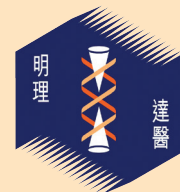


# PATHOLOGUE

The Hong Kong College of Pathologists, Incorporated in Hong Kong with Limited Liability



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## Message from the President

*This is the first issue of our College Newsletter in 2019. In 2018, College was busy with conducting the activities associated with the new training programme in Genetic and Genomic Pathology including the second open forum and First Fellow application. We are now processing numerous applications. An interview will be conducted in October 2019 to finalise the eligibility.*

*In 2018, the Hong Kong Academy of Medicine was also celebrating her 25th Anniversary with a series of fascinating events including the Congress of Medicine and Gala Dinner with an impressive drummer performance at the opening. Our Fellow, Prof. YUEN Kwok Yung was the Sir David Todd Orator in 2018. He presented his journey to becoming a renowned microbiologist with multiple showcases of Sherlock Holmes-style investigations for our community. In early 2019, the Academy also conducted a visit to Sichuan for our Young Fellows, so as to increase their understanding of the development of specialist care in Mainland China. Dr. MAK Siu Ming and Dr. CHENG Shui Ying, Ivy, represented our College to participate in this visit.*

*In March 2019, the Royal College of Pathologists of Australasia Quality Assurance Programs (RCPA-QAP) held one of their 30th Anniversary Quality Symposia in the Pao Yue Kong Auditorium at the Hong Kong Academy of Medicine Jockey Club Building. Local and overseas speakers came to give talks and opinions on how quality can be exercised as well as the future of quality in pathology. What an enjoyable experience-sharing opportunity!*

*In the recent issue of Topical Update, Dr. CHONG Yeow Kuan, Calvin reviewed the technological development of biochemical genetics on three major categories of inherited metabolic diseases. With the introduction of expanded newborn screening using mass spectrometry, there is increasing awareness of these conditions in the community.*

*Last but not the least, Dr. CHAN Sheung Wai, Gavin will share his concept of modern mortuary development, with a life paradigm shift from just being a place for the dead to a decent environment of bereavement for the family.*

*I would like to thank all the above Fellows who have contributed so much to the build a positive image of pathologists. Hope you enjoy this issue of Pathologue!*

Dr CHAN Ho Ming  
President  
May 2019



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# *The 14th Trainee Presentation Session*

The 14th Trainee Presentation Session was successfully held in the afternoon on 24th November 2018. The Trainee Presentation Session aims to provide a platform for our trainees to present their research findings. Four fellows from different disciplines were invited to be judges: Dr. LO Cheuk Lam, Regina (Anatomical Pathology, Queen Mary Hospital), Dr. MAK Wing Lai, Tony (Chemical Pathology, Princess Margaret Hospital), Dr. WONG Wai Shan (Haematology, Queen Elizabeth Hospital), and Dr. WU Ka Lun, Alan (Clinical Microbiology & Infection, Pamela Youde Nethersole Eastern Hospital). It was our great honour to have Professor Tony LANDGREN, Chairman of the Board of Education & Assessment of the Royal College of Pathologists of Australasia, to join the panel of judges.

The number of participants broke the record this year. A total of 26 trainees from different subspecialties actively participated in the Trainee Presentation Session. Due to the overwhelming response of participation in recent years, the poster presentation was introduced in 2014. Seven of the trainees were selected to deliver an oral presentation, while the remaining 19 trainees were invited to participate in the form of a poster presentation. Both oral and poster presentations are recognized education activities that fulfil the training requirements of our College. Participants of oral and poster presentations had 14 minutes (including 4 minutes Question & Answer) and 3.5 minutes (without Question & Answer session) on-stage presentation, respectively.

I would like to congratulate all participants for their excellent job and impressive presentation. On behalf of the Education Committee, I would also like to express grateful appreciation to our invited judges and helpers in assisting with the Trainee Presentation Session.



▲ *Trainees and judges exploring posters in the foyer*



The best presentation was awarded to Dr. CHAN Yim, Candace (Chemical Pathology, Princess Margaret Hospital). Her presentation was entitled, 'An outbreak of acute poisonings associated with an emerging ketamine analogue, 2-oxo-PCE.' The abstract of her study was:



► Dr. CHAN Yim, Candace, delivering her winning presentation on 'An outbreak of acute poisonings associated with an emerging ketamine analogue, 2-oxo-PCE.'

*Ketamine is a well-known drug of abuse of the arylcyclohexylamine class, the backbone of which is used for the synthesis of new psychoactive substances (NPS). In October 2017, a cluster of acute intoxications was encountered where patients presented with ketamine-like toxidrome. Upon initial toxicology screening, however, neither ketamine nor other causative agents were detected in the patients' urine. Instead, an unidentified substance was consistently detected. Further investigations led to the identification of an arylcyclohexylamine analogue, 2-oxo-PCE.*

*The study reports the analytical and toxicological profile of this emerging NPS. Acute intoxication cases involving 2-oxo-PCE confirmed October and November 2017 were reviewed. In total, 56 cases of 2-oxo-PCE associated poisoning were encountered. Laboratory analysis confirmed the presence of 2-oxo-PCE in the urine of all patients; nasal swab samples from three patients revealed the lone presence of 2-oxo-PCE. Urine bedside immunoassay for ketamine was found not to cross-react with 2-oxo-PCE. Whilst co-ingestion of other drugs of abuse (methamphetamine, cocaine, ketamine, cannabis) was commonly observed, in 25 cases (45%) 2-oxo-PCE was used alone. The main clinical symptoms associated with sole 2-oxo-PCE use include impaired consciousness, confusion, abnormal behaviour, hypertension and tachycardia. Convulsion was also observed with relatively high frequency. Three patients required intensive care. Clinical management of the patients was mainly supportive with occasional requirement of physical restraint or sedation, or in severe cases, intubation and alkaline diuresis. All patients recovered uneventfully. In conclusion, frontline clinical and laboratory personnel should be highly vigilant in the lookout for NPS, which may not be easily detected by conventional toxicology screening.*



▲ Dr. CHAN accepting the prize from Dr. LAI Koon Chi, Christopher; Chairman of the Education Committee of the Hong Kong College of Pathologists.

# Annual General Meeting 2018

The 27th Annual General Meeting (AGM) was held after the 14th Trainee Presentation Session on 24th November, 2018. Dr. POON Wai Ming was elected as Vice President in place of Dr. SHUM Shui Fung, Bobby, and Dr. LUNG David Christopher was elected as Honorary Treasurer in place of Dr. CHONG Yeow Kuan, Calvin. Dr. LEUNG Yuk Yan, Rock, Dr. LAM Woon Yee, Polly and Dr. LI Hiu Lui were re-elected as council members. One new Council Member was elected at the AGM: Dr. CHEN Pak Lam, Sammy.

We would like to take this opportunity to thank the immediate past Vice President, Dr SHUM Shui Fung, Bobby, immediate past Honorary Treasurer, Dr. CHONG Yeow Kuan, Calvin, and Council Member Dr. LO Yee Chi, Janice for their contribution to the College.



▲ *Council Members of The Hong Kong College of Pathologists 2018-19:*  
*Front row from left to right: Registrar, Dr. WONG Lap Gate, Michael; Immediate Past President, Professor CHEUNG Nga Yin, Annie; Vice-President, Dr. POON Wai Ming; President, Dr. CHAN Ho Ming; Vice President, Dr. CHAN Chak Lam, Alexander; Honorary Treasurer, Dr. LUNG David Christopher; Council Member, Dr. CHAN Kui Fat.*  
*Back row from left: Council Members, Dr. CHEN Pak Lam, Sammy, Dr. LAI Koon Chi, Christopher; Dr. LEUNG Ying Kit, Dr. MAK Siu Ming, Dr. LI Hiu Lui, Dr. LAM Woon Yee, Polly.*  
*Absent with apologies: Council Members, Dr. CHEONG Renee Constance Yue-Kew and Dr. LEUNG Yuk Yan, Rock*



### **Conferment Ceremony**

At the Conferment Ceremony, 5 Fellows and 17 Members were admitted to the College. The honorable guests included Professor Sophia CHAN, Secretary for Food and Health, of The Food and Health Bureau, Hong Kong Special Administrative Region (HKSAR); Professor LAU Chak Sing, President of The Hong Kong Academy of Medicine; Dr. the Honorable Pierre CHAN, Legislative Councillor of the HKSAR; and Dr. Doris TSE, Cluster Chief Executive, Kowloon West Cluster, Hospital Authority. We were also privileged to have special guests from The Royal College of Pathologists of Australasia (RCPA): Dr. Bruce LATHAM, President of the RCPA, Professor Tony LANDGREN, Chair of the RCPA Board of Education and Assessment; and Dr. Debra GRAVES, Chief Executive Officer (CEO) of the RCPA.



▲ Professor LAU Chak Sing, President of The Hong Kong Academy of Medicine, addressing newly admitted Fellows and Members as well as distinguished guests and the audience.



▲ Dr. KWOK Ka Ki.

▲ Dr. TSANG Chak Chi.

▲ Dr. SHUM Rocky.

▲ Dr. TSE Victoria Pui Wai.



► *Stage Party together with all the newly-admitted Members and Fellows of The Hong Kong College of Pathologists!*



▲ *From left to right, College President, Dr. CHAN Ho Ming, with Dr. Debra Graves, CEO of the RCPA, Professor Sophia CHAN, Secretary for Food and Health, HKSAR, Dr. Bruce LATHAM, President of the RCPA and Professor Tony LANDGREN, Chair of the RCPA Board of Education and Assessment.*



▲ *Dr. Bruce LATHAM, President of the RCPA, addressing the audience.*

**Congratulations to our  
newly admitted Fellows !**



### **T.B. Teoh Foundation Lecture**

The 27th T.B. Teoh Foundation Lecture was delivered by Dr. POON Wai Ming, Consultant Forensic Pathologist, Forensic Pathology Service, Department of Health, Hong Kong Special Administrative Region.

In his lecture, entitled 'The Practice of Forensic Pathology – Present and Future', Dr. Poon enlightened the audience on the special aspects and advances in forensic pathology over the years, and discussed the possible impact of Artificial Intelligence on the specialty.



▲ *T. B. Teoh Foundation Lecture being given by Dr. POON Wai Ming.*



► *President Dr. CHAN Ho Ming presenting a souvenir to Dr. POON.*



We would like to thank Dr. CHENG Shui Ying, Ivy, for being the Mistress of Ceremonies at the Annual General Meeting (AGM) and Conferment Ceremony. This year the College invited two medical students, CHAN Tsz Yeung and FOK Wan Ching, to take photos during the Trainee Presentation Session, AGM, Conferment Ceremony and T.B. Teoh Foundation Lecture. We would also like to express our gratitude towards our College Secretary, Ms. Adrienne YUNG and Ms. Heidi CHU for their support in organizing the AGM.



▲ Forensic Pathologists celebrating with their newly admitted Fellows.

▼ Celebrating newly admitted Members and Fellows from one unit.







▲▲ Chinese Banquet after  
the Annual General Meeting and  
Conferment Ceremony



► College President, Dr. CHAN Ho Ming  
presenting a souvenir to Dr. SHUM Shui Fung,  
Bobby in recognition of his contribution to the  
College





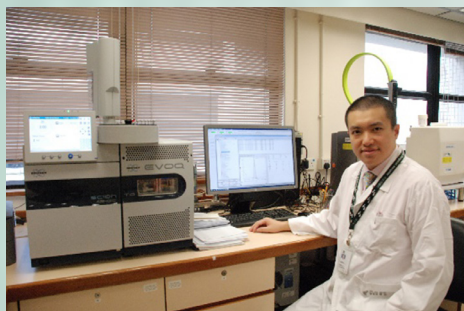
# TOPICAL UPDATE

Volume 14, Issue 1 January 2019

*The Hong Kong College of Pathologists, Incorporated in  
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**Editorial note:** In this topical update, Dr. Calvin Chong reviews and updates on the technological development of biochemical genetics for the three major classes of metabolic disorders, namely aminoacidopathies, organic acidurias and fatty acid oxidation defects. These conditions have increasing local awareness, in particular with the introduction of universal expanded newborn screening. With a rising clinical demand for confirmatory tests in biochemical genetics, the ways to achieve better analytical quality and capacity were discussed. We welcome any feedback or suggestions. Please direct them to Dr. Sammy Chen (e-mail: chenpls@ha.org.hk) of Education Committee, the Hong Kong College of Pathologists. Opinions expressed are those of the authors or named individuals, and are not necessarily those of the Hong Kong College of Pathologists.

## Biochemical genetics in the expanded newborn screening era



**Dr. CHONG Yeow Kuan, Calvin**  
*Department of Pathology,  
Princess Margaret Hospital*

### I. Introduction

Biochemical genetics refers to the diagnosis of genetic disorders with biochemical markers. Sir Archibald Garrod first described the biochemical features of alkaptonuria in 1902<sup>(1)</sup>, and is often named as the founding father of biochemical genetics. Throughout the past century, the practice of biochemical genetics has evolved from spot chemical tests towards the use of chromatography and mass spectrometry<sup>(2,3)</sup>. The recent implementation of the pilot study of expanded newborn screening means, on one hand, disorders are diagnosed earlier, and patients with such conditions would fare better, and on the other hand, this represents an increase in the use of confirmatory tests in biochemical genetics which is most acutely felt at the major chemical pathology laboratories.

The screening panel of the government-initiated pilot study included 8 aminoacidopathies, 7 organic acidurias, 6 fatty acid oxidation defects, and 3 other disorders<sup>(4)</sup>. Apart from the three disorders which required separate measurements, all three other major classes of disorders (viz. the aminoacidopathies, organic acidurias, and fatty acid

oxidation defects) are screened by the use of tandem mass spectrometric measurement of succinylacetone, amino acids and acylcarnitines, all done within a period of around two minutes.

Table 1 lists the confirmatory markers and tests for the screened conditions<sup>(5,6)</sup>: as can be seen, plasma amino acids, urine organic acids, and plasma acylcarnitines represents the main bulk work as confirmatory tests for these conditions. The present article aims therefore to explore the contemporary techniques available for these three major confirmatory tests in biochemical genetics and attempts to identify the approaches a laboratory may seek in order to cater for the increased demands.

### 2. Amino acids

Quantitative analysis of amino acids in plasma is the first-line confirmatory test for most aminoacidopathies. Amino acids are characterized by the presence of primary amine and carboxylic acid groups in one molecule, though some imino acids, namely proline and hydroxyproline, containing an imino (a functional group containing carbon-nitrogen double bond)



Condition	Confirmatory biochemical markers and tests
<b>Disorders of Amino Acids</b>	
Classical phenylketonuria	<i>Phe, Phe/Tyr ratio (PAA); Urine pterins and metabolites;</i>
PTPS deficiency	<i>CSF pterins</i>
Argininosuccinic acidemia	Argininosuccinic acid, Cit, and Arg (PAA); Orotic acid (UOA)
Maple syrup urine disease	Leu, Ileu, Val, and allo-isoleucine (PAA)
Citrullinemia type I	Cit, Argininosuccinic acid (PAA); Orotic acid (UOA)
Citrullinemia type II	Cit, Orn, Met, Arg, and Thr (PAA)
Tyrosinemia type I	Succinylacetone (UOA); Tyr (PAA)
Homocystinuria	Met, Hcy (PAA)
<b>Disorders of Organic Acids</b>	
Multiple carboxylase deficiency	Lactic acid, 3-OHIVA, MCA, 3-MCG, 3-OHPA, PG, TG (UOA)
Glutaric aciduria type I	GA and 3-OHGA (UOA); <i>Urine C5DC-carnitine and C5DC-glycine</i>
Methylmalonic acidemia	MMA, 3-OHPA, MCA, and PG (UOA)
Propionic acidemia	PG, 3-OHPA, and MCA (UOA)
Isovaleric acidemia	IVG and 3-OHIVA (UOA)
HMG-CoA lyase deficiency	3-hydroxy-3-methylglutaric acid; 3-MGA, 3-MGCA (UOA)
Beta-ketothiolase deficiency	2-methylacetoacetic acid, TG (UOA)
<b>Disorders of Fatty Acid Oxidation</b>	
Carnitine uptake deficiency	C0, C16, and C18-carnitines (PAC); <i>Urine fraction of excretion of carnitine</i>
CACT deficiency	C16, C18, C18:1-carnitines (PAC); Dicarboxylic acids (UOA)
CPT II deficiency	C16, C18, C18:1-carnitines (PAC); Dicarboxylic acids (UOA)
MCAD deficiency	C6, C8, C10-carnitines (PAC); HG, PPG (UOA)
VLCAD deficiency	C14:0, C14:1-carnitines (PAC); Dicarboxylic acids (UOA)
Glutaric acidemia type II	C4-C18 acylcarnitines (PAC); GA, 2-OHGA, 3-OHIVA, EMA, and dicarboxylic acids (UOA)
<b>Others</b>	
Congenital adrenal hyperplasia	<i>Plasma 17-hydroxyprogesterone, Urine steroid profile</i>
Biotinidase deficiency	3-OHIVA, MCA, 3-MCG (UOA) <i>Serum biotinidase</i>
Classic galactosemia	<i>Plasma galactose; Galactitol (UOA)</i>

▲ **Table 1: Confirmatory markers and tests for conditions covered in the government-initiated pilot study of expanded newborn screening. Specialized tests not discussed in the present article are italicized.**

as well as a carboxylic acid groups are also considered under the umbrella term amino acids in medical parlance and this use of terminology is adopted in the present article.

The analytical difficulties for amino acids are obvious when one examines their chemical structures: they are amphoteric, very small (glycine has a molecular mass of 75.067 g/mol), and most of them do not possess conjugated double bonds which gives absorbance in the ultraviolet spectra<sup>(7)</sup>. The first and second characteristics make separation difficult when reverse phase chromatography is used, whereas the last two give rise to problems in mass spectrometric detection and ultraviolet detection respectively. There are three major methods commonly used in clinical laboratories: amino acid analysers, high performance liquid chromatography (HPLC) with pre-column derivatization, and liquid chromatography-tandem mass spectrometry (LC-MS/MS). Irrespective of the methods employed, protein precipitation is necessary before analysis.

### **Amino acid analysers**

Amino acid analysers (AAAs) are standalone machines that employ cation-exchange chromatography using a lithium buffer system, with post-column derivatization with ninhydrin (at some 120-135 degree Celsius) and monitoring at two wavelengths<sup>(8)</sup>. It is considered the gold standard method for amino acid analysis. This method overcomes the difficulty in separation of amino acids by utilizing ion-exchange chromatography which relies on the charge of a molecule at a particular pH rather than the hydrophobicity and steric interactions of a molecule and that of detection by formation of purple complexes which absorb strongly at 570 nm for primary amino acids, and yellow complexes which absorb strongly at 440 nm for proline and hydroxyproline<sup>(9,10)</sup>. When compared with other methods, AAAs provide a higher degree of automation and require less expertise from the laboratory; the major issues with AAAs are the lengthy analytical run (120 minutes is common), the cost of the analytical instruments and the proprietary nature of the reagents. Analytical interference in AAAs are rare but do occur with dipeptides, which occur in proline deficiency<sup>(8,11)</sup>, and aspartylglucosamine, which occurs in aspartylglucosaminuria<sup>(8)</sup>, can be spotted by the 570/440 absorbance ratio.

### **HPLC and LC-MS/MS methods**

HPLC coupled with pre-column derivatization is used in the author's laboratory for PAA determination. The method relied on the pre-column (immediately before injection) derivatization of amino acids by orthophthalaldehyde (OPA) and 3-mercaptopropionic acid (MPA), followed by reversed phase chromatography and ultraviolet detection<sup>(12-14)</sup>. In the presence of a thiol reagent, OPA forms a fluorescent derivative

with primary amino acids with peak absorbance/excitation at 340 nm and emission wavelength at 450 nm<sup>(7,8)</sup>. The major drawback of this method is the inability to detect proline (and hydroxyproline) as OPA does not react with imino acids.

A newer derivatization reagent, AccQ-Tag (6-aminoquinolyl-N-hydroxysuccinimidyl carbamate), which converts both primary and secondary amino acids into stable fluorescent derivatives, has been used with ultra-high performance liquid chromatography with ultraviolet detection<sup>(15)</sup>. This derivatization reagent has the advantages of covering both primary and secondary amino acids and as the derivatization products are stable, advanced autosamplers which can pipet reagent from one vial to another is not required<sup>(16)</sup>. Complete chemistry kit-sets that use this derivatization reagent is commercially available<sup>(17)</sup>.

Liquid chromatography coupled with tandem mass spectrometry is also used locally for PAA determination. As these methods are often based on reversed phase chromatography, despite the use of mass spectrometric detector, derivatization is still often employed<sup>(7)</sup>. A method based on the proprietary derivatization reagent AccQ-Tag has been recently described in the literature with a run-time of 6 minutes<sup>(18)</sup>. Isotopic internal standards are required for LC-MS/MS-based methods and it is important to note that the impracticality of having isotopic internal standards for each and every analyte would mean that the robustness of the assay is considerably weaker than optical-based methods<sup>(19)</sup>.

Compared with methods based on optical detection, LC-MS/MS methods had a shorter analytical runtime and better specificity. This is counterweighed by the higher capital and maintenance cost of acquiring an LC-MS/MS (and not to mention the cost of having a backup LC-MS/MS system), as well as the technical expertise necessary to operate and troubleshoot an LC-MS/MS.

### **Choice of method and the future**

The question is different depending on the volume of testing as well as equipment availability. For laboratories that have existing HPLC-DAD equipment employing OPA derivatization there may be little incentive in adopting a new procedure: there is probably little competition for HPLC analyser time, and the inability to detect proline may not be a major issue as hyperprolinaemia type I is benign whereas hyperprolinaemia type II is classically diagnosed by the presence of N-(Pyrrole-2-carboxyl)-glycine in urine organic acids<sup>(20)</sup>.

On the other hand, for a laboratory seeking to provide amino acids analysis for the first time, the use of stable derivatization reagent such as AccQ-Tag mentioned



above has the advantage of not requiring higher-end liquid chromatographs and the availability of commercial kits means that development time is reduced. For a laboratory anticipating a high workload, a dedicated liquid chromatograph with UV detection appears to be the simplest solution as it is robust and inexpensive. On the other hand, for laboratories with lower service demand, mass spectrometric detection may in fact be more feasible as the notion of spare LC-MS/MS capacity means that the major downside of LC-MS/MS detection, viz capital cost and technical expertise, are sunk cost to the laboratory.

## 2. Organic acids

Organic acidurias are diagnosed most commonly by urine organic acid analysis with gas chromatography-mass spectrometry (GC-MS)<sup>(21)</sup>, and this technique is used by many local hospitals. Though the term organic acids refers to organic compounds with a carboxylic acid group, a broader spectrum of compounds, such as uracil and xanthine, are detected in practice. Urine organic analysis is usually qualitative though quantitative analyses of some compounds (e.g. orotic acid, methylmalonic acid) are often offered.

### **GC-MS based urine organic acid analysis**

For GC-MS analysis of urine organic acids, a creatinine-corrected amount of urine is subject to liquid-liquid extraction with ethyl acetate after acidification using hydrochloric acid, the organic extract is then dried and derivatized by N,O-bis-tri(methylsilyl)trifluoroacetamide (BSTFA) with 1% trimethylchlorosilane (TMCS)<sup>(22)</sup>. The resultant product is injected to the GC-MS operating in scan mode. For reliable detection of ketones, oximation with hydroxylamine hydrochloride can be performed prior to acidic extraction<sup>(23)</sup>.

An alternative way of performing urine organic acid analysis is to bypass the extraction step. As no acidic extraction step is done, a limited panel of amino acids, purine, pyrimidines, and mono- and di-saccharides can be detected in the same analytical run. This method has been in-use in the author's laboratory in the past 2 decades<sup>(24,25)</sup>, and allows the detection of a much wider range of metabolites in urine. With no extraction, the chromatograms are extremely complex due to co-elution of analytes and therefore this approach requires expertise in post-analytical data processing to be viable as a routine method.

The interpretation of GC-MS data for urine organic acid analysis is commonly based on examination of the chromatogram (Figure 1), followed by a combination of peak integration in the total ion chromatogram followed by library searching and examination of extracted ion chromatogram at particular retention times for analytes which gives lower responses (Figure 2). The raw analyte response is then

compared to locally-established age-specific reference intervals which allow clinical interpretation<sup>(23)</sup>.

### **LC-MS based urine organic acid analysis**

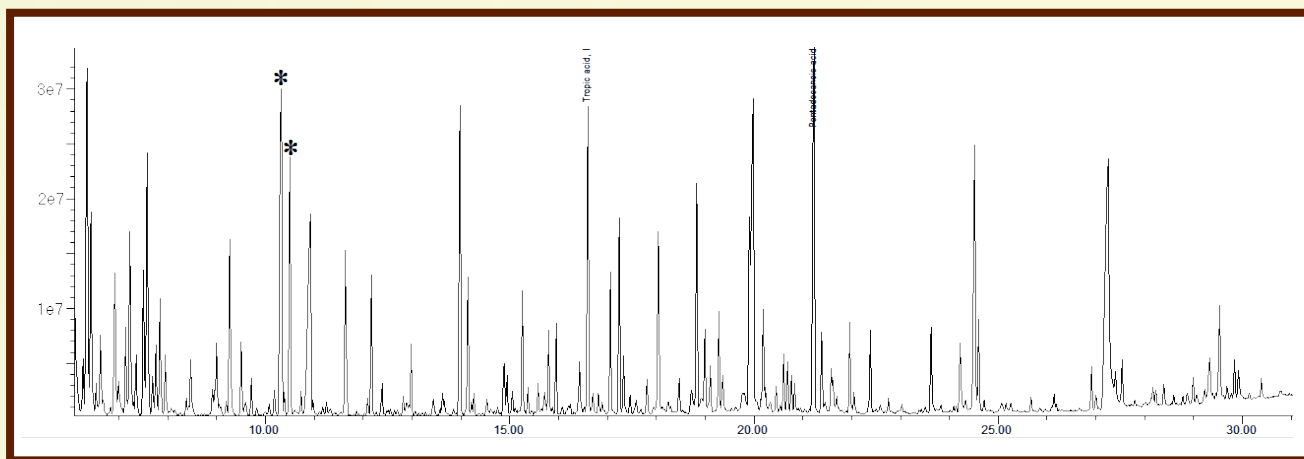
In the recent years, liquid chromatography-mass spectrometry-based methods have been published for analysis of urine organic acids. The benefit of liquid chromatography-mass spectrometry is clear: there is no need for the cumbersome derivatization step, and heat-labile analytes can be detected<sup>(26,27)</sup>. The major drawbacks of LC-MS based methods are the poorer separation of analytes and higher susceptibility towards ion suppression, as electrospray ionization is much less robust against matrix effects compared to electron ionization as used in GC-MS based methods<sup>(28)</sup>. The difficulty of developing an in-house LC-MS based method for urine organic acids lies in the procurement of a practically endless list of organic acid standards to establish the retention time and multiple reaction monitoring ratios. At the time of writing, there is at least one commercial kit that has been made available (Zivak Organic Acids LC-MS/MS analysis kit), but this commercial kit did not utilize isotopic internal standards even for critical analytes (such as hexanoylglycine and orotic acid) and one may wish to validate extensively the robustness of such assays against matrix effects,

### **Choice of method and the future**

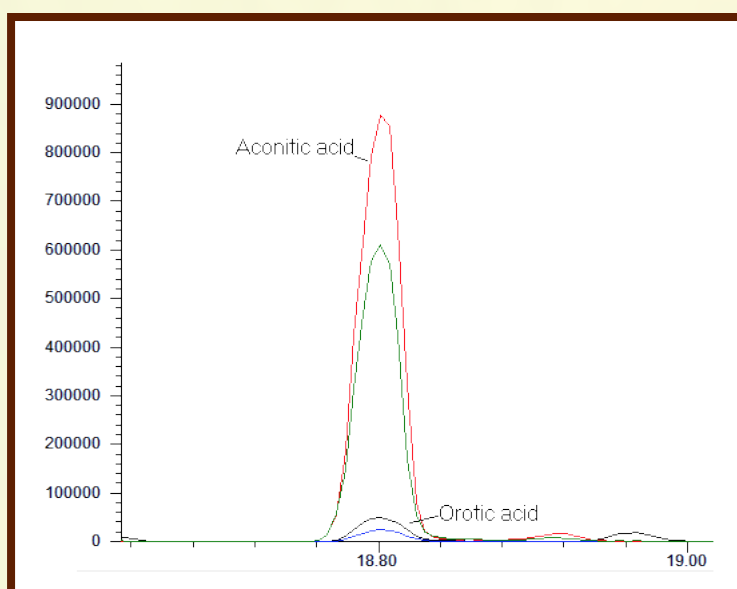
Out of the three assays discussed in the present article, urine organic acid analysis represents the most difficult of the three to establish in a laboratory. There is first the difficulty in establishing age-specific reference intervals, and then difficulty in acquiring a large number of chemical standards. A good starting point would be obtaining the bi-level quality controls for urine organic acid and special assays for urine from the ERNDIM network which consist of a number of critical analytes (<http://cms.erndimqa.nl/Control-Materials.aspx>). As for choice of internal standards, while traditional choices such as tropic acid and pentadecanoic acid are often employed, deuterated internal standards covering critical analytes are much more available nowadays (e.g. methylmalonic acid-d3 and orotic acid-1,3-15N2) and their addition may improve quantitation of these critical analytes, the former being commonly quantified and the latter being prone to analytical errors<sup>(29)</sup>.

For laboratories with existing GC-MS based urine organic acid assay, the question is probably whether to adopt LC-MS based solution. The belief that organic aciduria always results in sky-high level of abnormal metabolites in urine is flawed: the low-excretor phenotype of glutaric aciduria type I can serve as the classical example<sup>(30)</sup>. The difficulty may be mitigated somewhat if acylcarnitines and acylglycines are measured by the LC-MS based assays as glutaryl carnitine has been shown to be informative even for low-excretor GA I

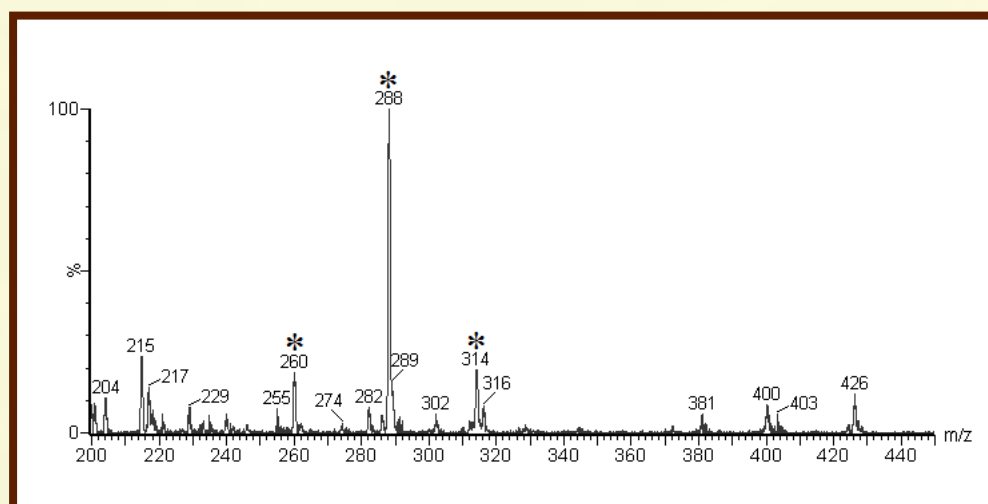




▲ **Figure 1:** Total ion chromatogram of a urine sample from a patient with malonic acidemia circulated in the ERNDIM qualitative organic acid program in 2016. The two abnormal peaks are indicated by asterisks: the first peak represents malonic acid (di-TMS derivative) and the second peak represents methylmalonic acid (di-TMS derivative).



◀ **Figure 2:** Extracted ion chromatogram showing the quantifier ions ( $m/z$  375, for aconitic acid in red; and  $m/z$  254, for orotic acid, in black) and qualifier ions ( $m/z$  285, for aconitic acid in green; and  $m/z$  357, for orotic acid, in blue). They are co-eluting in many GC-MS based urine organic acid assays.



▲ **Figure 3:** Cumulative precursor scan mass spectrum for a blood-spot sample distributed in the ERNDIM Qualitative Acylcarnitine Program in 2014. In this specimen, elevated signals at  $m/z$  ratio 260 (C6-carnitine), 288 (C8-carnitine), and 314 (C10:1-carnitine) can be seen. The pattern would be compatible with MCAD deficiency. (Mass spectra courtesy of Mr CK Lai, Chemical Pathology Laboratory, PMH)



patients<sup>(31)</sup>. Overall, it remains to be seen whether LC-MS based organic acid analysis could stand alone replacing, rather than complementing, GC-MS based assays.

### 3. Free carnitine and acylcarnitines

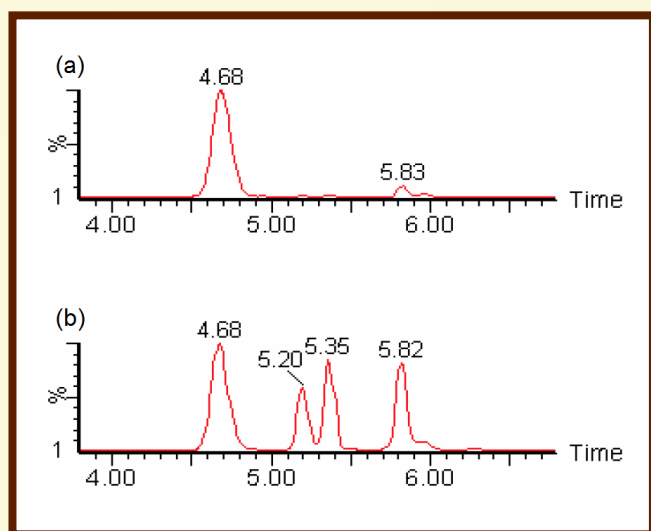
Quantitative analysis of free carnitine and acylcarnitines in plasma represents the first-line confirmatory test for fatty acid oxidation disorders. Analysis of free carnitine with calculation of fraction of excretion can be helpful in the diagnosis of carnitine uptake defect, as is measurement of urine glutarylcarnitine for the diagnosis of glutaric aciduria type I as discussed above<sup>(31)</sup>. Carnitine is a quaternary ammonium compound with a carboxylic acid group, as well as a hydroxyl group with which acyl groups are attached to form acylcarnitines; as such, it forms zwitterions under physiological pH. Derivatization to esters by incubation with an alcohol (typically butanol) has been employed<sup>(32)</sup> though locally underivatized analysis at acidic pH is commonly used<sup>(33)</sup>.

For underivatized analysis, plasma is first diluted with a mixture of isotopic internal standards, and acidified acetonitrile is slowly added to precipitate plasma proteins. The sample is vortexed, centrifuged, followed by evaporation of the supernatant to dryness and reconstituted for analysis by positive electrospray ionization-tandem mass spectrometry with or without liquid chromatography separation. Data can be collected in multiple reaction monitoring (MRM) mode, or

in precursor ion scan with accumulation of ions, commonly known as multichannel acquisition (MCA) mode<sup>(34)</sup> (Figure 3). The American College of Medical Genetics Guideline, published in 2008, suggested the use of precursor ion scan as it permitted the evaluation of the whole acylcarnitine profile, as well as the detection of drug artefacts, interfering compounds and assessment of derivatization<sup>(35)</sup>.

### Derivatization and chromatographic separation

In the analysis of carnitine and acylcarnitines, butyl-ester derivatization enhances the formation of positively-charged ion by reacting with carboxylic groups, and causes mass separation of dicarboxylic-carnitines and hydroxy-acylcarnitines (e.g. C4DC-carnitine and C5OH-carnitine), which are isobaric when underivatized<sup>(36)</sup>. Derivatization is typically performed at highly acidic conditions (e.g. 3N hydrochloric acid at 65°C for 15 minutes)<sup>(35)</sup>. On the other hand, chromatographic separation allows the separate determination of individual isomeric constituents (e.g. C4DC-carnitines include succinylcarnitine and methylmalonylcarnitine; and C5OH-carnitines include 3-hydroxyisovalerylcarnitine and 2-methyl-3-hydroxybutyrylcarnitine) (Figure 4). With meticulous chromatographic separation, most biologically relevant isomeric species could be separately quantified<sup>(37)</sup>.



◀ **Figure 4: SRM chromatogram of  $m/z$  262>85 showing chromatographic separation of different species of isobaric (C4DC/C5OH) acylcarnitines (viz. succinylcarnitine at 4.68 minutes, two diastereomeric peaks of methylmalonylcarnitine at 5.2 and 5.35 minutes, and 3-hydroxyisovalerylcarnitine at 5.82 minutes) could be individually identified and quantified with liquid chromatography-tandem mass spectrometry without derivatization. (a) sample with normal amounts of succinylcarnitine; (b) sample with abnormal amounts of methylmalonylcarnitines (Chromatograms courtesy of Mr CK Lai, Chemical Pathology Laboratory, PMH)**

#### Abbreviations:

**Plasma amino acids (PAA):** Arginine (Arg), Citrulline (Cit), Homocystine (Hcy), Isoleucine (Ileu), Leucine (Leu), Methionine (Met), Ornithine (Orn), Phenylalanine (Phe), Threonine (Thr), Tyrosine (Tyr), Valine (Val).

**Urine organic acids (UOA):** ethylmalonic acid (EMA), glutaric acid (GA), 2-hydroxyglutaric acid (2-OHGA), 3-hydroxyglutaric acid (3-OHGA), 3-methylglutaric acid (3-MGA), 3-methylglutaconic acid (3-MGCA), hexanoylglycine (HG), 3-hydroxyisovaleric acid (3-OHIVA), Isovalerylglycine (IVG), 3-methylcrotonylglycine (3-MCG), Methylcitric acid (MCA), Methylmalonic acid (MMA), 3-hydroxypropionic acid (3-OHPA), propionylglycine (PG), phenylpropionylglycine (PPG), Tiglylglycine (TG).

**Plasma acylcarnitines (PAC):** Free carnitine (C0).

**Others:** 6-pyruvoyl-tetrahydropterin synthase (PTPS), 3-hydroxy-3-methylglutaryl CoA (HMG-CoA), Carnitine-acylcarnitine translocase (CACT), Carnitine palmitoyltransferase II (CPT-II), Medium-chain acyl-CoA dehydrogenase (MCAD), Very long-chain acyl-coA dehydrogenase (VLCAD).



## Choice of method and the future

The question for the laboratory is, first, whether to employ the derivatization procedure. The advantage of derivatization is the mass separation of hydroxylacylcarnitines and carnitine derivatives of dicarboxylic acids; the problems associated with derivatization are the partial hydrolysis of acylcarnitines because of the high temperature and strongly acidic condition employed<sup>(33)</sup>. The other considerations are whether to employ chromatography and if so, how extensive should it be: it would then be a delicate balance between throughput, diagnostic specificity, and analyser-time that is available. With the improvement of separation capability of liquid chromatographs and sensitivity of modern mass spectrometers, it is suggested that a short UHPLC program combined with both scheduled MRM and precursor ion scan function would be a good compromise.

## 4. Conclusions

The three assays discussed above represent the bulk of workload for most metabolic laboratories. The planned

implementation of universal expanded newborn screening in Hong Kong means that the demand would increase, and the phenotype will be less defined, as tests are requested for patients who may not yet present with features of an inborn error and importantly, patients with only borderline elevation of analytes.

From a Bayesian point of view, this change in pre-test probability would mean that, if the analytical quality and interpretative capacity remains the same (which affect the likelihood ratio of positive results), the post-test probability would suffer from a negative impact. The quest for the metabolic bench of any major pathology laboratory is then to improve both throughput and quality of analysis at the same time, an impossible task, as the Duke of Norfolk wrote in 1538, "a man can not have his cake and eat his cake"<sup>(38)</sup>. The technology improvements as reviewed in the present article may aid in the analytical quality but the quest for improved interpretative capacity remains on the training and education of our present and coming generations of pathologists.

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# Hong Kong Academy of Medicine

## 25th Anniversary Congress

Established in 1993 by the Founding President, the late Professor Sir David TODD, the Hong Kong Academy of Medicine now has 15 constituent colleges with 67 specialties and subspecialties and more than 7,900 Academy Fellows who are eligible for registration in the Specialist Register of The Medical Council of Hong Kong or The Dental Council of Hong Kong.

The Academy celebrated its 25th Anniversary in 2018, with the year-long activities being kicked off by an opening ceremony officiated by Professor the Honourable Sophia CHAN, Secretary for Food and Health of the Hong Kong SAR, in February 2018. The Grand Finale was the 25th Anniversary Congress, which was held over 3 days in December followed by the Silver Jubilee Gala Dinner. The Congress was themed 'Beyond 25: New Paradigms in Healthcare' and attracted over 500 local and overseas participants. The Guest of Honour was the Honourable Carrie LAM, Chief Executive of the Hong Kong SAR.

Two distinguished speakers were invited to deliver plenary sessions; one entitled 'Medical Regulation: Supporting Technology, Protecting Patients' which was given by Dr. Humayun CHAUDHRY, President and Chief Executive Officer, The Federation of State Medical Boards of the United States, Inc. and Immediate Past Chair, International Association of Medical Regulatory Authorities. In his lecture, Dr. Chaudhry discussed the increasing involvement of technology in healthcare; the likes of which include artificial intelligence, telemedicine, genomics, advanced robotics, driverless cars, and 3-D printing. He emphasized the crucial role of the Academy as, being one of the world's medical regulators, it has to work with doctors to support these changes while assuring patient safety as they enter these uncharted waters together.

The David Todd Oration was given by our College Fellow, Professor YUEN Kwok Yung, Henry Fok Professor in Infectious Diseases, Chair of Infectious Diseases, Department of Microbiology, Li Ka Shing Faculty of Medicine, The University of Hong Kong. His lecture, entitled 'Emerging Infectious Diseases and Beyond', illustrated a selection of his encounters with microbial diseases throughout his career, including investigation of platelet transfusion-related bacteraemia in 1994, molecular biology for the diagnosis of cytomegalovirus in 1995, tackling penicilliosis in 1994, avian influenza A H5N1 in 1997, leading the fight against SARS in 2003, and last but not least, solving the intestinal zygomycosis outbreak related to contaminated allopurinol tablets in 2009.



▲ Our College Fellow Dr. CHONG Yeow Kuan, Calvin (second from right) performing at the Opening Ceremony of the 25th Anniversary Congress on 8th December 2018.



The workshops were varied and included aspects on paediatric metabolic diseases, infectious diseases, breast and colorectal cancer management, medicine in the digital era, and medical education and training.

◀ Our College Fellow, Dr. CHEUNG Sin, Joyce being conferred as Fellow of the Hong Kong Academy of Medicine.





▲ Professor Yuen giving the 2018 David Todd Oration.



▲▼ Distinguished guests from around the world attending the 25th Anniversary Congress.





# Royal College of Pathologists of Australasia Quality Assurance Programs 30th Anniversary Symposium

The Royal College of Pathologists of Australasia Quality Assurance Programs (RCPAQAP) held a series of worldwide symposia, entitled "RCPAQAP 30th Anniversary Quality Symposia" as part of their 30th Anniversary celebrations. On 9th March, our College was privileged to co-organize this one-day symposium in The Hong Kong Academy of Medicine Jockey Club Building.

Dr. CHAN Ho Ming, our College President, gave the opening speech which kick started the symposium. Professor Tony BADRICK, Chief Executive Officer of RCPAQAP who is also a renowned clinical biochemist, delivered his talks on the future of laboratory quality and the role of External Quality Assurance (EQA) in traceability. Dr. Louise WIENHOLT, an Immunologist by training, discussed quality issues in point of care testing. Mr. Derek HOLZHAUSER, Chief Information Officer at RCPAQAP shared with audience his expertise in cybersecurity in pathology settings, and updates in myQAP, a sophisticated digital pathology platform.

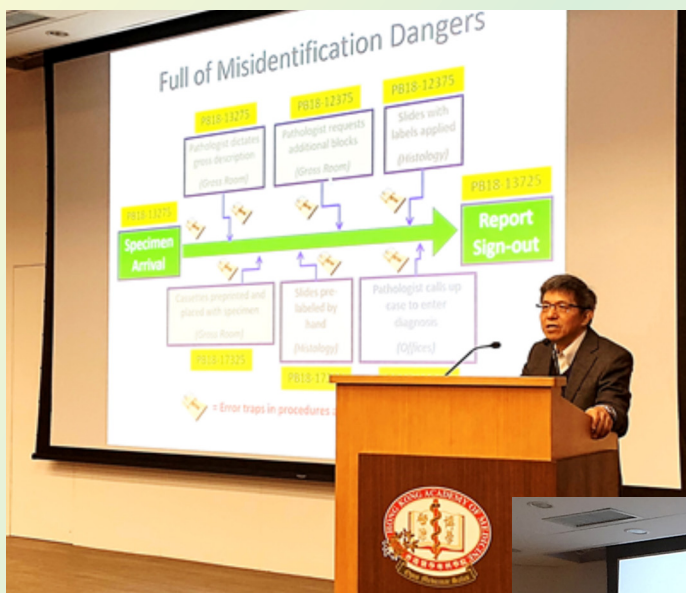
In addition to distinguished speakers from the RCPAQAP, a strong faculty of local speakers were also invited to share their expertise. Dr. CHAN Ngot Htain, Alice, Chairlady of our College's Quality Assurance Committee, gave the audience a birds' eye view of the laboratory quality system in Hong Kong. Dr. LEE Kam Cheong of Princess Margaret Hospital, presented his multi award-winning creation, the PATHOS system and how it improves quality and traceability by enhancing the digital workflow in anatomical pathology. Being the first hospital in Hong Kong to adopt the use of automated disk diffusion reading system, Microbiologist Dr. WU Ka Lun, Alan, of Pamela Youde Nethersole Eastern Hospital, demonstrated how implementation of this system can lead to more objective and efficient assessment of bacterial susceptibility to antibiotics. Dr. Miranda YAU, representing the Hong Kong Medical Laboratory Service Quality Assurance Program (HKMLSQAP), shared with audiences their mission as the proficiency test service providers in Hong Kong. Haematologist Dr. LEUNG Yuk Yan, Rock and Chemical Pathologist Dr. POON Wing Tat also discussed quality issues they have encountered in their respective disciplines.

The symposium was very well received and we look forward to the RCPAQAP's next visit to Hong Kong in two years' time!

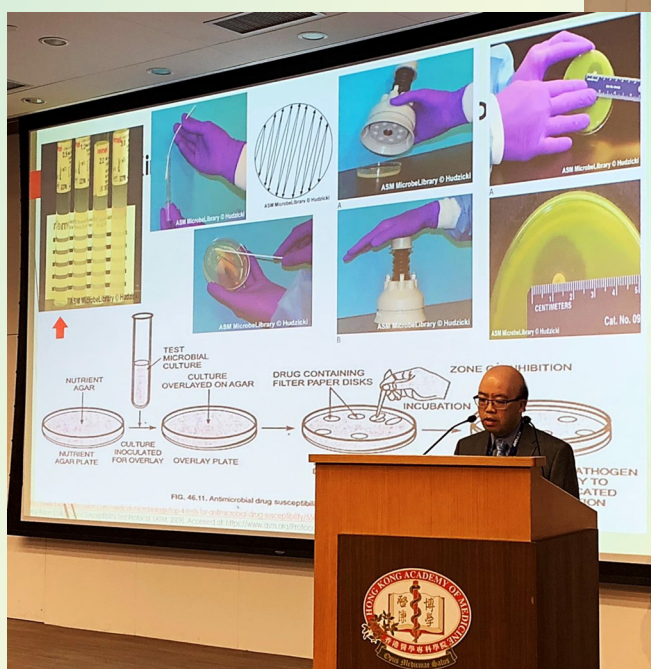
► *Dr Louise WEINHOLT welcoming the audience and speakers to the 30th Anniversary Symposium.*







▲▲▲ A faculty of local and overseas speakers at the symposium, including Dr LEE Kam Cheong, Professor Tony BADRICK, Dr WU Ka Lun, Alan, and Dr LEUNG Yuk Yan, Rock discuss quality issues with the audience.



# Featured Article -

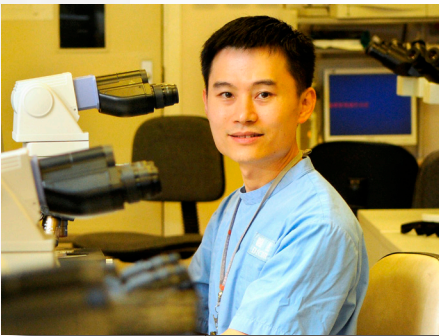
by Dr CHAN Sheung Wai, Gavin

## Life paradigm in a place of death:

### - To achieve a good-bye in the mortuary

*"Death leaves a heartache no one can heal,  
love leaves a memory no one can steal."*

- Anonymous



The function of a mortuary is classically limited to dead body management and autopsy. This 'place for the dead' is associated with stigma, coldness, fear, mystery and bad luck in Chinese culture and in some other countries.<sup>1,2</sup> Its role in bereavement support, life education, medical humanity, social science engagement and body/corneal donation is under-recognized. This 'life-affirming' strategy is an important paradigm shift in mortuary development and would help to break the taboo of mortuaries. It will have great potential to benefit the healthcare system and society.

With the advent of development projects in various hospitals, the life paradigm can be introduced and further explored in the future of mortuary services. In this article, two important areas of the 'life-affirming' strategies, including physical environment and information on body changes, are discussed which should be more readily applied in mortuaries.

#### Background

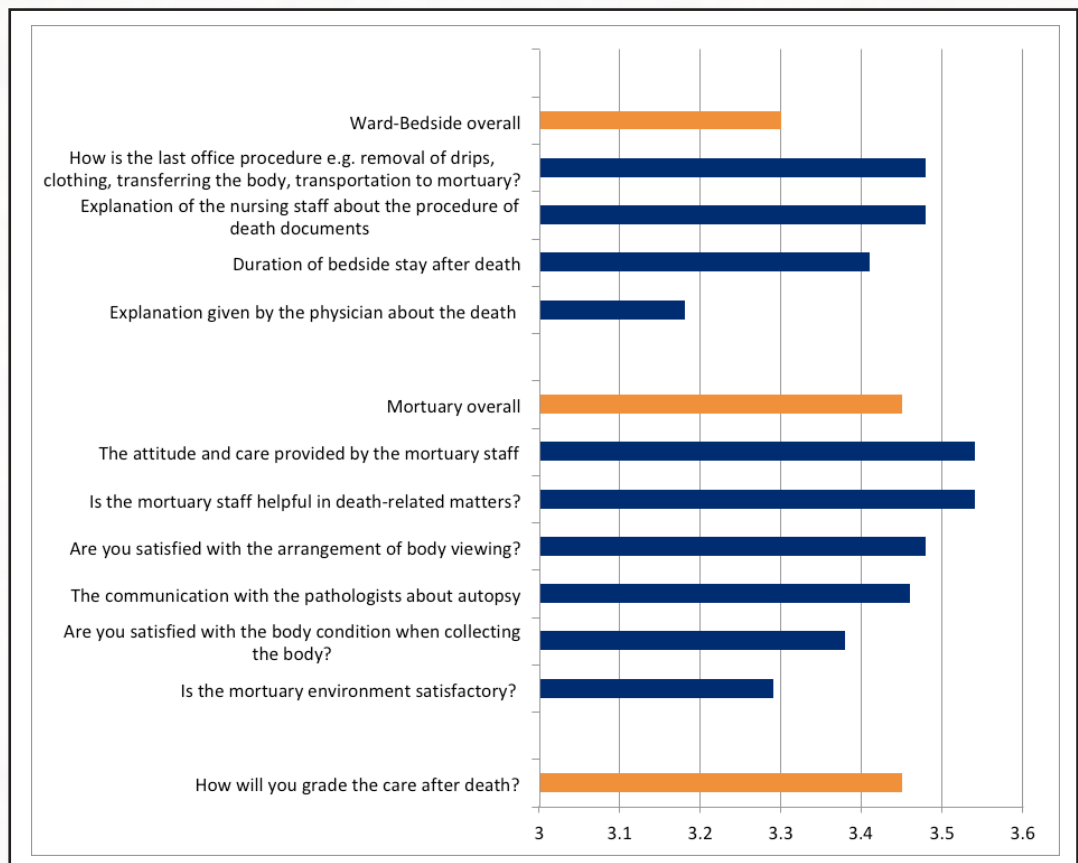
The Hospital Mortuary is essential to the healthcare system, providing vital services to deceased patients and bereaved families. The relatives will attend mortuaries for after-death procedures, and it is the area where early bereavement reaction is supported. After

a series of high-profile incidences related to mortuary practice,<sup>3,4</sup> information technology and barcode identification system with or without Radiofrequency Identification (RFID) have been implemented in various mortuaries to safeguard operational workflow. The safe release of bodies is the initial step to protect bereaved families from second trauma.

It appears that the safe release of bodies alone is not adequate for a quality service. The Department of Health of the United Kingdom has called for a review of service relating to death and bereavement in the past decade. In the paper entitled 'When a Patient Dies: advice on developing bereavement service in the NHS',<sup>5</sup> the need to enhance the care and support for bereaved families was advocated, where the respect and dignity of deceased patients and their families are key components. Good care after death is no longer regarded as an optional extra, but an essential component of quality healthcare.<sup>6</sup> The paradigm shift from 'dead body management' to 'life-affirmation' was regarded as an important symbolic change in modernizing mortuary services. The identification of the deceased person as a patient rather than a dead body is a fundamental change in the concept of a deceased, and such recognition helps to raise awareness of the significance of the mortuary staff as part of the continuity of care, and brings meaning and value to their work.<sup>2</sup>

Life-affirmation in mortuary services is under-recognised in Hong Kong. In 2015,





▲ *Figure 1: Score of hospital care after patient death: ward-bedside (pre-mortuary) and mortuary parts (adapted from reference 7)*

a survey was performed in the mortuary of Queen Mary Hospital on 190 bereaved families, to explore the adequacy of hospital care after death.<sup>7</sup> The survey enables us to better understand families' experiences, needs and expectations after patient death. Two deficiencies in the physical environment of the mortuary and body condition on collection were identified, suggesting a service gap which we need to pay attention to in future improvement plans (figure 1):

### **'Healing Environment' Concept**

Memories of mortuary experiences last long. The distress of bereavement can be made worse when relatives return to the mortuary for last office procedures. Dignity and respect to the families should also include providing an appropriate physical environment for care delivery. The importance of a supportive environment was recently recognized in the 2017 Hospital Authority (HA) Strategic Service Framework for Palliative Care:<sup>8</sup> 'the design of mortuaries is another area to be improved on, which will affect the experience and memories of patients' families/ carers. In particular, the

design for the circulation areas, viewing rooms and ceremony rooms in the mortuaries should convey a sense of reverence and respect for life. Modernisation of the overall design of mortuaries is also required to better suit the operational workflow and the needs of families/ carers'.

Improvement of the environment should be a key element of improving mortuary services in future hospital redevelopment of Hong Kong. The essential features of the design include:

- 1) The physical environment of the bereavement suite needs to be revamped and 'humanised'. A sympathetic, supportive and de-institutionalised (home-like) environment with easy chairs and adequate lighting can alleviate grief and improve privacy. Artistic works can be judiciously introduced to distract grievance, and connection with nature (e.g. sunlight or plants) could echo the affinity between life and nature. A heart-warming and caring atmosphere with soft music would

help to relieve bereavement reaction, and make people feel calm and being cared-for (figure 2).

- 2) In some hours of the day, the mortuary is a place of hustle and bustle packed with various persons and activities. The attending persons are composed of mortuary staff, bereaved families, pathologists, police and funeral staff. Activities include body identification, viewing requests, release of bodies, coroner's autopsy interviews, organ dissection, autopsy and corneal donation. The workflow logistics and pathways of the entrances and exits need to be carefully designed to streamline the flow of people, to ensure safe body handling, to enhance privacy, to respect grief reaction and to improve families' experiences. A well-designed physical environment will also be conducive to service delivery and increase staff morale.
- 3) It is imperative for bereaved families to view and spend time with the deceased patients. Viewing room facilities should be in place for viewing purposes, and it is best for one of them to be segregated from the body storage area to accommodate special religious needs of specific groups of bereaved families.
- 4) After death, 63% of Next of Kin will accompany the deceased to the mortuary.<sup>7</sup> The journey of relatives after patients' death needs to be assessed. Long, dim and dirty corridors leading from the ward to the mortuary, termed 'walk of shame',<sup>9</sup> must be avoided to enhance a respectful and dignified experience for the relatives (figure 3).



▲ Figure 2: A newly renovated mortuary with home-like facility and connection with nature



▲ Figure 3: 'Walk of Shame': long dim corridor leading from ward to mortuary is to be avoided for better relatives' journey after patient death

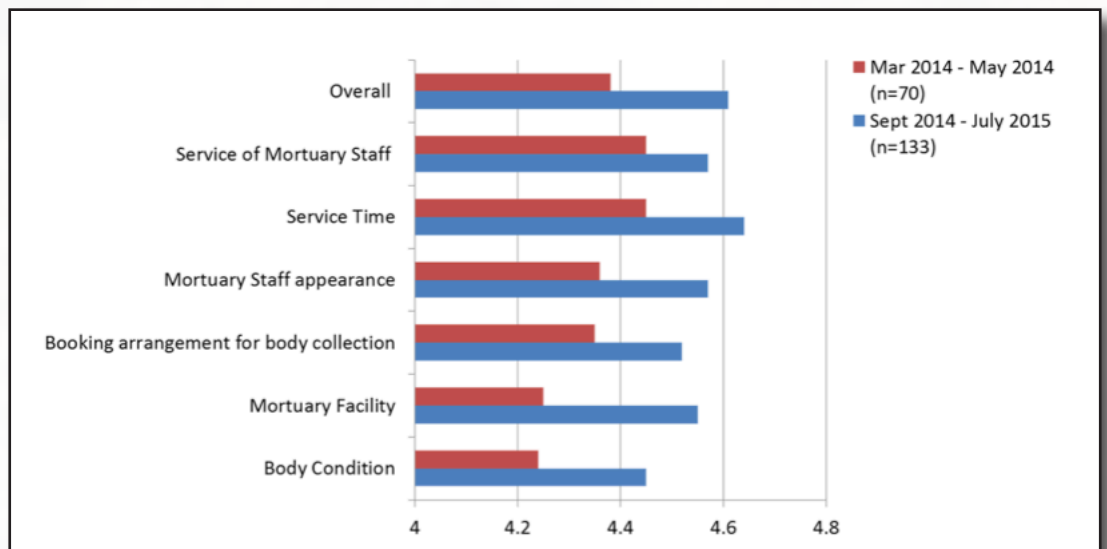
### Body condition on Body collection

While the low score on the mortuary environment is understandable, the dissatisfaction on the body condition appears intriguing in view of the fact that the bodies have been refrigerated and preserved at adequately low temperatures. This finding however echoes two similar surveys performed independently by another hospital, Pamela Youde Nethersole Eastern Hospital (PYNEH), during Mar 2014-July 2015. In the surveys, body condition had the lowest scores amongst all the service parameters for two consecutive years (figure 4):

According to the 2016 HA Internal Audit Report, the average length of stay of bodies in the mortuary was around 20 days in Hong Kong. Can the dissatisfaction of body conditions be related to the unpreparedness of the relatives for the body changes? Body changes after death are a normal phenomenon, which include body color changes, leakage of body fluids, skin peeling, dehydration, infection and muscle stiffening/ softening which may open the eyes and mouth..... It should be noted that even when refrigerated at low temperatures, variable degrees of body changes are inevitable after 20 days. The longer the storage, the more severe will be the post-mortem decomposition. Relatives need to be prepared for these changes before they view or identify the body.

In order to allow better understanding





▲ **Figure 4: Results of two surveys on mortuary service in PYNEH during 2014 and 2015 (unpublished data; courtesy of Dr. WL TANG & Mr. A LAM, Department of Clinical Pathology, PYNEH).**

and acceptance of post-mortem body changes, information of such post-mortem changes could be included in the hospital information leaflets given to bereaved families (Figure 5). This may also encourage relatives to collect the bodies sooner in order to minimize the decay. The mortuary staff may also remind relatives of these normal body changes due to long-stay of bodies, so that the relatives may accept the changes more readily when collecting the body.

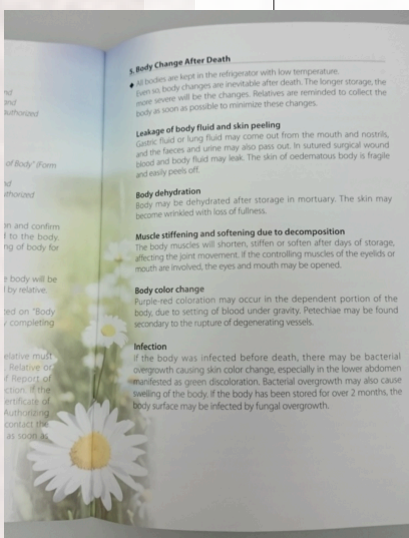
## Conclusion

Hospital care does not end with patients' death. The Mortuary forms an integral component of the health service, providing seamless care, support and respect to the families. The 'life-affirming strategy' is an important paradigm shift in mortuary services. A 'healing environment' with the provision of person-centered, humanistic care can alleviate bereavement reaction and should be introduced in future hospital designs during hospital development. Information on the post-mortem body changes may prepare the relatives for post-mortem changes when they collect the bodies, and potentially

improve their experience. A 'life-affirming' strategy will help to demystify and de-stigmatise the mortuary, and pave the way for better service provision for bereaved families.

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◀ **Figure 5: Information on post-mortem body changes in the hospital pamphlet 'Important Notes for Relatives of the Deceased'**

## Panda's Home: Hong Kong Academy of Medicine Sichuan Study Tour, February 2019

This February, twenty-eight doctors from various colleges under the Hong Kong Academy of Medicine (HKAM) took part in the HKAM Sichuan Study Tour, and I am honoured and privileged to have been one of them.

This tour was a once in a lifetime experience. I wasn't quite sure what to expect initially. But as we met the rest of the team, things seemed to just fall into place. The entire trip was incredibly well organized. We learned a great deal about the Chinese healthcare system and their beliefs in primary care and disease preventive measures. In particular, we went to the West China Hospital and Sichuan Center for Disease Control and Prevention, and saw first-hand the way they solved health disparities and problems with access to medical care that many communities faced after the natural disaster in Sichuan. It makes you appreciate the practical resources that went into a system that is able to cope with the problems of a huge population as well as overcoming various other difficulties.

I remember one of the pathologists from West China Hospital, Sichuan University, approaching us and sharing her experiences of working in anatomical pathology, where they receive over 280,000 surgical specimens per year. Their highly specialized workflow made the available manpower more efficient so as to cope with the diversified and huge workload.

In between hospital and clinic visits, we also had ample opportunities to learn about the history and culture of Sichuan. Everyone was nice and supportive. Well, perhaps not everyone enjoyed the Sichuan spice, as my gastrointestinal tract was burning! This program far exceeded

my expectations and I would absolutely recommend it to future Young Fellows interested in finding out more about the healthcare system and management in China.



◀ College Young Fellows, Dr. MAK Siu Ming and Dr. CHENG Shui Ying, Ivy with Dr. LAU Chor Chiu, Vice-President (General Affairs) of the Hong Kong Academy of Medicine





▲ Group photo of HKAM representatives who joined the study tour and their hosts in front of the West China Hospital, Sichuan.



▼ Visiting the Institute for Disaster Management and Reconstruction in Chengdu, Sichuan. This establishment was opened in 2013 and was jointly set up by the Sichuan University and the Hong Kong Polytechnic University following the magnitude 8 earthquake in May 2008.



# Revision of the

## “Policy on Interruption of Training due to Long Leave”

This is to announce that the following paragraph in the section of “Policy on Interruption of Training due to Long Leave” in the Regulations on Postgraduate Training and Examinations has been revised as follows, **with effect from 11 January 2019**:

*Trainee shall report any long leave more than 90 continuous calendar days to the Training & Examinations Committee as soon as possible and not later than the deadline of the Annual Report submission. The period of such leave in excess of 90 calendar days shall not be counted as recognised training. (This policy applies to all trainees of all disciplines registered on or after 1 June 2009.)*

A soft copy of the addendum regarding this issue will be emailed to all active trainees and educational supervisors in due course.

We wish you all a successful 2019!

Training & Examinations Committee