THE HONG KONG COLLEGE OF PATHOLOGISTS
SCIENTIA ILLUMINAT MEDICINAM
香港病理學專科學院

# Topical Update – The Hong Kong College of Pathologists

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

**Volume 11, Issue 2** **August 2016**

## Editorial note:

In this topical update, Dr Chloe Mak reviews the history and development of newborn screening, in particular for Hong Kong. Both benefits and limitations of expanded newborn screening were discussed. The latest pilot screening programme, as stipulated in the Policy Address by Chief Executive, was also illustrated. 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.

# Newborn Screening: Past, Present and the Future

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### Introduction

Newborn screening (NBS) is one of the most successful public health programs in the 20th century. In fact, the idea of mass screening was totally new to the society before 1960's. When Dr Ivar Asbjørn Følling discovered the disease phenylketonuria (PKU) leading to mental retardation in many children [1] and Dr Robert Guthrie invented a simple and reliable screening test using bacterial inhibition test for blood phenylalanine [2] together with the understanding of disease pathogenesis and effective treatment to prevent mental retardation initiated during early asymptomatic phase [3], the proposal of NBS was born. However, criticisms were vigorously received over the uncertainties of disease nature, assay validity and long-term treatment effectiveness. To begin with, NBS for PKU was tested as a pilot service in Massachusetts in 1962 [4]. World Health Organization (WHO) issued two landmark reports about population screening: "The Principles and Practice of Screening for Disease" [5] and "The WHO Scientific Group on Screening for Inborn Errors of Metabolism (IEM), Geneva" [6]. The latter report elaborates more on screening for IEM.

After the success of PKU screening in preventing mental retardation, the legislation for mandatory

screening was made in 1975 in USA. More disorders were added to the panel, such as congenital hypothyroidism (incidence 1 in 2,200) in 1976, congenital toxoplasmosis (1 in 27,800) in 1986, hemoglobinopathies (1 in 2,900) and congenital adrenal hyperplasia (1 in 19,200) in 1990, biotinidase deficiency (1 in 42,000) in 1992, medium-chain acyl-CoA dehydrogenase deficiency (1 in 21,000) and cystic fibrosis (1 in 2,900) in 1999 in the New England Newborn Screening Program of the University of Massachusetts Medical School [7]. The Centers for Disease Control (CDC) launched the Quality Assurance Program for NBS laboratories since 1978 and now with more than 200 laboratories worldwide participated.

The first wave of NBS started in other countries soon, such as Canada in 1963, Singapore in 1965, Japan in 1967, Australia in 1967, Portugal in 1979, while in other Asian areas NBS was mostly initiated after 1980s: Mainland China, Hong Kong, India, Malaysia and Taiwan in 1980s; Bangladesh, Indonesia, South Korea, Philippines and Thailand in 1990s; Mongolia, Myanmar, Palau, Pakistan, Sri Lanka and Vietnam in 2000s [8-10]. The approach adopted was one-test-one-disease and the panel was limited to a few conditions usually including PKU, congenital hypothyroidism, maple syrup urine disease, homocystinuria, galactosemia, cystic fibrosis and/or congenital adrenal hyperplasia.

## Expanded Newborn Screening for Inborn Errors of Metabolism

IEM is a huge group of clinically and genetically heterogeneous metabolic disorders (Table 1). There are more than 1,000 diseases mainly affecting children. The cumulative incidence was reported up to 1 in 800 [11, 12]. Some IEM are amenable to timely treatment with good prognosis. Traditionally, the diagnosis replies on one or more tests for one disease. However, the advent of tandem mass spectrometry (TMS) applications in amino acids and acylcarnitines detection enables the one-test-many-diseases breakthrough in NBS for IEM [13-15]. TMS accurately identifies analytes by their fingerprint molecular mass-to-charge ratios with commendable specificity and sensitivity. It only requires 0.3 mL whole blood to test for more than 30 diseases in a single dried blood spot. The analytical time takes about two minutes for one sample allowing a high-volume throughput with rapid turnaround time in a NBS setting. Table 2 shows the advantages and disadvantages of TMS applications in NBS.

**Table 1. Classifications of IEM**

1. Disorders of amino acid and peptide metabolism
2. Disorders of carbohydrate metabolism
3. Disorders of fatty acid and ketone body metabolism
4. Disorders of energy metabolism
5. Disorders in the metabolism of purines, pyrimidines and nucleotides
6. Disorders of the metabolism of sterols
7. Disorders of porphyrin and haem metabolism
8. Disorders of lipid and lipoprotein metabolism
9. Congenital disorders of glycosylation and other disorders of protein modification
10. Lysosomal disorders
11. Peroxisomal disorders
12. Disorders of neurotransmitter metabolism
13. Disorders in the metabolism of vitamins and (non-protein) cofactors
14. Disorders in the metabolism of trace elements and metals
15. Disorders and variants in the metabolism of xenobiotics

http://www.ssiem.org/centralstore/resources/SSIEMClassificationIEM2011.pdf

**Table 2 Advantages and Disadvantages of TMS Applications in NBS**

Advantages

1. Detection of multiple analytes in the same analytical run
2. Small blood volume required (0.3 mL whole blood)
3. Fast analytical time about two minutes per sample
4. High throughput capacity
5. Accurate identification of molecular compounds by their fingerprint mass-to-charge ratios
6. Highly sensitive and specific with low false positive rate
7. Availability of commercial kits for acylcarnitines and amino acids

Disadvantages

1. High capital cost
2. Skillful expertise
3. Lack of full automation

In 1998, the New South Wales Newborn Screening Program was the first center to implement expanded NBS based on electrospray ionization TMS [16]. In the next year, the New England Newborn Screening Program introduced an optional metabolic panel for 19 IEM [7]. Twenty IEM patients were identified after 2.5 years screening of 200,000 newborns [17]. The prospective study showed that screened patients had shorter hospitalization and required less extra parental care. There was no significant difference in parental stress among NBS screened true positive, false positive results and normal control groups. In the same year, Germany started its extended screening with an unrestricted approach and since 2005 streamlined into 10 conditions [18]. Japan piloted TMS-based NBS from 1997 to 2007 with screening of 606,380 babies [19] and 65 IEM patients were identified with overall incidence of 1 in 9,330. Mainland China piloted TMS based NBS in Shanghai from 2003 to 2007 with 116,000 newborns screened [20]. Twenty patients were positive for six IEM with mainly PKU, maple syrup urine disease, methylmalonic acidemia and propionic acidemia. The overall incidence of IEM was 1 in 5,800. There were significant differences in the disease spectrum between northern and southern Chinese [21]. For example, classical PKU with phenylalanine hydroxylase deficiency accounts for the majority of PKU in northern Chinese, whereas, 6-pyruvovyl-tetrahydropterin synthase deficiency was much more common among southern Chinese. There were around 1,300 new cases of PKU screened in China each year. Glucose-6-phosphate dehydrogenase (G6PD) deficiency was very prevalent in Guangzhou with incidence of 1 in 28 but not in Northern Chinese [22]. In addition to expanded NBS in some advanced provinces covering more than 30 IEM, congenital hypothyroidism and PKU are mandatorily screened throughout the whole mainland stipulated in the law of maternal and infant health (launched in 1994) and its action program (launched in 2000) [22].

The International Atomic Energy Agency had devoted a total of $6.7 million USD to assist developing countries developing the infrastructure for NBS, in particular for congenital hypothyroidism [23]. In 2008, the Working Group of the Asia Pacific Society for Human Genetics on Consolidating Newborn Screening Efforts in the Asia Pacific Region was formed with representatives from 11 countries, viz. Bangladesh, China, India, Indonesia, Laos, Mongolia, Pakistan, Palau, Philippines, Sri Lanka and Vietnam. [24].

In 2006, the American College of Medical Genetics (ACMG) announced a consensus statement to standardize the NBS panel and decision matrix with recommendations of a core panel of 29 disorders and 25 additional secondary targets disorders [25]. It also provides the act sheets and confirmatory algorithms on each condition (http://www.ncbi.nlm.nih.gov/books/NBK55827/).

Wilson-Jungner criteria have been recently revisited in the context of genomic and modern medicine. The emphasis has been shifted towards more on the benefits to the affected baby and the family from early diagnosis and the availability of a satisfactory medical system for subsequent patient management [26]. Whether curative treatment is available or not, this is not a mandatory pre-requisite for NBS implementation.

**Newborn Screening in Hong Kong**

In Hong Kong, two metabolic conditions have been screened on a population basis namely congenital hypothyroidism and G6PD deficiency since March 1984 under the Neonatal Screening Unit of Clinical Genetic Service, Department of Health. The local incidence of CH is about 1 in 2,500, while that of G6PD deficiency is 4.5% in male and 0.3% in female newborn [27]. The program significantly lowered the mortality and morbidity. Apart from antenatal education through the Maternity and Child Health Centers, the Department of Health also provides follow-up and counseling to affected families.

The third was neonatal hearing screening. Language development is significantly improved if the hearing loss is treated before the age of 6 months. A local feasibility study was performed in 1999 screening 1,064 infants with an incidence of permanent deafness 1 in 355 [28]. A two-stage

program was implemented in all Hospital Authority hospitals with maternity service since 2007 [29].

In 2008, a Coroner inquest was called into the acute death of a 14-year-old boy with a postmortem genetic diagnosis of glutaric acidemia type II (multiple acyl-CoA dehydrogenase deficiency) [30]. The Coroner's report recommended that "*the Department of Health, the Hospital Authority, the Faculty of Medicine of various universities and others concerned should carry out a feasibility study to see whether universal check may be carried out on all newborn babies for congenital metabolism defect"* (http://www.judiciary.gov.hk/en/publications/coroner_report_july08.pdf).

In 2012, the University of Hong Kong conducted the first territory-wide pilot study funded by the SK Yee Medical Fund Foundation (http://hub.hku.hk/cris/project/hkugrant107939). The study tested the feasibility of expanded NBS in public hospitals with an OPathPed model [31]. In 2013, a private NBS for IEM service commenced in the Chinese University of Hong Kong, sponsored by Joshua Hellmann Foundation for Orphan Disease (http://www.obg.cuhk.edu.hk/fetal-medicine/fetal-medicine-services/jhf-newborn-metabolic-screening-program/).

In 2015, the Policy Address by the Chief Executive announced that a working group was established between the Department of Health and Hospital Authority to study the feasibility and logistics of expanded NBS for IEM in the public healthcare system (http://www.info.gov.hk/gia/general/201501/14/P201501140477.htm).

The feasibility study in the form of a pilot study was officially initiated on 1 October 2015 and lasts for 18 months, testing in two public hospitals with the collaboration between the Department of Health and the Hospital Authority. The aim of this pilot study is to demonstrate the feasibility of implementing NBS for IEM while developing and optimising education on IEM to public and healthcare professional, the screening tests, laboratory algorithms, clinical management and follow-up algorithms and programme evaluation. Twenty four conditions are included (Table 3). Educational materials were distributed to public and healthcare professionals (figure 1). A video was broadcasted in antenatal clinics and postnatal wards (Cantonese: https://youtu.be/RHK1NOGZkDs; Mandarin: https://youtu.be/MLLxJf7RvEQ and English version: https://youtu.be/JPPFfzUavGQ).

**Table 3 Screening Panel of Government-initiated Pilot Study**

**Disorders of Amino Acids**
Classical phenylketonuria
6-pyruvoyl-tetrahydropterin synthase deficiency
Argininosuccinic acidemia
Maple syrup urine disease
Citrullinemia type I
Citrullinemia type II
Tyrosinemia Type I
Homocystinuria

**Disorders of Organic Acids**
Multiple carboxylase deficiency
Glutaric acidemia type I
Methylmalonic acidemia
Propionic acidemia
Isovaleric acidemia
3-hydroxy-3-methylglutaryl-CoA lyase deficiency
Beta-ketothiolase deficiency

**Disorders of Fatty Acid Oxidation**
Carnitine uptake deficiency
Carnitine-acylcarnitine translocase deficiency
Carnitine palmitoyltransferase II deficiency
Medium-chain acyl-CoA dehydrogenase deficiency
Very long-chain acyl-CoA dehydrogenase deficiency
Glutaric acidemia type II

**Others**
Congenital adrenal hyperplasia
Biotinidase deficiency
Classic galactosemia

## Pros and Cons of Expanded Newborn Screening

NBS for IEM enables early diagnosis and treatment, prevents morbidity and mortality, avoids unnecessary investigations, alleviates family's anxiety, predicts prognosis and provides

valuable information for family planning and genetic counseling. In addition, some maternal diseases with treatment implications can also be detected during NBS, such as primary carnitine deficiency, PKU and vitamin B12 deficiency. The storage of DBS on a population scale can be a valuable asset in quality assurance, biomedical researches and forensic investigations.

NBS is shown to be cost-effective. Although randomized clinical trial on clinical utility and cost-effectiveness is difficult due to the rarity of individual IEM, cost-effectiveness in PKU [32-34], congenital hypothyroidism [35-37] and MCADD [34, 38, 39] were well documented. Table 4 shows some examples of studies on the outcome comparison between screened and unscreened patients.

There are also limitations in expanded NBS. First, because of the short history of expanded NBS developed only in the last two decades, long-term evaluation is still lacking. Recently, the Southeastern Newborn Screening Genetics Collaborative and the Public Health Informatics Institute collaborated to address the long-term issue through international effort. Second, patients with early symptom onset before release of NBS result would not benefit. False negative can happen to patients with mild or atypical presentation or use of non-standardized cutoff values and testing strategies. Third, since TMS allows one-test-multiple-diseases, some diseases which are not required by the program would also be unraveled. Conditions which are benign or with doubtful pathological significance may be identified, for examples, 3-methylcrotonyl-CoA carboxylase deficiency and short-chain acyl-CoA dehydrogenase deficiency. Detection and disclosure of carrier status such as in sickle cell disease and cystic fibrosis may create confusion to the parents [40, 41]. Fourth, although screening is available and even mandatory in some countries, treatment is not and not all screened positive children received proper treatment. Some treatments require special drugs and milk formulae. The clinical follow-up system may not be as well established as the screening program. Fifth, NBS results can be false positive or inconclusive. The overall sensitivity and specificity of TMS-based NBS is already commendable more than 99% with false positive rate from 0.07% to 0.33%, positive predictive values from 8% to 18% [20, 42-46]. False positive may lead to unnecessary hospitalizations and parental anxiety [47]. Measures such as better education and communication, algorithmic interpretation rules and two-tier testing system, can be implemented to reduce false positive rates and potential adverse effects.

## Conclusion

NBS represents the highest volume of genetic testing. It is more than a test and it requires a comprehensive healthcare system from pre-analytical, analytical to post-analytical phase involving expertise from public health, healthcare management, clinical, pathology and information technology. The field of NBS and IEM is still expanding. More disorders are under evaluation and covered such as severe combined immunodeficiency [48, 49] and X-linked adrenoleucodystrophy [50]. Various different or new technologies are applied to enhance the diagnostic performance, increase throughput, allow more automation and decrease costs [51-54]. Although a genomic approach for NBS is technically feasible, it entails a lot of difficult technical, clinical, social and ethical issues with hazards more than good [55]. On the other hand, using SNP array approach to detect a large panel of well-known pathogenic mutations on a wide spectrum of disorders would be more pragmatic. Expanded NBS is shown to be economically valid with significant reduction in critical care and chronic medical care expenditures. Last but not the least, NBS saves lives.

## Table 4 Outcome comparison between screened and unscreened IEM patients

| Reference | Study | Results |
|---|---|---|
| Wilcken et al. [56]. | Screening more than two million babies | The handicap rate 1 in 74,074 in the clinical group versus 1 in 232,558 in NBS group |
| Boneh et al. [57] | Six babies with glutaric acidemia type I detected by NBS | These patients benefited from mild protein restriction and carnitine supplement. All patients except one had normal cognitive and gross motor development, versus in unscreened patients with glutaric acidemia type I leads to acute encephalopathy and debilitating dyskinetic dystonia. |
| Klose et al. [58]. | 57 patients clinically diagnosed with organic acidemias and fatty acid oxidation defects | Sixty-three percent of these patients presented within the first year of life and 54% suffered from acute metabolic crises with eight deaths.<br>Majority of these metabolic crises (93.5%) and death (87.5%) could have been prevented by expanded NBS and early treatment. |
| Schulze et al. [44]. | 250,000 neonates for 23 metabolic diseases and 106 patients with positive screening results followed for 42 months | Seventy patients received proper treatment and remained asymptomatic. Six patients developed symptoms and three died. Nine patients presented earlier than the availability of screening results. Overall, 1 in 4,100 babies benefited from the early screening and subsequent treatment. |
| Cipriano et al. [59]. | Decision-analytic model analyzing 21 diseases taking into account of the disease severity, analytical sensitivity and specificity, need of confirmatory tests, specialist management, start-up and operating costs, hospital-related costs and potential deflation of future costs and benefits. | Bundling PKU together with 14 diseases was the most cost-effective strategy with $70,000 Canadian dollars per life-year gain. |
| Seymour et al. [60] | Systemic reviews published by the Health Technology Assessment in United Kingdom | Recommended screening for PKU, biotinidase deficiency, congenital adrenal hyperplasia, MCADD and glutaric acidemia type I. |
| Pollitt et al [61]. | Systemic reviews published by the Health Technology Assessment in United Kingdom | Considered screening as many conditions as possible with the emphasis on the benefits of early diagnosis to the patients and the family. The availability of effective treatment was not a compulsory pre-requisite. |
| Filiano et al. [62]. | Cost-benefit study | The lifetime costs for one cerebral palsy patient from infancy to 65 years old were $167,000 to $1 million USD as at 1998. The costs included medical charges, developmental services, special education and lost wages. Projected yearly savings of $36,600,000 (USD as at 1998) could be achieved through expanded NBS. The saving was twice of the incremental cost for NBS. |
| Couce et al. [63] | 10-year clinical follow-up of 137 IEM patients picked up by expanded NBS | The incidence was 1 in 2,060 newborns. With the long-term management, death rate was only 2.92% and majority of the survivors (95.5%) were asymptomatic after a mean observation of 54 months. |
| Linder et al. [64] | 373 IEM patients detected from a cohort of 1,084,195 newborns studying the efficacy and outcome of 10-year experience in expanded NBS | Presymptomatic diagnosis and treatment of other IEM achieved the same clinical benefits as in PKU. |

**Figure 1 Education materials of the Government-initiated Pilot Study**

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