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NEWBORN SCREENING REVISITED

Newborn blood spot screening: New opportunities, old problems

R. J. Pollitt

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Summary Newborn screening is evolving very rapidly. Geographical coverage is expanding, particularly for common disorders such as congenital hypothyroidism. New technologies, particularly tandem mass spectrometry and high throughput mutation analysis, have increased greatly the range of disorders which could be covered. However, these new possibilities are being exploiting at very different rates in different countries. This is due in part to the different ways in which generally-accepted screening criteria, based on the ten principles of Wilson and Jungner, are being interpreted and applied to policy. The appropriate management of some of the conditions newly-detectable by screening also remains controversial and there is a pressing need to align screening policy and clinical practice. Critical analysis and careful collection of data on an international basis are required to resolve these issues.

Introduction

Newborn blood-spot screening began in the early 1960s with the pioneering work of Robert Guthrie on phenylketonuria. A key feature, and the most enduring, was the use of filter paper cards to collect the blood samples and transport them to a central laboratory. Guthrie worked tirelessly on both the organizational and analytical aspects of newborn screening and some two decades later was able to list twenty blood-spot screening tests that had been developed in his laboratory (Guthrie 1980; Table 1). Very few of these have passed into general use. Surveys of current screening practice in Europe (Loeber 2007), North and South America (Borrajo 2007; Therrell and Adams 2007), the Middle East and North Africa (Saadallah and Rashed 2007) and the Asia Pacific region (Padilla and Therrell 2007) showed that of the disorders on Guthrie's list, only phenylketonuria, galactosaemia and sickle cell diseases were being screened for widely, and maple syrup urine disease and homocystinuria less frequently. Either most of the others are considered too rare to justify stand-alone screening or their early detection appears to offer little clear benefit. Nowadays policy-makers would probably denigrate much of Guthrie's later work as 'technology driven'. However, he and others in that era, Dick Koch for example (Koch 1980), had a clear vision of the way that screening should be integrated with follow-up and clinical management. Unfortunately, a quarter of a century later, this vision has still not been fully realized in their home country (Buist and Huntingdon 2007; Howell and Engelson 2007).

From 1973 onwards developments in immunoassay technology began to increase the scope of blood-spot screening. Detection of congenital hypothyroidism by assay of thyroxine, thyrotropin, or combination of the two, is now the most widely practised screen in the world. It is being actively promoted in developing countries by the International Atomic Energy Agency through its Technical Cooperation Programme (Solanki 2007) and supported by other initiatives such as the Japan International Cooperation Agency (Fukushi

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R. J. Pollitt (✉)
Newborn Screening Laboratory, The Children's Hospital, Sheffield S10 2TH, UK
e-mail: Rodney.pollitt@sch.nhs.uk

2007). Immunoassay-based screening for cystic fibrosis and congenital adrenal hyperplasia is also practised fairly widely.

New opportunities

Developments in DNA analysis are having an increasing impact on newborn screening. Mutation analysis as a second tier in screening for cystic fibrosis was first suggested by Bowling and colleagues in 1990 and has since become widespread (Wilcken 2007). Mutation analysis is also proving of benefit in screening for congenital adrenal hyperplasia (Torresani and Biason-Lauber 2007). New technologies capable of testing for numerous mutations simultaneously are increasingly being applied to both screening and diagnosis (Dobrowolski et al. 2003, 2007). In theory it would be possible to employ gene scanning to screen for almost any inherited disorder, but the concept of genetic privacy and exaggerated beliefs in the predictive power of DNA analysis raise both ethical and legal difficulties (Dhondt 2007). Many of the issues raised by the application of genomics to diagnostics and preventive medicine, summarized by Valle and Manolio (2008), are pertinent to newborn screening.

The potential of tandem mass spectrometry (MS/MS) for newborn blood-spot screening was first raised by Dave Millington (Duke University, North Carolina) in a paper presented at the 1989 SSSIEM meeting in Munich (Millington et al. 1990), though only later, with the development of electrospray atmospheric pressure ionization, did the method become sufficiently rapid and robust for routine use in screening. It has now been widely applied to amino acids disorders, including several for which Guthrie had developed stand-alone assays (Table 1), and a range of organic acidaemias and disorders of fatty acid oxidation. With its increased precision and accuracy, MS/MS is now considered the method of choice when screening for phenylketonuria (Chace et al. 1993). Several groups, including that from North Carolina (Frazier et al. 2006), have published results from large-scale MS/MS 'extended screening' programmes. Protocols are still being refined, with the use of metabolite ratios rather than fixed cut-off values as the primary screen for propionic acidaemia and methylmalonic acidaemias, for example (Lindner et al. 2008), and the development of second-line MS/MS tests (Matern et al. 2007).

Rapid-throughput short-column liquid chromatography with MS/MS detection can increase specificity when screening for congenital adrenal hyperplasia (Janzen et al. 2007; Matern et al. 2007). MS/MS methods are also proving useful for assay of enzymatic activities in dried blood spots. Several lysosomal enzymes can be assayed simultaneously using novel substrates (Gelb et al. 2006; Zhang et al. 2008) and pilot studies of screening are in progress. MS/MS may also be used to assay specific proteins by determining characteristic peptides after enzymatic digestion. Potential applications include measuring haemoglobin variants (Daniel et al. 2007) and screening for Wilson disease (de Wilde et al. 2008).

These technical advances give clear opportunities to improve existing newborn screening programmes and

Table 1 Newborn screening tests using dried blood spots developed in Robert Guthrie's laboratory (adapted from Guthrie 1980). The disease nomenclature is that used in the original publication

Analyte	Condition
Bacterial inhibition assays	
Phenylalanine[a]	Phenylketonuria
Leucine[a]	Maple syrup urine disease
Tyrosine[a]	Tyrosinaemia
Methionine[a]	Homocystinuria
Histidine[a]	Histidinaemia
Lysine[a]	Hyperlysinaemia, saccharopinuria
Glutamine[a]	Urea cycle disorders
Glycine[a]	Hyperglycinaemia (ketotic and non-ketotic)
Proline[a]	Hyperprolinaemia
Metabolite bacterial inhibition assays	
Valine[a]	Valinaemia
Galactose	Galactosaemia (transferase and kinase)
Enzyme auxotroph assays	
Argininosuccinate lyase	Argininosuccinic aciduria[a]
Orotidine-1′-phosphate decarboxylase	Orotic aciduria
Fluorescent spot tests	
C′1-Esterase inhibitor	Angioneurotic edema
α-1-Antitrypsin	Emphysema (adult), liver disease (infant)
Arginase	Hyperargininaemia[a]
Adenosine deaminase	Severe combined immunodeficiency disease
Autoradiography	
HGPRT[b]	Lesch-Nyhan syndrome
Radial diffusion immunoassay	
β-Lipoprotein	Hyper-β-lipoproteinaemia (type II)
Electrophoresis	
Haemoglobin	Sickle cell anaemia (SS and SC disease)

[a] These metabolites/disorders can be measured/detected by tandem mass spectrometry of amino acids.
[b] HGPRT, hypoxanthine-guanine phosphoribosyltransferase.

to extend the range of disorders covered. However, they have also brought into renewed prominence two problem areas: (1) wide disparities in national and more local (state) screening policies and lack of congruence with clinical practice; (2) the need to adapt diagnostic and clinical management protocols originally developed for clinically-presenting cases to the prospective management of screening-diagnosed cases.

Screening policy

Countries with well-established newborn screening programmes and at similar levels of social and economic development have adopted widely divergent policies on which diseases should be covered. Differences may be understandable for stand-alone screens such as those for biotinidase deficiency or congenital adrenal hyperplasia where variations in prevalence, medical practice, or funding priorities come into play. What is less understandable is why, in countries where MS/MS screening has been already been introduced for specific conditions such as phenylketonuria or medium-chain acyl-CoA dehydrogenase deficiency, the range of additional disorders that are considered acceptable varies so widely (Pollitt 2007). Some technically less-developed countries where there is a high incidence of metabolic disease are making rapid progress in implementing comprehensive MS/MS screening programmes (amino acids and acylcarnitines) in collaboration with existing centres elsewhere (Khneisser et al. 2009; Lindner et al. 2007). However, a recent survey of European practice has shown that only 10 of the 22 countries for which information was available were using MS/MS to expand the range of disorders covered by screening, the number of additional disorders ranging from one (medium-chain acyl-CoA dehydrogenase deficiency) to 20 (Bodamer et al. 2007). In some countries increased public expectations and political lobbying by parent groups have led to a rapid expansion in disease coverage. In others a more exacting scientific approach, with emphasis on universal criteria and quantitative data, has predominated. The United States and the United Kingdom, respectively, may be taken as representing these extremes (Pollitt 2006).

In the United States some aspects of the American College of Medical Genetics recommendations (2006) have proved controversial and the President's Council on Bioethics (2008) has very recently advocated a more restrained approach: 'States (should) mandate newborn screening only for diseases that meet traditional criteria, including the availability of effective treatment' but 'should be encouraged to implement pilot studies for newborn screening of conditions that do not meet traditional criteria', participation requiring voluntary informed consent of the infant's parents. The parent-led Save Babies Through Screening Foundation (http://www.savebabies.org/about/mission.html) currently advocates a screening panel consisting of over 50 disorders.

In the United Kingdom the National Screening Committee has formalized the Wilson and Jungner principles into firm criteria and requires high-quality evidence and numerical data to evaluate them (Downing and Pollitt 2008). For rare diseases it is impossible to provide the requisite data within a realistic timeframe, particularly as many of the conditions show clinical and genetic heterogeneity. MS/MS screening is limited to phenylketonuria and medium-chain acyl-CoA dehydrogenase deficiency with the ruling that screening for other disorders 'should not be offered'. This policy seems misaligned with clinical practice as many of the disorders currently proscribed are treated vigorously and successfully once they have been diagnosed, but there are often long-term sequelae due to diagnostic delay. Any case that provokes the reaction 'if only we had known earlier' should raise the question of whether newborn screening would have been practicable.

Diagnostic and clinical management protocols: the challenge of non-diseases

In many of the disorders under consideration the relationship between biochemical phenotype and clinical evolution is far from absolute and it is not possible to predict outcome in individual screening-detected cases if left untreated. Even with phenylketonuria it has proved impossible to develop a clear evidence-based consensus on how to manage mild or borderline non-classical cases (van Spronsen 2009). MS/MS screening has uncovered relatively common genotypes of medium-chain acyl-CoA dehydrogenase deficiency and isovaleryl-CoA dehydrogenase deficiency that have so far not been encountered in clinically-presenting cases. Presumably such patients retain significant residual enzyme activity and would require an exceptional degree of stress to provoke metabolic decompensation. The underlying molecular mechanisms linking genotype to phenotype remain unclear (Gregersen et al. 2008). Analogously, carnitine transporter defect (OCTN2 deficiency) has been found in healthy mothers through routine newborn screening or screening of cord blood samples (Walter et al. 2009), though in

other cases this condition has presented as a life-threatening event or proved fatal. 3-Methylcrotonyl-CoA dehydrogenase deficiency may be discovered similarly and seldom presents clinically. It is impossible to stratify risk precisely in babies diagnosed by screening to have such conditions, but it is important to adopt appropriate management protocols and not to over-react (Wilcken 2008). As in the analytical phase of screening, clinical management of the resultant cases requires a careful balance between benefit and harm. Again at this point there is considerable heterogeneity in practice between and even within countries. The expanding technical possibilities for high-throughput screening as well as for molecular-genetics-based screening call for harmonization of expanded programmes based on evidence-based algorithms for all key aspects: diagnostic quality, confirmation of suspected diagnosis, management and care. Considering that the incidence of some of the individual disorders can be as low as 1:1 000 000, this can only be done through extensive organized international collaboration. That would be well worth the effort.

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