stores. The reason for choosing this index was because the diagnosis of iron deficiency is associated with microcytosis (abnormally small red cells with declined MCV), hypochromia (pale red cells with declined MCH), and anisocytosis (unequal sizes of red cells with increased RDW). Besides, this study also investigated the diagnostic potential of conventional RBC parameters (Hgb, MCV, MCH, MCHC, RDW and RBC) to detect iron depletion. Pearson's correlation analysis was used to see the correlation between haematological parameters including CCI and serum ferritin. By drawing ROC curves and AUC calculations for various parameters, our study showed that the maximum AUC values for CCI in female subjects was found at cut-off >52.75 (sensitivity=80.58%, specificity=63.03%), while it was RDW in male subjects at cut-off >14.9% (sensitivity=100%, specificity=90.57%) and it was Hgb in the diagnosis of iron depletion at cut-off ≤12.9 (sensitivity=83%, specificity=58.46%). MCHC and RBC yielded the lowest AUC values in all subjects, indicating lower diagnostic value in assessing iron depletion. Some similar results were obtained in the study by Vuk *et al* which they also proved CCI and RDW to be better predictors of iron depletion over other parameters in female and male subjects respectively.[10] Several studies have demonstrated the use of particular haematological parameters and formulas for detection of iron depletion.[20] Boulton *et al* suggested using CCI with the same formula was able to detect impaired haemoglobin synthesis in individuals with sudden iron deficiency.[20]

Our results proved there was a potential diagnostic value of CCI in assessing iron stores, suggesting the possibility of its use in clinical practice. Although there were comparable results in this study regarding its correlation with serum ferritin, this parameter may be highly useful in analyzing CBC while using serum ferritin as unique indicator of iron deficiency. The investigations of most studies mentioned above were based on a population of blood donors. Therefore, it is worth to re-access the values in a local transfusion service prospective as further investigation to confirm the findings from this study.

Conclusions

WHO's serum ferritin cut off may not be suitable in Hong Kong, which is the main reason why there is an increased of deferred blood donations. This phenomenon is possibly due to differences in body size, diet, lifestyle and heredity among our Chinese population. In addition, this study illustrated a potential diagnostic value of CCI in assessing iron stores, but further studies are suggested to reconsider and confirm the results reported and also further investigate their value in a local blood donor population specifically.

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Use of biomarkers for the early detection of chronic kidney disease

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Abstract

Background: Early stage of chronic kidney disease (CKD) is asymptomatic, thus a simple and reliable diagnosis based on levels of biochemical parameters is important to reduce the progression of renal deterioration and burden of end-stage renal failure requiring Renal Replacement Therapy (RRT).

Objectives: To evaluate the correlation of some simple biochemical parameters with estimated Glomerular Filtration Rate (eGFR), and to assess their association with the progress of CKD.

Methods: A retrospective study was undertaken of which 1470 (689 females, 781 males) sets of data with gender, age, race, and concentrations of biochemical parameters including fasting glucose, serum creatinine, total bilirubin, direct bilirubin and uric acid of random subjects were included. They received consultation from their doctors from Jan 2017 to December 2017. Data were retrieved from the database of a private laboratory in Hong Kong. The eGFR values were calculated by the Chronic Kidney Disease Epidemiology Collaboration (CKD-EPI) equation and categorized into 6 CKD stages. The correlation between biochemical parameters and eGFR was assessed by linear regression.

Results: Significant positive correlation ($p < 0.05$) was found between eGFR values and total bilirubin concentrations. Significant negative correlation ($p < 0.001$) was found between eGFR values and concentrations of fasting glucose, serum creatinine and uric acid. The correlation between eGFR values and direct bilirubin concentrations was insignificant ($p > 0.05$). Hypobilirubinemia was much more severe in patients with increased fasting glucose concentrations as it dropped from 12.8 μmol/L to 6.6 μmol/L comparing to 12.0 μmol/L to 11.8 μmol/L in patients with normal fasting glucose concentrations.

Conclusion: Concentrations of fasting glucose, serum creatinine, total bilirubin and uric acid could be potential markers for the early detection of CKD.

Journal of Hong Kong Institute of Medical Laboratory Sciences 2019-2020 Volume 16 No 1 & 2

Key words: *Chronic kidney disease (CKD), correlation, estimated Glomerular Filtration Rate (eGFR)*

Introduction

Chronic kidney disease (CKD) has become the worldwide public health concern over decades.[1] CKD was the 6th leading cause of death in 2018 in Hong Kong.[2] A simple and reliable approach based on concentrations of some simple biochemical parameters for identification of relevant biomarkers for early detection of CKD and other renal diseases is necessary. This helps to manage the renal deterioration and release the burden of end-stage renal failure therapy that requiring renal replacement therapy (RRT).

CKD is defined as any renal-related diseases with the duration of more than three months leading to progressive renal function loss until renal failure.[3] This could be confirmed by measuring glomerular filtration rate (GFR) that is categorized into stage III or above, or if any of urine abnormalities identified, such as the presence of red blood cells (RBC) and protein.[3] GFR, on the other hand, can be divided into six categories ranging from stage I to stage V based on the GFR level to reflect degree of renal function (**Table 1**).[4]

Stage I indicates normal or high kidney function. Stage II indicates early stage of CKD, which is asymptomatic thus it is hard to be diagnosed at this stage. Stage III is subdivided into IIIA and IIIB. Patients with stage IIIA CKD can be controlled by medication to relieve the symptoms, such as glucosuria and proteinuria caused by hypertension but those with stage IIIB CKD needs extra medical care to maintain the normal function of kidneys. Moreover, renal replacement therapy may be required for stage IIIB patients.

GFR can be estimated by the Cockcroft-Gault Equation, without computer programming, which takes age (in years), weight (in kg) and serum creatinine level (in mg/dL) into account. However, an over-estimation of 16% in GFR was reported using the formula.[5] The confounding effect of sex and race on creatinine concentration and muscle mass could account for the inaccuracy.[6]

New equations such as Chronic Kidney Disease Epidemiology Collaboration (CKD-EPI) equation, and the Modification of Diet in Renal Disease (MDRD) study equation have been widely used for GFR estimation in CKD diagnosis because of their high accuracy. Amongst MDRD and CKD-EPI equations, CKD-EPI was reported to be more accurate especially for patients with cardiovascular disease (CVD) caused by type II diabetes.[7]

Category	GFR / ml/min/1.73m^2	Kidney function
Stage I	≥ 90	Normal or high
Stage II	60 – 89	Mildly decreased
Stage IIIA	45 – 59	Moderately decreased
Stage IIIB	30 – 44	Moderately to Severely decreased
Stage IV	15 – 29	Severely decreased
Stage V	< 15	Renal failure

Table 1. CKD classification with respect to GFR[4]

New equations such as Chronic Kidney Disease Epidemiology Collaboration (CKD-EPI) equation, and the Modification of Diet in Renal Disease (MDRD) study equation have been widely used for GFR estimation in CKD diagnosis because of their high accuracy. Amongst MDRD and CKD-EPI equations, CKD-EPI was reported to be more accurate especially for patients with cardiovascular disease (CVD) caused by type II diabetes.[7]

As complications such as cardiovascular diseases can occur along with the progression of CKD, early detection and treatment is important for improvement of cardiovascular health status of CKD patients.[21] However, diagnosis of CKD at early stage is very difficult, as early symptoms of CKD are not apparent or even asymptomatic. Patients are usually reluctant to attend their doctors for advice as initialization of dialysis is still unacceptable by the majority.[9] Therefore, CKD was usually diagnosed until the end stage.

The use of glycosuria, proteinuria, serum creatinine level and uric acid level as markers to detect the kidney function has been widely reported.[11-14] Hence, the aims of this study are to evaluate the correlation of some biomarkers with estimated Glomerular Filtration Rate (eGFR) and to assess their association with the progress of CKD.

Materials and Methods

A retrospective study was undertaken that data with gender, age, race and concentrations of biochemical parameters, including fasting glucose, serum creatinine, total bilirubin, direct bilirubin and uric acid were collected from 1470 random subjects, who attended their doctors from 4th January, 2017 to 30th December, 2017. The data were retrieved from the laboratory database of a private laboratory in Hong Kong.

The eGFR values were calculated by the Chronic Kidney Disease Epidemiology Collaboration (CKD-EPI) equation and categorized into six CKD stages based on the classification of Kidney Disease Improving Global Outcomes (KDIGO) guidelines.[19]

The association between different biochemical parameters and eGFR values was assessed by linear regression using SPSS (IBM SPSS Statistic 23) to examine whether there was any significant change in concentrations among those biochemical parameters with the progression of CKD under the condition of p value < 0.05 as statistically significant difference. Reference ranges of tested parameters were provided by Abbott Architect c8000 Chemistry analyzer.

In order to investigate the effect of blood glucose levels on hypobilirubinemia, all cases were redistributed into Diabetes Mellitus (DM) and non-Diabetes Mellitus (non-DM) groups based on the assumption that all patients who had a fasting glucose concentration greater than the upper limit of reference range (6.04 mmol/L) were categorized as DM patients.

Results

Age, sex and CKD stage distribution

Among 1470 cases, there were 781 (53%) of male cases and 689 (47%) of female cases aged from 19 to 96 Mean= 49.4; 1 SD= 14.8. Table 2 shows the details in age and CKD stage distribution.

N=1470				
Age range	*No. of cases		CKD stage	*No. of cases
<20	1 (0.1%)		I	972 (66.1%)
20-29	124 (8.4%)		II	446 (30.3%)
30-39	296 (20.1%)		IIIA	36 (2.4%)
40-49	333 (22.7%)		IIIB	8 (0.5%)
50-59	379 (25.8%)		IV	8 (0.5%)
60-69	205 (13.9%)		*Percentages have been rounded and may not add up to 100%	
70-79	63 (4.3%)			
80-89	58 (3.9%)			
90-99	11 (0.7%)			

Table 2. Distribution of age and CKD stages for all cases (n=1470)

Correlation between fasting glucose with eGFR

The concentrations of fasting glucose steadily increased (**Figure 1**) from 4.72 mmol/L to 6.77 mmol/L with the progression of CKD (**Table 3**). There was a significant relationship between fasting glucose concentration and eGFR) (linear regression: F = 161.666, p = 3.2666E-35, adjusted r^2 = 0.099, y = 6.94 – 0.02x), thus there was an increase in the concentrations of fasting glucose with the progression of CKD. Significant negative correlation (p < 0.001) was found between eGFR values and concentrations of fasting glucose.

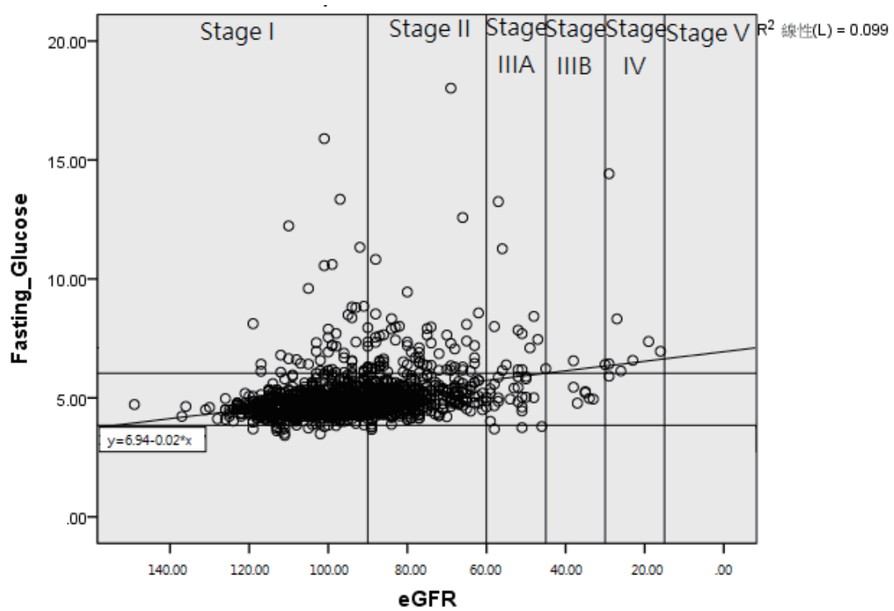

Figure 1. Linear regression of fasting glucose concentrations (in mmol/L) vs eGFR (in ml/min/1.73m^2) with respect to different stages of chronic kidney disease (n=1470). The horizontal lines represent the reference range of fasting glucose at 3.85 and 6.04 mmol/L respectively. The slope represents the correlation equation calculated by SPSS.

	Stage I	Stage II	Stage IIIA	Stage IIIB	Stage IV	Ref. Range	Unit
Fasting Glucose	4.72 (972)	5.07 (446)	5.51 (36)	5.24 (8)	6.77 (8)	3.85 - 6.04	mmol/L

Table 3. Mean values of fasting glucose concentrations (number of cases) with respect to CKD stages (n=1470).

Correlation between Serum creatinine with eGFR

The concentrations of serum creatinine increased rapidly (**Figure 2**) from 77.0 to 196.4 μmol/L for male and 62.3 μmol/L to 152.4 μmol/L for females with the progression of CKD (**Table 4**).

There was a significant relationship between serum creatinine concentration and eGFR (linear regression: F = 1168.408, p = 7.0556E-189, adjusted r^2 = 0.443, y = 0.0141 – 0.69x), thus there was an increased concentrations of serum creatinine with the progression of CKD. Significant negative correlation ($p < 0.001$) was found between eGFR values and concentrations of serum creatinine.

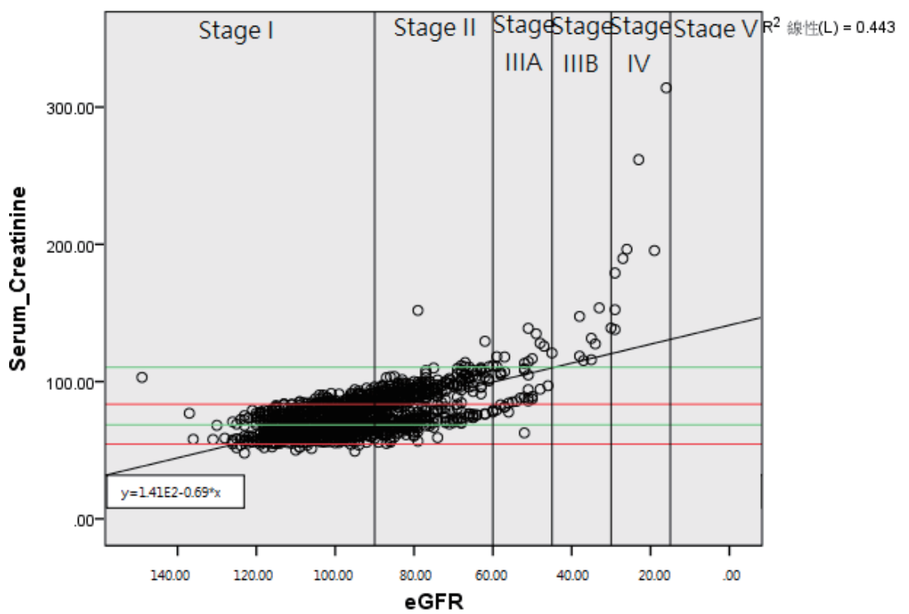

Figure 2. Linear regression of serum creatinine concentrations (in μmol/L) vs eGFR (in ml/min/1.73m^2) with respect to different stages of CKD (n=1470). The red horizontal lines represent the lower and upper limits of reference range of serum creatinine for females at 54.5 and 83.44 μmol/L respectively while the green horizontal lines represent the lower and upper limits of reference ranges of serum creatinine for males at 68.5 and 110.44 μmol/L respectively. The slope represents the correlation equation calculated by SPSS.

	Stage I	Stage II	Stage IIIA	Stage IIIB	Stage IV	Ref. Range	Unit
Creatinine/M	77 (483)	91.6 (273)	113.85 (18)	150.6 (2)	196.4 (5)	68.5 - 110.44	μmol/L
Creatinine/F	62.3 (489)	70.2 (173)	85.75 (18)	115.9 (6)	152.4 (3)	54.5 - 83.44	μmol/L

Table 4. Mean values of serum creatinine concentrations (number of cases) with respect to CKD stages for males (M) and females (F) (n=1470).

Correlation between Total bilirubin with eGFR

The concentrations of total bilirubin decreased mildly (**Figure 3**) from 12.05 μmol/L to 8.6 μmol/L with the progression of CKD (**Table 5**).

There was a significant relationship between total bilirubin concentration and eGFR (linear regression: F = 4.496, p = 0.034, adjusted r^2 = 0.003, y = 11.43 + 0.02x), thus the concentrations of total bilirubin decreased with the progression of CKD. Significant positive correlation ($p < 0.05$) was found between eGFR values and concentrations of total bilirubin.

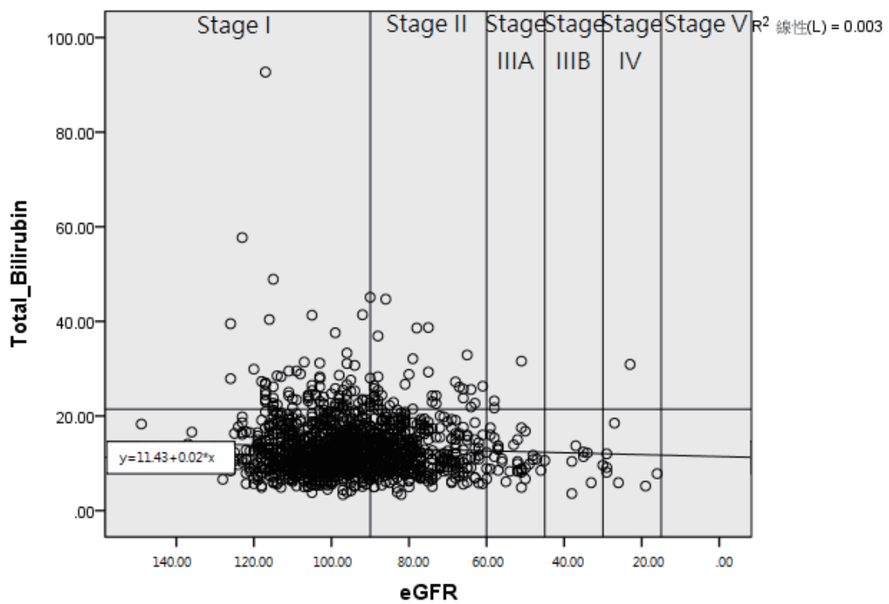

Figure 3. Linear regression of total bilirubin concentrations (in µmol/L) vs eGFR (in ml/min/1.73m^2) with respect to different stages of CKD (n=1470). The horizontal line represents the lower cut-off value of reference range of total bilirubin at ≤ 21.44 µmol/L. The slope represents the correlation equation calculated by SPSS.

	Stage I	Stage II	Stage IIIA	Stage IIIB	Stage IV	Ref. Range	Unit
Total bilirubin	12.05 (972)	12.15 (446)	10.7 (36)	10.9 (8)	8.6 (8)	≤ 21.44	µmol/L

Table 5. Mean values of total bilirubin concentrations (number of cases) with respect to CKD stages (n=1470).

Correlation between Direct bilirubin with eGFR

The concentrations of direct bilirubin remained relatively stable (**Figure 4**) between stage I to IIIB, then it was dropped to 3.5 µmol/L in stage IV (**Table 6**). There was an insignificant relationship between direct bilirubin concentration and eGFR (linear regression: F = 1.270, p = 0.260, adjusted r^2 = 8.643E-4, y = 4.51 + 0.00313x), thus no correlation relationship (p > 0.05) was found between eGFR values and concentrations of direct bilirubin.

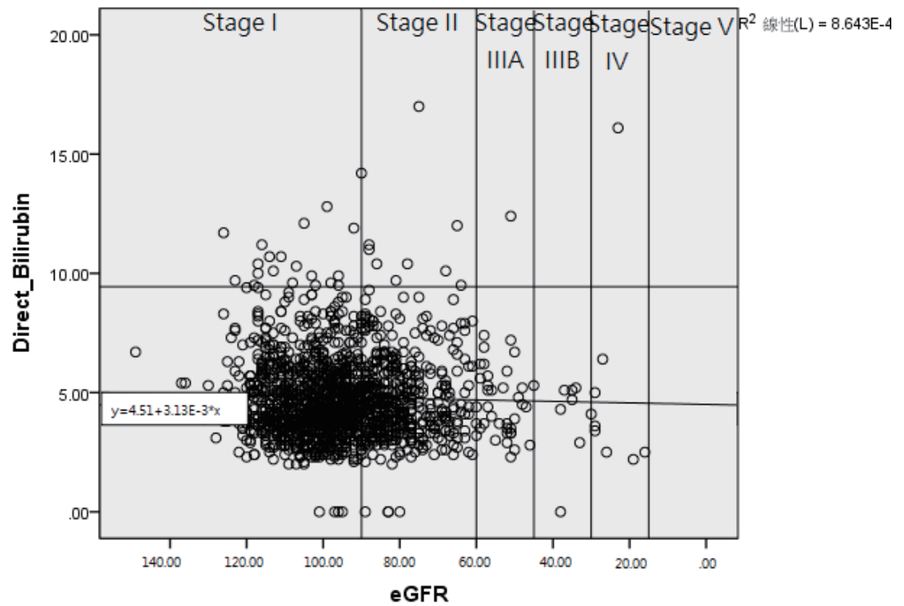

Figure 4. Linear regression of direct bilirubin concentrations (in µmol/L) vs eGFR (in ml/min/1.73m^2) with respect to different stages of CKD (n=1470). The horizontal lines represent the lower cut-off value of the reference range of direct bilirubin at ≤ 9.44 µmol/L. The slope represents the correlation equation calculated by SPSS.

	Stage I	Stage II	Stage IIIA	Stage IIIB	Stage IV	Ref. Range	Unit
Direct bilirubin	4.4 (972)	4.5 (446)	4.45 (36)	4.7 (8)	3.5 (8)	≤ 9.44	µmol/L

Table 6. Mean values of direct bilirubin concentrations (number of cases) with respect to CKD stages (n=1470).

Correlation between Uric acid with eGFR

The concentrations of uric acid increased steadily (**Figure 5**) from 0.38 µmol/L to 0.47 µmol/L for males, and from and 0.28 µmol/L to 0.42 mmol/L for females with the progression of CKD (**Table 7**). There was a significant relationship between uric acid concentration and eGFR (linear regression: F = 70.663, p = 9.89E-17, adjusted r^2 = 0.046, y = 0.45 – 0.0011x), thus the concentrations of uric acid increased with the progression of chronic kidney disease. Significant negative correlation (p < 0.001) was found between eGFR values and concentrations of serum uric acid.

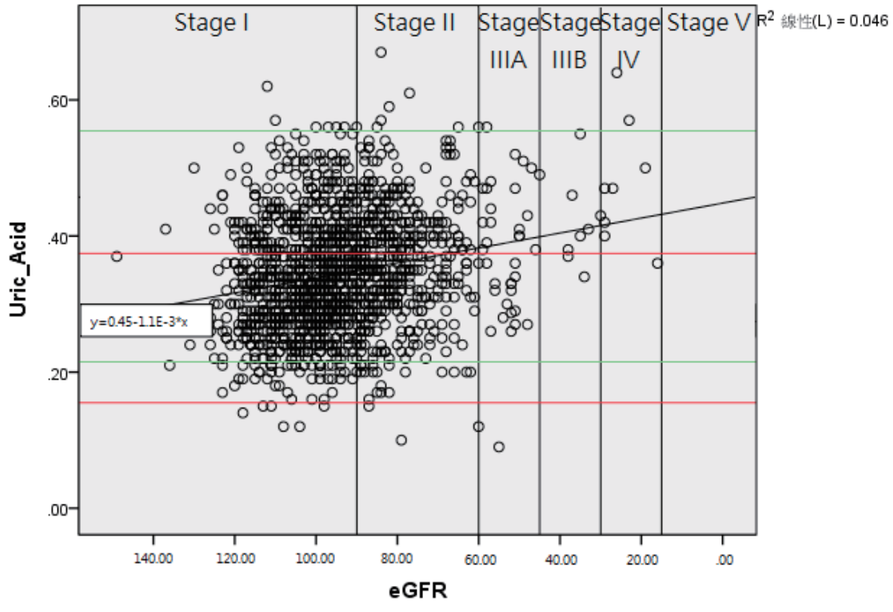

Figure 5. Linear regression of uric acid concentrations (in mmol/L) and eGFR (in ml/min/1.73m^2) with respect to different stages of chronic kidney disease (n=1470). The red horizontal lines represent the lower and upper limits of the reference range of uric acid for females at 0.155 and 0.3744 mmol/L respectively while the green horizontal lines represented the reference range of uric acid for males at 0.215 and 0.5544 mmol/L respectively. The slope represents the correlation equation calculated by SPSS.

	Stage I	Stage II	Stage IIIA	Stage IIIB	Stage IV	Ref. Range	Unit
Uric acid (M)	0.38 (483)	0.4 (273)	0.415 (18)	0.39 (2)	0.47 (5)	0.215 - 0.5544	mmol/L
Uric acid (F)	0.28 (489)	0.31 (173)	0.33 (18)	0.4 (6)	0.42 (3)	0.155 - 0.3744	mmol/L

Table 7. Mean values of uric acid concentrations (number of cases) with respect to CKD stages for males (M) and females (F) (n=1470).

Hypobilirubinemia in Diabetes Mellitus (DM) and non-Diabetes Mellitus (non-DM) patients

The result indicated that the concentrations of total bilirubin dropped more severely in patients with increased fasting glucose concentrations as it dropped from 12.8 µmol/L to 6.6 µmol/L comparing to 12.0 µmol/L to 11.8 µmol/L in patients with normal fasting glucose concentrations (**Table 8**).

	Stage I	Stage II	Stage IIIA	Stage IIIB	Stage IV	Ref. Range	Unit
Total bilirubin	12.05 (972)	12.15 (446)	10.7 (36)	10.9 (8)	8.6 (8)	≤ 21.44	µmol/L
Total bilirubin (DM)	12.8 (53)	11.1 (64)	10.3 (13)	6.6 (2)	9.1 (7)	≤ 21.44	µmol/L
Total bilirubin (NDM)	12 (919)	12.2 (382)	12.9 (23)	11.8 (6)	8.1 (1)	≤ 21.44	µmol/L

Table 8. Mean values of total bilirubin concentrations (number of cases) with respect to CKD stages vs all cases (n=1470), DM cases (n=139) and non–DM cases (n=1331) respectively.

Discussion

Glucose is totally reabsorbed by the proximal tubules in nephron under normal circumstances towards the bloodstream, where the blood flows from the arteriole through the glomerulus via a clump of capillaries, and circulating throughout other body parts at a rate of fixed amount of glucose at approximately 375 mg/min.[15] Renal deterioration leads to decreased glucose reabsorption rate due to impaired kidney function, resulting in glycosuria. CKD is also associated with insulin resistance and this could lead to increased blood glucose levels with the progression of CKD.[16] The results of this study indicated CKD patients at stage IV had a higher chance of having diabetes mellitus. It was found that the mean concentration of glucose in this study was 6.77 mmol/L, which exceeded the upper limit of reference range at 6.04 mmol/L.

Creatinine is the metabolite of creatine, which is used for the recycling of adenosine triphosphate (ATP) and creatine phosphate (PCr).[17] Creatine can be found in various tissues that has high ATP demand, including kidneys, skeletal muscle and also brain cells. Unfortunately, creatinine can also be secreted by tubular secretion leading to the overestimation of eGFR. Creatinine is finally being removed from the blood by glomerular filtration in kidney. The concentration of creatinine would increase rapidly if the kidney function is impaired since the clearance of creatinine become the rate determining step while liver functions normally to metabolize the creatine. As a result, creatinine is a non-specific marker that passively reflects the kidney function. However, the level of creatinine can be affected by many factors, such as muscle mass, gender, race and even high protein diet. Some non-specific interferences like Jaffe reaction can also interfere the creatinine concentration, thus it is hard to accurately estimate the GFR based on creatinine level.[18] Furthermore, creatinine based GFR estimation had a blind spot at 40 to 70 ml/min/1.73m^2 and undetectable until 50% kidney function loss, leading to delayed diagnosis of CKD at later stages.[19]

Bilirubin is the yellow compound that is involved in the catabolic pathway of heme.

This pathway is essential to clear the waste product that arise from destruction of abnormal and aged red blood cells.[20] Heme will firstly be broken down into biliverdin, a green bile pigment, under the presence of heme oxidase (HO), and hence be further broken down into bilirubin by biliverdin reductase.[21,22] Bilirubin can be further broken down into stercobilin and urobilin and are excreted in bile and urine.[23] Stercobilin is a bile pigment as the end product of heme catabolism, which is responsible for the presence of brown color of the feces and being excreted from the body while urobilin is another pigment that responsible for the coloring of urine.[24,25] Hypobilirubinemia is defined as exceptional low level of total bilirubin in serum. A possible cause of hypobilirubinemia could be bilirubinuria, a common symptom which occurs in end-stage renal disease (ESRD) due to impaired glomerular filtration leading to failure of retaining the bilirubin in the bloodstream, so bilirubin diffuses into urine and being excreted. Besides, bilirubin has its complementary antioxidant and anti-inflammatory roles by oxidizing itself back into heme[26], it is more effective than several known antioxidants[27], such as vitamin C and vitamin E analogue, thus bilirubin is a potent and important antioxidant. The abnormal loss of bilirubin can cause the oxidative stress, chronic inflammation and endothelial dysfunction to the kidney and cardiovascular system[28-30], leading to gradual kidney dysfunction, progression of CKD also contribute to more loss of bilirubin and increase the oxidative stress thus they formed a vicious cycle.[28]

The result of this study showed that there was a steadily decrease in the concentration of 12.05 μmol/L in stage I to 8.6 μmol/L in stage IV. This is in agreement with the previous study that the decrease in concentration of total bilirubin is a sign of early stage of CKD.[31]

The severity of hypobilirubinemia is more critical in co-existing of CKD and diabetes mellitus. Under hyperglycemic condition, bilirubin not only inhibits and attenuates the lipid peroxidation and attenuates LDL oxidation under normal circumstances, but also neutralizes the generation of reactive oxygen species (ROS) such as superoxide, by chronic hyperglycemia caused by diabetes mellitus, which results in drastically decrease in the concentration of bilirubin.[32] This study found that there was a 48% drop from 12.8 μmol/L to 6.6 μmol/L in the concentration of total bilirubin in diabetes group comparing to 1.7% drop from 12 μmol/L to 11.8 μmol/L in the group with normal glucose concentration which is in consensus with previous findings.[33,34]

Direct bilirubin is a water-soluble form after conjugation of glucuronic acid in liver with the presence of bilirubin uridine diphosphate glucuronosyltransferase (UGT) enzyme.[36] The concentration of direct bilirubin may increase mildly due to the auto-renoprotective measurement. This study found that there was a mildly increase in the concentration of direct bilirubin from 4.4 μmol/L in stage I to 4.7 μmol/L in stage IIIB before declined to 3.5 μmol/L in stage IV, which is supported by the findings of

Moolchandani *et al.*.[34] However, correlation relationship between direct bilirubin and eGFR was insignificant. Recent study has shown that the change of direct bilirubin concentration is more significant at end stage.[34] Such change is not proven in current study, and there it warrants further investigation.

Uric acid is a nitrogen waste product that is metabolized from purine compounds and are excreted in urine via kidneys.[35] Under normal circumstances, around 250 to 750 mg of uric acid should be excreted daily, which accounts for about 70% of daily disposal.[37] Hyperuricemia and gout are examples of complications that can be observed with heavily decreased kidney function that lead to accumulation of uric acid in blood with the progression of CKD.[37] The study also found that there was a sharp increase of uric acid concentration in both males and females. Moreover, the mean concentration of uric acid for females in stage IIIB exceeded the upper limit of reference range at 0.3744 mmo/L, while it was within the reference range for males even at stage IV, and this indicated that the increase in uric acid concentration was more obvious in females although estrogen had an effect to increase the renal clearance rate of urate in women.[3]

There were several limitations and improvements in this project. First of all, data retrieved for this project were found mainly on the age groups of 30 to 69 (82.5%), and had only a few cases in age groups of 70 or above, it is better to retrieve more data sets from the age groups of 70 and above for a comprehensive correlation result. Secondly, majority of the data sets used in this project were stage I (66.1%) and II (30.3%) of CKD, but the changes of some parameters' concentrations such as direct bilirubin, were proved to be more obvious at later stages,[24] thus more data sets from end stages, stage IV and V, are necessary for a comprehensive correlation. Thirdly, there were some cases assumed to have diabetes mellitus based on the assumption that all patients who had a fasting glucose concentration above the upper limit of reference range at 6.04 mmol/L were categorized as DM patients. As a result, patient clinical information is needed to confirm the diabetic status of patients. Finally, patients' medication history is useful because some drugs may interfere with the measurements of biochemical parameters.

Cystatin C could be a possible replacement of creatinine for CKD detection as it is renal specific and detectable in mild changes, the level remained stable even at inflammation state, it can be contributed for early diagnosis. However, only limited number of laboratories are capable of cystatin C testing because of high cost of testing and lack of equipment for its measurement. Therefore, creatinine is still selected as CKD marker although there are many well-known defects.[39] On the other hand, urine albumin level could also be included for correlation with respect to eGFR. It is one of the markers for proteinuria, commonly found in patients with renal diseases. There are three types of albuminuria, normoalbuminuria,

microalbuminuria and marcroalbuminuria, which depends on the amount of albumin found in urine by the measurement of either albumin excretion rate (AER, in terms of mg/24 hours) or albumin to creatinine ratio (ACR, in terms of μg/mg). Although both AER and ACR have the same reference values so that no matter which measurement is being used, they have systematic differences, so they are not interchangable.[40,41] Under normal circumstances, only lesser than 30 units of albumin can be excreted in urine defined as normolbuminuria.[40] If there are more than 30 units but lesser than 300 units of albumin found in urine, it is categorized as microalbuminuria and it is the early sign for CKD detection.[40] Finally, if there are more than 300 units of albumin found in urine, it is calssified as marcoalbuminuria, it is commonly found in late stage CKD as the glomerulus are damaged and nearly permeable to all molecules to pass through.[40]

In conclusion, concentrations of fasting glucose, serum creatinine, total bilirubin and uric acid could be useful markers for the early detection of CKD as they had correlation relationships with respect to eGFR. Hypobilirubinemia could be an extra marker for diabetes mellitus patients as an early sign of occurrence of CKD, and those who were suspected of having CKD should be referred to nephrologists for follow-up.

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Assessment of Iron Status in Non-anaemic Adults: A Pilot Study

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Abstract

Ferritin measurement is a reliable indicator of body iron store and is generally used for detecting iron deficiency in anaemic patients as well as non-anaemic blood donors. However, ferritin could be affected by various habitual, nutritional, physiological and pathological conditions. Consequently, haematological parameters in a complete blood count (CBC) including haemoglobin (Hgb), haematocrit (Hct), red blood cell (RBC), mean corpuscular volume (MCV), mean corpuscular haemoglobin (MCH), mean corpuscular haemoglobin concentration (MCHC) and RBC distribution width (RDW) are often requested to assess iron status.

The aim of this study was to investigate the relationship of serum ferritin concentration with various CBC parameters that may help in assessing iron deficiency in non-anaemic subjects.

A retrospective study was undertaken that the data was collected from the database of two private laboratories in Hong Kong including ninety-seven non-anaemic subjects (80 females and 17 males, age ranged from 11 to 84) with ferritin, CBC, who attended the laboratories from Jan 2, 2014 to March 31, 2016.

This study showed that Hgb and Hct are significantly correlated with ferritin ($p < 0.05$) in male, female and the combined subjects. Inconclusive relationships between RBC, MCV, MCH and MCHC with ferritin were found. RDW is not correlated with ferritin.

In conclusion, Hgb and Hct are helpful for detecting iron deficiency, however, further studies should be performed to prove whether using ferritin, Hgb and Hct as a combination could improve the detection sensitivity of diagnosing iron deficiency in both non-anaemic subjects and routine blood donors.

Key words: *Ferritin, Iron, Complete Blood Count, Haematological Parameters*

Introduction

Iron is an essential element for haemoglobin biosynthesis. Iron depletion can eventually cause iron deficiency anaemia. Iron deficiency is the most common cause of anaemia worldwide. Ferritin is the major iron-storing protein in our body. Measurement of serum ferritin concentration is the best indicator of body iron status. [1,2] Therefore, ferritin is very useful for detecting non-anaemic iron deficiency in blood donors. However, ferritin is an acute phase protein affected by various habitual, nutritional, physiological and pathological conditions including ethanol intake, daily diet, stress, cancer, infection, inflammation and hemochromatosis.[3,4] When the above mentioned conditions are excluded, serum ferritin concentration is well correlated with haemoglobin.[5] However, other haematological parameters in a complete blood count (CBC) including haematocrit (Hct), red blood cell (RBC), mean corpuscular volume (MCV), mean corpuscular haemoglobin (MCH), mean corpuscular haemoglobin concentration (MCHC) and RBC distribution width (RDW) have seldom been further investigated.

The aim of this study was to investigate the relationship of serum ferritin concentration with various CBC parameters as some of these parameters may probably be helpful in assessing non-anaemic iron deficiency in blood donors.

Materials and Methods

Subjects

A retrospective study was conducted that ninety-seven non-anaemic subjects; results of serum ferritin, complete blood count, age and sex were retrieved from two private laboratories (Laboratory A and B) in Hong Kong from Jan 2, 2014 to March 31, 2016. Inclusive criteria for non-anaemic subjects were subjects with haemoglobin concentrations within the adult sex-specific haemoglobin reference intervals. The haemoglobin reference intervals for female and male adults from Laboratory A and B are 11.5 – 16.0 g/dL (female); 13.5 – 18.0 g/dL (male), and 11.5 – 15.5 g/dL (female); 13.0 – 17.0 g/dL (male) respectively. The haemoglobin reference intervals as recommended by the World Health Organization (WHO) are ≥ 12.0 g/dL for non-pregnant women and ≥ 13.0 g/dL for men at 15 years of age and above.[6] The exclusion criteria are (1) subjects with haemoglobin concentrations outside the upper or lower cut-off values of the male and female reference intervals; and (2) subjects with serum ferritin concentrations outside the upper or lower cut-off values of the male and female reference intervals. The serum ferritin reference intervals for female and male adults from Laboratory A and B (provided by the manufacturer) are the same [female (5 – 204 µg/L) and male (22 – 275 µg/L) adults].

Measurement of serum ferritin concentrations

Serum ferritin concentrations were measured by the automated immunology analyzer (Abbott Architect i2000, Abbott Laboratory, IL, U.S.A.) using the Architect Ferritin[7] reagent by both laboratories.

*Measurement of c*omplete blood count (CBC)

CBC were measured by the automated haematology analyser on EDTA blood samples from the subjects. Sysmex XT-1800i (SYSMEX Corporation, Japan) haematology analyser was used by Laboratory A while Sysmex XN-3000L (SYSMEX Corporation, Japan) and Beckman Coulter DxH 800 (BECKMAN COULTER, U.S.A.) were used by Laboratory B. The following haematological parameters in a CBC test were assessed: haemoglobin (Hgb), haematocrit (HCT), RBC, MCV, MCH, MCHC and RDW.

Statistical Analysis

All data were classified into three groups: Combined group (female + male data), female group and male group. Pearson's product-moment correlation between the selected hematological parameters and ferritin concentration were performed. The strength of association between two parameters is strong when the Pearson's correlation coefficient (r), is greater than 0.6; moderate correlation when r is between 0.3 to 0.6. [2] The level of statistical significance was set as $p < 0.05$. Linear regression between selected hematological parameters and ferritin concentration were also conducted for confirmation. The IBM SPSS statistics version 22 and Excel 2016 were used for statistical analysis. Results of this study had no influence on the clinical management and outcome of all subjects.

Results

Ninety-seven subjects with age [mean (range): 39.9 (11-84)] were selected. Eighty (82.5%) of them were female subjects [age: mean (range): 40.3 (11-84)] and seventeen (17.5%) of them were male subjects [age (range): 37.7 (17-58)]. Figure 1 shows the distribution graph of female and male among 97 non-anaemia subjects. Serum ferritin concentrations of male subjects were much more widely scattered and higher than female subjects. Moreover, female subjects had serum ferritin concentrations of 5 to 150 µg/L. Both Pearson's product-moment correlation and linear regression analysis were performed between selected haematological parameters and serum ferritin concentrations (dependent variables). Both analyses produced the similar results of r, r^2 and p values. The statistical results are summarized in Table 1.

Figure 1. Distribution graph of female and male subjects

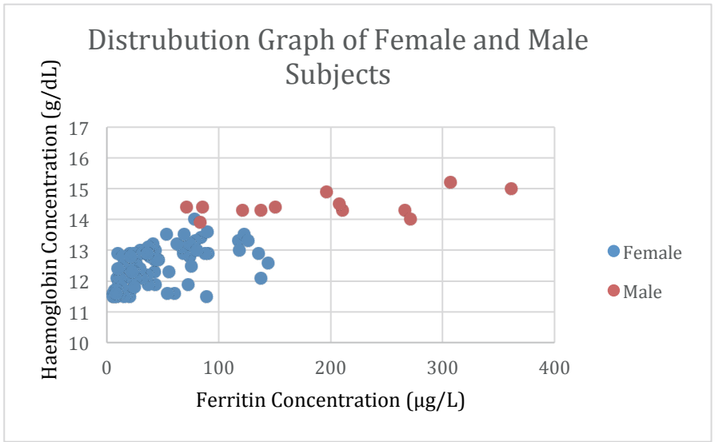

Table 1. Correlation between haematological parameters and serum ferritin concentrations by Pearson's product-moment correlation.

	Combined (Female + Male)			Female			Male		
	r	**r^2**	**P value**	**r**	**r^2**	**P value**	**r**	**r^2**	**P value**
Hgb (g/dl)	**0.801**	0.6419	**0.000**	**0.526**	0.2771	**0.000**	**0.849**	0.7204	**0.000**
Hct (%)	**0.777**	0.6033	**0.000**	**0.448**	0.2011	**0.000**	**0.760**	0.5778	**0.000**
RBC (x10^12/L)	0.462	0.2138	**0.000**	0.060	0.0036	0.597	**0.731**	0.5337	**0.001**
MCV (fL)	0.240	0.0577	**0.018**	0.252	0.0636	**0.024**	0.125	0.016	0.631
MCH (pg)	0.224	0.0503	**0.027**	0.241	0.0580	**0.032**	0.239	0.057	0.356
MCHC (g/dl)	0.212	0.0449	**0.037**	0.232	0.0540	**0.038**	0.133	0.018	0.610
RDW (%)	-0.153	0.0234	0.135	0.187	0.035	0.097	0.025	0.001	0.924

The results of this study showed that Hgb and Hct had high degree of correlation with serum ferritin in the combined, female and male groups ($r = 0.448 – 0.849$; $p = 0.000$). In the combined and female group, weaker positive correlations in decreasing order were RBC, MCV, MCH and MCHC.

In male group; Hgb, Hct and RBC were significantly correlated with serum ferritin ($p < 0.05$). MCV, MCH, MCHC and RDW were not correlated with serum ferritin.

Hgb and Hct were highly correlated with ferritin in all of the three groups. While MCV, MCH and MCHC had much weaker correlations with serum ferritin.

In addition, serum ferritin concentrations of 97 selected subjects of this study were compared with the cut-off values adopted by WHO[1] , Vuk *et al.*[2] and reference intervals used by two laboratories of this study to calculate the number of iron deficiency subjects. This study showed that 18 out of 97 (WHO; 19%), 15 out of 97 (Vuk *et al.*; 15%) and 10 out of 97 (Two laboratories of this study; 10%) would be classified as iron deficiency and not being considered for blood donation (Table 2).

Table 2. Comparisons between serum ferritin concentration of 97 subject data with different ferritin cut-off values.

Study	WHO	Vuk *et al.*	Two laboratories of this study
Ferritin Cut-off values	15 µg/L	12 µg/L	10 µg/L
No. of iron deficient subjects	18 out of 97	15 out of 97	10 out of 97
% of iron deficient subjects	19%	15%	10%

Discussions

Haemoglobin is the oxygen-transporting protein in red blood cells which carries oxygen from the lung to other tissues in the body and facilitates the return transport of carbon dioxide at the same time. It consists of four subunits with each having a polypeptide chain attached to a heme group. The heme group is an iron-containing structure and therefore iron is an essential element for synthesis of haemoglobin.[8] The amount of haemoglobin synthesized by the bone marrow is directly related to iron stores in the body. Ferritin is an iron storage protein which reflects body iron stores. Ferritin measurement is a reliable indicator of body iron store and generally used for detecting iron deficiency in anaemic patients as well as in non-anaemic blood donors. The use of serum ferritin concentration for the assessment of iron status in population have been recommended by World Health Organization (WHO).[1] Individuals with low serum ferritin concentration reflects iron deficiency while individuals with high serum ferritin concentration could be due to iron overload. However, ferritin as an acute phase protein can be affected by various habitual, nutritional, physiological and pathological conditions. In this study, haematological parameters in a complete blood count (CBC) including haemoglobin (Hgb), haematocrit (Hct), red blood cell

(RBC), mean corpuscular volume (MCV), mean corpuscular haemoglobin (MCH), mean corpuscular haemoglobin concentration (MCHC) and RBC distribution width (RDW) were used to compare with serum ferritin to see whether other CBC parameters could be useful for assessing iron status.

This study found that the Hgb and Hct had high degree of correlation with serum ferritin in all of the combined, female and male groups. This findings are consistent with previous studies. [5,9]

However, the use of RBC as a marker for assessment of iron status was inconclusive. Although RBC was positively correlated with ferritin in the male group.

MCV, MCH and MCHC had much weaker correlations with serum ferritin. Thus they are not very useful in the assessment of iron status.

The cut-off for serum ferritin concentrations recommended by WHO that reflects the status of depleted iron stores is set at 15 µg/L for both female and male subjects who are five years old or older. [1] A cut-off at 12 µg/L was adopted by Vuk *et al*, 2017, [2] while it was set at 10 µg/L adopted from the current manufacturers. Unpublished data from Hong Kong Red Cross Blood Transfusion Service (HKRCBTS) reported in February 2018 that one in eight (12.5%) blood donors were deferred for blood donations by using the current WHO ferritin cut-off at 15 µg/L. [10] The main reason for deferred blood donations was due to poor diet choices which results in low iron intake as well as low haemoglobin concentration. In this study, there were 18 out of 97 (19%), about one in six non-anaemic subjects that would be rejected for blood donations by using the WHO ferritin cut-off at 15 µg/L. On the other hand, rejected donations would be 15 out of 97 (15% or one in seven) and 10 out of 97 (10% or one in ten) if the cut-off from Vuk *et al*, 2017, [2] and current study respectively were adopted. It seems that it is more suitable to adopt a lower cut-off of serum ferritin concentration for Hong Kong population. Thus, more non-anaemic subjects would be considered as qualified blood donors. Therefore, it is essential to establish the reference intervals of serum ferritin concentrations from local Chinese population.

Conclusions

The findings of this study showed that there was significant correlation between Hgb, Hct and serum ferritin ($p < 0.05$) in all of the female, male and combined groups. Further studies should be performed to prove whether using ferritin, Hgb and Hct as a combination could improve the detection sensitivity of diagnosing iron deficiency in both non-anaemic subjects and routine blood donors.

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Technical Communication

Technical Communication on Diagnosis of Coronavirus Disease 2019 (COVID-19)

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Abstract

Coronavirus Disease 2019 (COVID-19) is a disease caused by severe acute respiratory syndrome coronavirus 2 (SARS-CoV-2). It is highly infectious and the World Health Organization has declared a pandemic and a global public health emergency on 30th January 2020. Rapid and accurate tests are essential to control the source of infection, cut off the routes of transmission and identify suspected and confirmed cases of infections. Reverse transcriptase polymerase chain reaction (RT-PCR) is now the preferred method of SARS-CoV-2 detection. In spite of the high accuracy of RT-PCR, They need expensive equipments, reagents and trained technologists to run the test. Serological tests are supplementary to molecular tests. Since it takes time to develop humoral response, they are not suitable for detecting acute infection. They are useful in calculating prevalence, mortality rate and are helpful for vaccine development. Antigen tests are the least sensitive in SARS-CoV-2 detection. However, it is cheap and easy to perform and have a high potential of mass production. It is hopeful that the sensitivity of these tests can be so that people can perform the test at point of care or office. Moreover, quarantine measures can be so that infection can be contained and the morbidity and mortality rate could be reduced.

Key words: *COVID-19, SARS-CoV-2, Reverse Transcription PCR (RT-PCR), Diagnosis*

Introduction

Coronavirus Disease 2019 (COVID-19) pandemic is a novel infectious disease caused by severe acute respiratory syndrome coronavirus 2 (SARS-CoV-2). Until 10th of June 2020, the disease has affected 213 counties. There are over thirty million infected cases with >950,000 deaths globally. United States have confirmed >6,500,000 cases with >190,000 deaths until 20th of September 2020, and had the most cases and deaths in the world. In Hong Kong there are >5,010 confirmed cases with >103 deaths until 10th September 2020.[1]

Epidemiological studies have shown that there are 3 factors involved in viral spreading of SARS-CoV-2:[2] sources of infection, routes of transmission and susceptibility. The virus is transmitted mainly by aerosol and is the main source of contagion, which happens mainly through contaminated droplets, hands, or surface. Viral particles, which are present in secretions from an infected respiratory system, infect others through direct contact with mucous membranes.[3,4]

People with COVID-19 have reported a wide range of symptoms from mild to severe illnesses. Symptoms may include fever, chills, dry cough, shortness of breath or difficult breathing, sore throat, running nose or congestion and others symptoms resembling influenza and common cold.[5] About 18% of those with COVID-19 may develop serious symptoms such as breathing difficulty[5] and 73% of cases are mild, with a fatality rate of 0.9% [6] About 5% of fatal cases have critical diseases marked by respiratory failure, septic shock, and multi-organ failure.[7] The main treatment is supportive and there is no vaccine for this disease available in the moment. Compared with severe acute respiratory syndrome coronaviruses (SARS-CoV and MERS-CoV respectively), SARS-CoV-2 has higher transmissibility and infectivity but lower mortality rate.[8]

Viral detection tests of COVID-19 infection are currently available. Besides symptoms and contact tracing with those infected, laboratory testing accounts for the definite diagnosis of the infection.[9] The U.S. Centers for Disease Control and Prevention (CDC) currently does not recommend the use of computerized tomography (CT) scan or low oxygen levels for diagnosis of COVID-19.[10] Molecular methods are more suitable than synchronic testing for accurate diagnoses. [10] Detection of SARS-CoV-2 includes methods

that detect the gene expression of the virus (RT-PCR), the viral antigens and the antibodies produced in response to the infection.[11]

Specimens for Testing

The most important factor for diagnosis of pneumonia caused by SARS-CoV-2 is the collection of right specimens from the patients at the right time.[11] The virus can be detected from lower and upper respiratory sites including Nasopharyngeal (NP), Oropharyngeal (OP), throat, sputum and bronchial fluid (BAL).[11,12,13]

Upper respiratory samples are highly recommended for testing of SARS-CoV-2. Recent study has reported that pharyngeal swabs were used much more frequently than NP swabs.[7] However, SARS-CoV-2 was detected only in 32% of pharyngeal swabs which was significantly lower than that in nasal swabs (63%).[14] Lower respiratory samples including sputum, BAL or tracheal aspirate are more recommended for patients with productive cough or intubation.[11] BAL showed the highest positive detection rate of SARS-CoV-2 (93%) followed by sputum (72%).[14] It was claimed that nasal and throat swabs were less suitable for RT-PCR testing since these specimens contain considerably less viral RNA than sputum[13], so that the viral load may not be detectable. It should be noted that the use of saliva, NP swabs, or pharyngeal swabs as specimens may not be reliable to detect the viral load during early infection as well as later infection. [11,12,13]

The amount of detectable viral load depends on the days after onset of infection. In the first 14 days after onset, the virus could be reliably detected on sputum, lower respiratory samples and nasal swabs.[13] The viral loads are unreliable in throat swabs 8 days after onset of symptoms.[13]

The detection sensitivity for each type of specimen depends on the stage of infection, viral clearance and degree of viral multiplication.[15] In later infection, the main site of the viral multiplication may be shifted from upper respiratory tract to lower respiratory tract.[11] Sputum or BAL should be used since they could yield the highest viral loads during diagnosis of COVID-19.[13,15] However, collection of BAL samples requires the help of medical professionals and the collection procedure would cause uneasiness and discomfort to patients. Nevertheless, a lower respiratory tract sample should be collected during the intubation procedures. It would be better

to collect samples from various sources such as stool and blood samples besides respiratory samples at different timepoints.[14] Do not use calcium alginate swabs or swabs with wooden shafts, as virus may be inactivated in these samples which may affect PCR test results, and should be stored at Universal Transport Medium (UTM) swabs and sent to the laboratory as soon as possible ideally under refrigerated condition.[12]

There are evidence indicated that transport medium such as VTM/UTM will stabilize the virus without meaningful degradation and specimens can be stored up to 72 hours at 4˚C.[11] If a delay in testing or shipping is expected, specimens should be stored at -70˚C or below.[12] Liquid Amis medium may also be used for viral transport when VTM/UTM are not available. Specimens can also be stored in liquid Amis for up to 72 hours at 4˚C.[12] The FDA has recommended the use of Phosphate Buffered Saline (PBS), including molecular grade PBS when available, and other similar formulations including Dulbecco's PBS to collect and transport samples for RT-PCR SARS-CoV-2 assay.[16] Sterile normal saline can also be used according to the FDA recommendation.[12]

PCR Detection

Development of molecular detection method depends upon understanding of proteomics and genomic composition of the pathogens. According to reports from China and the World Health Organization (WHO), SARS-CoV-2 virus strains were isolated and sequenced previously.[17] It doesn't contain DNA but only RNA. SARS-CoV-2 has a single-strained positive sense RNA genome that is about 30,000 nucleotides in length.[18] Virions are mostly spherical. There are 27 proteins with 4 structural proteins including the pronounced spike surface glycoprotein (S), small envelope protein (E), matrix protein (M) and nucleocapsid proteins (N).[18]

Coronavirus are RNA virus and genome of coronaviruses whose size ranges between approximately 26,000 and 32,000 bases, includes a variable number (from 6 to 11) of open reading frames (ORFs).[19] Prior to SARS-CoV-2, six coronaviruses were known to cause diseases in humans, including SARS-CoV and MERS-CoV.[18] SARS-CoV-2, like SARS-CoV and MERS-CoV, is a β-coronavirus.[18] Comparison of genomes from these three strains showed that they are almost identical, with only five nucleotide differences in the

genome of ~29.8 kb nucleotides.[18] SARS-CoV-2 resembles the SARS-like bat coronavirus (MG772933) than SARS-CoV.[18] It is suggested that intra- and inter-species transmission of CoVs, and genetic recombination events contribute to emergence of new CoV strains.[20]

For molecular PCR methods, RNA is extracted and purified from patient samples with the kit reagents provided. Then Reverse Transcription PCR (RT-PCR) converts the extracted RNA into mRNA and then real-time PCR amplifies the resulting gene products. Reverse Transcriptase real-time PCR (qPCR) provides advantages during the PCR testing of this process. It is automated and high throughput, enabling more reliable instrumentation and so it is the preferred diagnostic method for SARS-CoV-2.[19,21,22]

In real-time RT-PCR, the amplification of DNA is monitored in real time as the PCR reaction progresses and target genes are amplified. This is done using a fluorescent dye or a sequence-specific DNA probe labeled with a fluorescent molecule and a quencher molecule. An automated system then replicates the amplification process for about 40 cycles until the viral cDNA can be detected, usually by a fluorescent or electrical signal.[23,24]

Amongst the SARS related genomes, three regions were found to be the conserved sequences: the RNA-dependent RNA polymerase gene (RdRP) in the open reading frame ORF1ab region; the E gene (envelope protein gene); and the N gene (nucleocapsid protein).[25,26] RdRP and E genes provide higher sensitivity for detection, while N gene provided lesser analytical sensitivity.[21] PCR can be performed on either a one-step or a two-step assay based on the detection of ORF1ab genes, E genes, N genes or the RdRP genes. The one-step assay adopts a one-step protocol; reverse transcriptase and PCR amplification are consolidated into one reaction. In the two-step protocol, the reaction is done sequentially in two separate procedures. The challenge for the one-step protocol is the difficulty in optimizing the reverse transcriptase and amplification steps as they occur simultaneously.[24] The two-step protocol is more sensitive but time-consuming.[24]

There are at least 11 nuclear-acid based method detection kits approved in China. The qRT-PCR is the most predominantly used method for diagnosis of COVID-19 using respiratory samples.[24] Molecular sequencing methods are necessary to determine the mutation of the virus but are

not useful for diagnosis.

WHO recommended first-line screening with detection of the E gene followed by a confirmation assay detecting the RdRPgene.[11,21] In fact, due to the potential genetic drift of the virus, at least two or more molecular targets should be included in the molecular assay.[21,27] In some assays, three targets are considered, when two or more targets are detected, the test is determined to be positive.[27]

The Biofire Coronavirus Disease 2019 (COVID-19) test is intended to be used for qualitative detection of SARS-CoV-2 by a nested multiplexed real-time Polymerase Chain Reaction (PCR) method for nasopharyngeal swab in transport medium.[28] The BioFire test is authorized for use under the US FDA Emergency use Authorization.[29]

The necessary chemicals for sample preparation, reverse transcription, polymerase chain reaction and detection in order to amplify and detect the nucleic acid from the SARS-CoV-2 virus are all stored in a close system in disposable film array pouch and the system lysed the sample by bead beading. Using magnetic bead technology, the nucleic acid is extracted and purified from the sample. Then, the nested multiplex PCR is performed after performing reversed transcription and a single, large volume, multiplex PCR (PCR1). Second stage PCR (PCR2) is then performed to amplify sequences with the PCR1 product. Lastly, the result for each target assay on SARS-CoV-2 test is generated and interpreted by analyzing endpoint melting curve data. When two or more of the three targets (ORFlab x 2 and ORF8) are detected, the test is considered as positive. If only one target is detected, the test is considered as equivocal and it should be repeated. If same result is obtained in the second test, it should be considered as positive. When no target is detected, the test is considered as negative.

During the test, the sample is mixed with sample buffer and hydration solution in the tube, which is then incubated in the film array instrument and starts the PCR reaction cycles.

Two sample controls are included in each run:

1. Ribonucleic acid (RNA) process control
   The control consists of a RNA transcript from the yeast Schizosaccharomyces probe and is carried through all stages of the run. A positive result from this RNA control sample indicates the PCR reaction cycle is normal throughout the stages.

2. PCR2 Control
   The PCR2 control consists of the raw gene material for PCR2 reaction. A positive result from this control indicates that PCR2 reaction cycle is normal.

However, external control is still recommended for use as quality control and test verification.

The positive predictive agreement is tested to be 100%. However, negative results do not absolutely preclude SARS-CoV-2 infection and the test should not be used for the final and sole decision of patient management. When a negative result was reported, clinical observations, patient history and epidemiology information should be considered or even repeated testing should be conducted.

The Biofire test has not been validated for testing samples other than nasopharyngeal swab in transport medium. It can only be performed once at a time that a batchwise handling with many specimens is not feasible. It should only be used as a stat test or a confirmation test.

The Xpert Xpress SARS-CoV-2 test, which is a RT-PCR molecular diagnostic test, aids in the detection of SARS-CoV-2.[30] The test contains probes, primers and internal controls used in RT-PCR for qualitative detection of virus RNA in upper respiratory specimens, including nasopharyngeal, oropharyngeal, nasal, or mid-turbinate swabs and / or nasal wash / aspirates. It is an automated in vitro test using the GeneXpert instrument system. The system integrates sample preparation, nucleic acid extraction, nucleic acid amplification, and detection of target sequences by a single real-time PCR.[30] As it is self-contained and so cross contamination is minimized.

A Sample Processing Control (SPC) and a Probe Check Control (PCC) are included in the kit. SPC ensures the inhibitors and the RT-PCR conditions are working. PCC ensures all reaction components are working and monitors the dye stability as well as probe integrity. External control should be included to confirm their liability of the test.

Samples incubated in transport medium are mixed and then transferred to the sample chamber. The cartridges are then loaded into the GeneXpert Instrument System to perform real-time RT-PCR with pre-set protocol to detect gene expression of SARS-CoV-2.

Results will be displayed and interpreted in the Renew Results Window automatically by the GeneXpert system. Gene expression level is expressed based on the detection of two gene targets – the N2 and E genes. When both gene targets have Cycle threshold (Ct) values with the valid range detected, the result is considered as SARS-CoV-2 positive. When only the E gene target is detected, this constitutes a presumptive positive result. The test should be repeated and if it is still presumptive positive, additional confirmatory testing should be considered.[30]

The performance of the system was evaluated using contrived clinical NP swab in viral transport medium at 2x limit-of-detection (LoD), 3x LoD and 5x LoD level.[28] Our results of Ct mean values of E and N2 targets are similar to the previous reported findings.[30] As with other SARS-CoV-2 nucleic acid test, false negative results could still be obtained.[30] Negative reports should be interpreted with caution, in which patient history and diagnostic information should be considered to determine patient infection status. GeneXpert test is easy to perform and is precise; however, the test cannot be performed batchwise and may be not suitable for high volume laboratory.

Isothermal nucleic acid amplification

qRT-PCR needs the temperature change on each cycle. The reaction temperature of each qRT-PCR cycle changes, therefore sophisticated instrument with thermal cycling function is required which could be costly. Isothermal nucleic acid amplification is an alternative strategy that keeps temperature constant during the test and without the need of a thermal cycler.[31,32] Based on this technology, several methods have been developed.

Reverse-Transcription Loop-mediated Isothermal Amplification (RT-LAMP)[24]

The RT-LAMP test uses reverse transcriptase to convert the viral RNA to cDNA, which is subsequently amplified by the DNA-dependent DNA polymerase for rapid colorimetric or turbidity detection. It has been developed as a rapid and cost-effective test, such as the ID NOW COVID-19 test from Abbott Diagnostics.[24] RT-LAMP requires a set of four primers specific for the target gene/region to enhance the sensitivity and combines LAMP with a reverse transcription step for detection of RNA. The amplification product can be detected by florescence or turbidity caused by magnesium pyrophosphate precipitate in the reaction mixture as a byproduct of amplification.

Transcription Mediated Amplification (TMA)[33]

TMA is an isothermal amplification technology and patented single tube test. An isothermal single-tube nucleic acid amplification system is used for retroviral replication and amplify specific regions of either RNA or DNA much more efficiently than RT-PCR. The enzymes reverse transcriptase and T7 RNA polymerase rapidly amplify the target RNA/DNA. This method allows clinical laboratory to perform nucleic acid test (NAT) assays for blood screening within fewer steps, less processing time, and faster results obtained. It can be also used for identification and diagnosis of multiple pathogenic organisms. In contrast to other techniques such as polymerase chain reaction (PCR) and ligase chain reaction (LCR), this method involves both RNA transcription (via RNA polymerase) and DNA synthesis (via reverse transcriptase) to produce an RNA amplicon (the source or product of amplification) from a target nucleic acid. This technique can be used to target both RNA and DNA. Since RNA is more labile in a laboratory environment, this reduces the possibility of carry-over contamination, TMA produces 100-1000 copies per cycle (PCR and LCR exponentially doubles each cycle). This results in a 10 billion-fold increase of DNA (or RNA) copies within 15-30 minutes. The copies obtained after retroviral replication can be used to amplify specific region of RNA or DNA much more efficiently than RT-PCR.[33] Hence, this technique allows high throughput screening of multiple samples as well as multiple pathogens simultaneously. It provides a sensitivity up to 3 orders of magnitude and was able to distinguish SARS-CoV RdRp cDNA from SARS-CoV-2 RdRp cDNA.[33]

Clustered Regularly Interspaced Short Palindromic Repeats (CRISPR)[31,34]

CRISPRs represent a family of nuclei acid found in prokaryotes and extracted by a set of bacterial enzymes.[31] CRISPR and CRISPR associated (Cas) protein (CRISPR/Cas) structures were first identified in E. coli, with the function of combating any invading pathogens. The technique starts with extracting RNA from a patient sample, and uses a fast nucleic acid amplification method called loop-mediated

amplification to extract and purify sufficient RNA for for later detection. There are two commercially available assays that independently explore the possibility of using the gene-editing CRISPR methodology to detect SARS-CoV-2. One of them uses Cas12 and Cas13 that is capable of excising reporter RNA sequences in response to activation by SARS-CoV-2-specific guide RNA while the other relies on the cleavage of reporter RNA by Cas12a to specifically detect viral RNA sequences of the E and N genes, followed by isothermal amplification, resulting in a visual readout with a fluorophore.

Rolling Circle Amplification (RCA)[35]

RCA is different from conventional DNA amplification techniques such as polymerase chain reaction (PCR).[23] RCA is an isothermal nucleic acid amplification technique where the polymerase continuously adds single nucleotide to a primer annealed to a circular template that results in a long concatemers DNA containing tens to hundreds of tandem repeats complementary to the circular template. It is a process of unidirectional nucleic acid replication that can rapidly synthesize multiple copies of circular molecules of DNA or RNA, such as plasmids, the genomes of bacteriophages, and the circular RNA genome of viroids.[35] As an isothermal DNA amplification technique, rolling circle amplification was developed to simplify natural rolling circle replication. It can be performed in constant temperature with minimal reagent and avoid the generation of false positive results.

Nucleic Acid Hybridization using microarray[32]

The nucleic acid microarray relies on generation of cDNA from viral RNA using reverse transcription and subsequent leveling of the cDNA with specific probes.[23] The labeled cDNAs are loaded to wells of microarray plates that contain solid-phrase oligonucleotides. After washing, they will remain bound if hybridization occurs, thus signaling (mostly fluorescence) of the specific viral nuclei acid present can be detected. This assay can identify mutation associated with the CoV virus and approach 100% accuracy.[23]

Serological Testing

COVID-19 serological testing relies on binding affinity of the targeted antibodies to SARS-CoV-2 antigens. Blood samples, plasma or serum is collected and incubated

with the viral antigens in a platform. If patient has developed the specific antibodies in their blood, the corresponding antibodies will be recognized and bound to the antigens, so that a detectable signal can be quantified to determine the presence of viral antibodies in the samples. Examples of these platforms include Rapid Diagnostic Test (RDT), enzyme-Linked immunosorbent assay (ELISA) and Chemiluminescent immunoassay (CLIA).[36,37]

Rapid Diagnostic Test (RDT)[37]

RDT is a qualitative lateral flow assay that is small and portable which can be used at point of care. Like pregnancy test, the color lines indicate positive or negative results. The test results quantify the presence of IgG or IgM antibody or antigen.

Enzyme-Linked Immunosorbent Assay (ELISA)[37]

ELISA assay is done on a coated plate coated with a viral protein. Patient samples such as whole blood, plasma or serum are incubated with the coated protein. Antibodies in the patient samples will bind to the antigens if the target protein (antigens) is matched. The bound antibody-protein complex can be detected with another antibody to produce a color or fluorescence that can be quantified.

Chemiluminescent immunoassay (CLIA)[37,38]

CLIA is a quantitative assay using whole blood, serum or plasma from patients. Magnetized, protein-coated microparticles is used in this chemiluminescent microparticles immunoassay. The patient samples are mixed with the microparticles, reaction buffer and specific enzyme-labeled antibodies that will form a luminescent signal. Antibodies present in the patient samples will bind with the viral protein to form a complex and enzyme-labeled antibodies are added and bind to this complexes. The luminescence formed from the chemical reaction is then used to quantify the antibodies present in the sample. The tests can also detect multiple types of antibodies, while quantitative assays are preferred for kinetic monitoring.

Some studies have demonstrated that the spike (S) and nucleocapsid (N) proteins are the primary viral antigens against which IgM and IgG antibodies are raised.[39,40] The immune response of the humoral immunity in response to the viral infection involves production of antibodies IgM and IgG. IgM and IgG antibodies specific to SARS-CoV-2 will be detectable in blood about 5 days after initial infection.[7] Studies have found that IgM can be detected in the patient samples on day 5-7 and peak on day 28 after SARS-CoV-2 infection, while IgG can be detected on day 5-10 and peak on day 29.[41] Although IgM manifests earlier than

IgG, its level drops quickly. On the other hand, IgG can persist for a longer time following infection which imply potential protective role.

Although antibodies are not the perfect marker of acute infection, they can persist for many years in bloodstream and they are ideal for detecting past infections. It is still not clear how long is the latent period after infection and is the detailed immune response.[37,42,43] It is still unknown how long the immunity offered by antibody could last.[37,43] World Health Organization (WHO) claimed that currently there is no evidence to conclude that people who have recovered from COVID-19 could have protective antibodies to prevent re-infection.[37,44]

Neutralizing antibodies (NAb) can protect a cell from pathogen invasion by neutralizing any biological effect of the infectious agent.[42,44] Other binding antibodies which do not neutralize the virus will bind to the infectious agent but the pathogen remains infectious. A positive antibody test cannot indicate that a person being tested has generated NAb.

Neutralizing antibodies are conventionally detected by Plaque Reduction Neutralization tests (PRNTs).[44] However, this test requires the use of living virus and it must be performed in biological class 3 safety cabinet facilities. The neutralization test using class 2 safety cabinet has been developed using Pseudotyped Vesicular Stomatitis virus expressing SARS-CoV-2.[44]

The surrogate Virus Neutralization Test (sVNT) also detects NAbs, without using any living virus, and can be completed in 1-2 hours in a laboratory with only class 2 safety cabinet. Using purified receptor-binding domain from the S protein and the host cell receptor ACE2 (angiotensin-converting enzyme 2)[42], the test mimic the virus-host interaction through an ELISA. This receptor-binding domain ACE 2 interaction can be neutralized with specific NAbs in patient or animal sera.[42]

Nevertheless, there are limitations to the above-mentioned tests. False positive results of antibody test may occur. Antibodies that bind to other common coronaviruses may cross react with SARS-CoV-2 virus. Therefore FDA requires laboratory to state clearly in the serology reports that false positive results due to antibodies to common coronaviruses may occur.[40,44]

Also, antibody measurement requires standardization and purification of viral

DNA. A representative amount of patient DNA samples is required so that the viral DNA is detectable.

Another limitation is that antibody tests can indicate an individual who has been infected the COVID-19 disease but cannot confirm an individual with a current COVID-19 infection. Mortality rate of the disease and herd immunity in the population can be determined from the results of antibody tests.[24] Moreover, it can be used as a tool for vaccine development.[24]

USA CDC has a guideline for using antibody testing as part of surveillance efforts to identify the US population who has been infected with SARS-CoV-2 and how the virus is spreading through the population over time.[45] The reliability of current antibody tests for the novel coronavirus deserves further confirmation. They have to be standardized, refined and validated by more researches on many different samples.[37,43] Moreover, some of the serology tests have been approved for research use only but not approved for use as a public health diagnostic tool.[37]

Antigen Tests

Widespread testing for COVID-19 is critical for public health policy making and implementation of quarantine measures. Although qRT-PCR is accurate and quite promising, it requires expensive equipment, chemicals, and trained specialists and professional laboratories.

Antigen tests build most, if not all, those steps into a paper like strip that gives a simple positive or negative result. The patient sample is collected using a nasal swab or throat swab or related specimen. They then dip the swab into a chemical liquid to dissolve the mucus and the virus has to be broke up to release the detectable protein. The resulting solution is added to a test strip. If the solution contains viral proteins, they travel, through capillary force, along the strip and eventually get trapped by the monoclonal antibodies specific to viral antigen.[46] The results are read by a device that measures fluorescence signal. Antigen test uses a secondary antibody with a colored or fluorescent molecule. This secondary antibody binds to the antigen-antibody complex. If the test is positive, the strip can also generates a detectable color signal fluorescence change.[46]

Choosing the right antibodies are challenging to develop an antigen test.[47]

Antibodies must be specific to the desired viral protein such as the SARS-CoV-2 spike proteins uses to enter cells, but at separate sites. The two antibodies which are specific for the same viral protein must not interfere with each others.[47] The antibodies also cannot cross react to protein from other coronavirus that have unique own spikes.

Another limitation is that the signal may not be detectable sometimes.[47] There are often not sufficient antigen proteins present in the nasal swab in order to be quantifiable. This is especially common in asymptomatic people and who have very little nasal discharge. Since antigen test does not amplify the protein signal, so they are less sensitive than antibody tests, with a sensitivity of only 50% to 90%.[47]

Compared to PCR tests, antigen tests are not as sensitive and specific which would give false negative or positive results.[48] The test results should be supported with additional PCR test to confirm the infection status.[49]

One of the main advantages of antigen test is the fast turnaround time, which can provide results in minutes, though it cannot detect all infectious entities.[48,49] Since the antigen test doesn't require additional equipment, they can be conducted more easily and used as a point-of-care test in emergency care medical centres, doctor's office and even at schools, health centres and companies.[47] Another important advantage is scalability when the monoclonal antibody is found to be effective and easy to be manufactured in bulk, that millions of test kits can be provided each day. This allows mass diagnosis check and can rapidly identify positive cases that control the infection.

Conclusion

Interpreting the result of diagnostic test for COVID-19 depends on the accuracy of the test and the accessibility of the test. Considering PCR test's high specificity with moderate sensitivity, a positive result for the virus has greater weight than a negative test. A single negative test should not rule-out possibility of infection from a patient with strong symptoms. The negative results could be due to affected by sampling methodology, viral load in samples and mutation of viral genomes.[450,51,52] The false negative rate of the test is reported to be between 2% and 29%.[53,54]

Diagnosis of COVID-19 infection should also consider travel history and detailed contact Moreover, it is important to understand the epidemiology of emerging CoV, including the burden of asymptomatic infections.

Many diagnostic methods for COVID-19 rely on known techniques developed over the 20 years. It also depends on the ongoing efforts to develop effective diagnostic tools for COVID-19. Real-time reverse transcription-PCR assay has been the gold standard for detection of COVID-19 infection while serology techniques are being introduced as supplementary tools.

The sensitivity of various COVID-19 tests is reported to be within 71%-98%.[54] The use of repeated PCR testing as gold standard would underestimate the true false negative rate. As not all patients in the studies having repeated testing and those clinically diagnosed COVID-19 were not considered as actually having COVID-19.

Sensitivity of antibody detection is generally lower than the molecular method and it is mostly used for retrospective diagnosis.[44] It is quite difficult to interpret the result as the immune response may vary over the population. However, it is useful to calculate the infection rate and morbidity rate and it provides valuable information about the cause and degree of immune response as well as durability of immunity. It can also contribute to vaccine development and mortality rates of clinical trial.

Antigen tests, however, have scalable capacity that can be produced with lower cost and with a larger scale. They are available at point of care without the need of expensive instrument and facilitate rapid diagnosis. If sensitivity of the test could be upgraded, they can greatly assist in controlling the COVID-19 outbreak since timely diagnosis is essential for quarantine measure and integrated intervention to control the outbreak.

Laboratory diagnosis of COVID-19 may be non-specific.[50] The WBC count is usually within or lower than the reference range. There may be lymphopenia; a lymphocyte count of less than one thousand in one microliter of blood is associated with severe disease and may cause rapid progression to Acute Respiratory Distress Syndrome (ARDS).[6] Inflammatory markers, including ESR and C-reactive protein are generally high but procalcitonin levels are normal. The Prothrombin Time, AST/ALT, creatinine, D-dimer and LDH may be high and higher levels are associated with severe disease.[36,50]

It is suggested that older age and co-morbidities are factors that are correlated to bad clinical outcomes and severity of diseases.[36] There are approximately 20% of those hospitalized patients admitted to ICU, and the mortality for hospitalized patients is more than 10%.[36] Timely diagnosis is essential for quarantine and integrated interventions to control the outbreak if they are tested positive for the virus. Frontline medical staff are at high risk and should pay extra attention to effective use of personal protective equipment (PPE).

WHO estimates that it will take 18 months for the COVID-19 vaccine to be available.[55] No specific antiviral drugs for treating COVID-19 are currently available and proved effective. Symptomatic treatment and supportive care are usually given to infected patients. [39,56] It is now important to control the diseases and using existing drugs or supportive treatment. Therefore, effective drug and vaccine development are warranted in future.

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Journal of Hong Kong Institute of Medical Laboratory Sciences 2019-2020 Volume 16 No 1 & 2

Combination usages of GI PCR with BCID to improve the turnaround time of positive blood culture report

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Abstract

Background: New advance BCID Panel FilmArray® greatly improve the turnaround time of reporting the organism causing sepsis once the blood culture bottles show positive. However, since it cannot include all the bacteria in the panel, an all-negative results from a positive blood culture may happen sometimes.

Materials and Methods: From 01.01.2018 to 01.10.2018, a total of 89 positive blood cultures were reported. In our practice, once the positive culture pop-up message was generated by VITRUO ® system, a BCID PCR will be followed. Than subculture and direct Gram stain would be performed.

Results: Among the 89 cases, 3 cases were reported positive as Enterobacteriae only with no further identification. One case gave all-negative in BCID panel. For those 3 cases giving positive culture of Enterobacteriae, Gram stain showed gram-negative bacilli and patient record was reviewed. Two cases had sent stool for culture with positive in Salmonella and one of it had sent serum for Widal test with high titer in "O" and "H" antigen. The other one that showed all negative results in BCID gave a topical Gram stain with gram-negative curved shape bacilli that was highly suspected of Campylobacter morphology. All the four cases used the positive blood culture sample to perform GI PCR panel Film Array®. All the four cases give positive results in GI PCR. Three of them were reported Salmonella and the other one reported as Campylobacter. All these results matched with the final culture results.

Conclusion: Combination usage of different PCR panel can help to give a preliminary report in a very short turnaround time. However, results interpretation should be done carefully for different sample types.

Case report of pyogenic meningitis

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Abstract

Background: Meningitis usually gives general symptoms at the beginning onset of illness. Those symptoms such as fever, vomiting, severe muscle pain may lead the doctor swap the diagnosis as influenza infection especially during the influenza peak seasons.

Materials and Methods: A male patient, 42 years old, admitted to hospital on 27.10.2018 00:20 with upper respiratory symptoms. He was prescribed with Augmentin and Zithromax. Nasopharyngeal swab for respiratory full panel PCR, sputum for bacterial culture and sensitivity test, TB smear and PCR were performed. The same day afternoon at 14:30, the general practitioner (GP) referred the patient to a neurology specialist and took a lumbar puncture of cerebrospinal fluid for further investigation.

Results: The results for Respiratory PCR panel, TB smear and PCR were all negative. Cerebrospinal fluid showed Xanthochromia and turbid appearance. WBC count: 11118/μL; RBC: 295/μL; Total protein: 4936 mg/L and Glucose: 4.24 mmol/L. Gram stain of cerebrospinal fluid showed no organism seen. TB PCR of cerebrospinal fluid gave negative result. However, the FilmArry®Meningitis/Encephalitis (ME) panel showed positive of Streptococcus pneumoniae. After report released to doctor, the antibiotic given to the patient was changed to Rocephin IV 2g Q12h. Diagnosis was made after 1 hour and 24 mins after receiving the cerebrospinal fluid.

Conclusion: Rapid PCR is a useful tool for clinical diagnosis. Cerebrospinal fluid is critical clinical samples. Two hundred microliter (μL) of cerebrospinal fluid can give a rapid and accurate diagnosis. The false negative result by Gram stain in this case could be due to human error or too much white blood cells that interference the smear. It takes about 18-24 hours to have the culture result. ME PCR is no doubt a very useful tool in cerebrospinal fluid samples.

Abstract

Expression of ANA patterns by Indirect Fluorescent Assay in a local Hong Kong population from private sector

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Abstract

Objectives: To investigate the association among age, sex and Antinuclear Antibody (ANA) patterns expressed in Hong Kong population for improving the autoimmune diagnosis by using ANA HEp-2 slides under fluorescent microscope.

Materials and Methods: A total of 65 blood samples from both male and female were collected and examined under fluorescent microscope. Haemolysed samples were excluded. In this research, a HEp-2 complete Kits with the methodology of indirect fluorescent antibody (IFA) was utilized for the detection and semi-quantification of antinuclear antibodies to distinguish positive ANA patterns classified in International Consensus on ANA Patterns (ICAP) for human body.

Results: In this experiment, we obtained that the number of positive samples was 6 (9%), the number of suspected (weak) positive samples was 4 (6%) and the number of negative samples was 55 (85%). In 6 positive samples, they were all females aged between 59 – 89. 5 in 6 positive samples in the result were over age 69 (83%). The positive ANA patterns included AC2 – Speckled, AC3 – Centromere, AC6 – Discrete Nuclear Dots, AC7 – Discrete Nuclear Dots, and AC8 – Nucleolar patterns separately. No AC-29 - DNA topoisomerase I was examined and resulted.

Conclusion: We found that Hong Kong have a higher distribution of ANA positive frequency for female (9.23%) than male. Our data indicated that ANA patterns were found in female aged above 69 is more likely to suffer from autoimmune disease related to ANA, providing an important foundation for considering and studying the trend in the elderly who are easier to have an ANA related autoimmune disease in Hong Kong.

(This abstract was accepted in the session title of B Cells and Antibodies in Autoimmunity as poster presentation in the IMMUNOLOGY 2020 of AAI and published in The Journal of Immunology on 1 May, 2020.)

Prevalence of PSA level among men in Hong Kong

Daniel Kwun-Hung Lau[1], Ying Duan[1], Ching-Yan Chan[1], Shuk-Yu Chan[1], Ming-Tsun Cheung[1], Yu-Tak Chan[1], Yat-Kan Ha[1], Man-Chun Wong[2], Peter Kin-Nam Yau[2], and Man-Yin Che[3]

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Abstract

Objectives: To investigate the prevalence of prostate cancer risk associated with age and Prostate Specific Antigen (PSA) level detected in Hong Kong population for improving the earlier detection of prostate cancer in early stage.

Materials and Methods: A total of 67 random blood samples from males were collected and examined by Architect i1000SR to obtain quantitative laboratory values of Total PSA in samples serum via chemiluminescent microparticle immunoassay (CMIA). The age was between 25 and 89 years old. In this research, according to World Health Organization (WHO) providing international classification of disease (ICD), the applicable code for prostate carcinoma screening is ICD-10 Z12.5. We utilized ICD as the international standard for reporting prostatic diseases to compare data globally, and health condition if obtaining abnormally PSA level (e.g. >4.0 ng/mL according to National Cancer Institute (NIH) recommendation) coding as ICD-10 R97.20.

Results: In this experiment, for population characteristics, we obtained that the number of positive samples was 4 (5.97%) aged between 56 – 75 (Mean positive PSA = 10.43 ng/mL). For aged 56-60 (Mean positive PSA = 14.32 ng/mL), the numbers of positive samples were 2 (2.99%). For aged 66-70 and 71-75 (Mean positive PSA = 6.54 ng/mL), the numbers of positive samples were 1 respectively (1.49%). No PCA3 mRNA, Free PSA and p2PSA were examined in this screening.

Conclusion: We found that Hong Kong have a higher distribution of PSA positive frequency starting from men age of 56. Our data indicated that the older males have an increased risk of prostate cancer associated with elevation of Total PSA level, providing a vital and statistical foundation for comparing and studying the positive trend for the older males who are more susceptible to get high Total PSA level to prevent progression of prostate cancer in men.

Published for the **Hong Kong Institute of Medical Laboratory Sciences**
Formerly **Hong Kong Medical Technology Association (Founded 1966)**
Flat 1711, Block C, Bell House,
525-543 Nathan Road, Kowloon, Hong Kong.
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