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NEWBORN SCREENING

Neonatal screening: from the 'Guthrie age' to the 'genetic age'

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Summary Newborn screening has 'traditionally' been performed to detect metabolic or endocrine diseases that are severe, frequent and treatable, according to criteria established in the late 1960s. Technological advances in laboratory testing over the past ten years open new possibilities. However, many new problems have to be explored before the establishment or expansion of a newborn screening programme. The purpose of this paper is to present some of the major problems that screening programmes will face in the near future.

Abbreviations

CAH	congenital adrenal hyperplasia
CF	cystic fibrosis
HPA	hyperphenylalaninaemia
MCADD	medium-chain acyl-CoA dehydrogenase deficiency
MS/MS	tandem mass spectrometry
PKU	phenylketonuria

Introduction

The development of neonatal screening programmes derives from the efforts of Robert Guthrie, who developed a bacterial inhibition assay for phenylalanine (1962–1963). He was not only a scientist who designed many assays for the screening of metabolic disease, but he was also an ambassador of neonatal screening around the world. The success of the implementation of programmes in many countries as early as the mid-1960s, was mainly due to the sampling system that made systematic screening possible: 'While I recognize how pivotal the phenylalanine assay was for the development of newborn screening, I have always considered the filter paper blood specimen to be my most important contribution' (Guthrie 1992).

The 'principle of early disease detection' formulated by Wilson and Jungner in 1968 has been the main frame used for considering whether a screening programme is applicable. Many guidelines and recommendations have been published (WHO, ISNS, ...). In summary, screening should concern severe conditions that are relatively prevalent, that are treatable (or controllable), and for which a test exists and is measurable on a large scale. Despite such a list of prerequisites to be fulfilled before starting a programme, most programmes have been set up without 'evidence-based' evaluation. For example, the screening for phenylketonuria (PKU) was based on a prediction that dietary treatment would prevent mental retardation: 'It is reasonable to presume that the best results of dietetic treatment of phenylketonuria will be obtained if treatment is started in infancy and particularly in the neonatal period' (Bickel et al 1953). Fortunately the screening of PKU has been a success, and during the last decades of the past century programmes were extended with the same 'benefit' to other conditions (congenital hypothyroidism, congenital adrenal hyperplasia, ...).

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Recent years have been very rich in new perspectives, moving neonatal screening from the 'Guthrie age' to the 'genetic age'. New or promising methods offer new challenges for public health programmes designed to improve the prevention of severe handicaps. Multiplex technologies have been developed to cover a wide range of parameters that can detect several 'orphan' diseases. Tandem mass spectrometry (MS/MS) is the current technology that provides such multi-analyte determination; in the near future, DNA micro-array technologies will permit the analysis of hundreds of genes (and more!) in a single assay.

The lessons from screening programmes

Analysing the different screening systems around the world, implementation problems can be identified and can be considered before implementing a new or an additional programme. Some points illustrate the way the original Wilson and Jungner principles can be or should be 'revisited':

1. *The condition screened for should be an important health problem*. This can be judged by incidence as well as by the consequences for the affected individual. However, very often a clear definition of the condition is lacking.

The experience of looking at an abnormal concentration of a given analyte has shown that many different conditions can be responsible for an abnormal result (e.g. hyperphenylalaninaemia (HPA) and PKU, mild HPA, transient HPA, biopterin deficiencies, ...). Without a clear definition of the search condition, comparison of the 'performance' of screening programmes is not possible. This lack of precise definition may explain why most programmes have experienced the finding of higher incidence than that expected from clinical surveys. In many programmes, cut-off values have been progressively lowered, resulting in an increased number of false-positives. In programmes like congenital adrenal hyperplasia (CAH) and cystic fibrosis (CF) screening, a clear distinction of true-negative and true-positive results is not evident, and we might consider, especially for future programmes, that the concept of '*a priori* false-negatives' is an undeniable parameter to be taken in account. With the introduction of genetic testing in the flowchart of newborn screening, ethnic variations of gene frequency and genotype–phenotype correlations need to be revisited. The choice of mutations in a screening panel might integrate the ethnic specificity of the screened population but also avoid the search for 'mild' mutations.

The recent introduction of MS/MS in screening strategies illustrates that the criteria of 'good candidates for systematic screening' have to be revisited. The number of reports published recently is an indicator of lack of a unanimous point of view.

2. *Thereshould be an accepted treatment for patients with recognized disease*. This principle raises the question of the 'benefit' of the early detection (immediate benefit for the newborn, genetic implication for the family, parental plans, ...). The screening of a number diseases is often proposed (type I diabetes, fragile X syndrome, hereditary hemochromatosis, ...), but preventive treatment is not possible and abnormal findings only reflect susceptibility for disease (Levy 2003). In contrast, more and more medicines become available. Enzyme therapies are now available (or under development) for lysosomal storage disease, which consequently can benefit from early screening (Li et al 2004).
3. *Facilities for diagnosis and treatment should be available*. Screening must be considered as an integrated public health service for the newborn. Effective programmes must provide the infrastructure for universal access and rapid follow-up (Clinical and Laboratory Standards Institute 2006). The screening is not limited to the collection of blood spots and their analysis: it should consider all the components from public education to the possibility of efficient follow-up of patients, with a particular attention to 'patients' who will become adults (i.e. PKU girls).

The introduction of new technologies will also have impact on the global organization of programmes. Concentration on 'supra-regional' laboratories, new structures for confirmatory testing and reorganization of clinical centres might be necessary to provide the same quality of service to all the babies of a country.

4. *There should be a recognized latent or early symptomatic stage*. This is a complex item taking in account asymptomatic/symptomatic neonates, specific/nonspecific symptoms, and delay of clinical manifestation.
5. *There should be a suitable test or examination*. The test should have *no* false-negatives and a *minimum* of false-positives. This involves the choice of the parameter and of the threshold value, but also the flow chart of the screening process (two-stage, three-stage strategy, ...). For example, with MS/MS technology the choice of

pertinent threshold is a crucial point to avoid having to manage 'non-diseases' (Pollitt et al 1997). DNA analysing systems (i.e. microelectronic chips) provide the possibility of multiplex hybridization, and completely automated platforms can handle a large number of samples for identification of a large panel of mutations. However, inclusion of mutation analysis raises a number of issues: mild or intermediate forms of diseases and capricious genotype–phenotype correlations. In addition, the panel of screened mutations might be based on their frequency in the population.

The choice of efficient cut-off values is always a crucial point. For example, when screening for CAH with 17-OH-progesterone testing, programmes have to manage a high rate of false-positives, particularly in premature and severely ill babies. The dilemma is to detect true-positive cases but with a timely availability of screening results to make therapy possible before the time of a risk of salt-wasting crisis. MS/MS of steroids or detection of CYP21 mutations have been proposed. The time at which the blood is collected also has to be taken into account, since this parameter can alter the screening performance (e.g. PKU and early discharge).

6. *The test should be acceptable to the population*. For a long time, parents were informed about the screening tests carried out on their child in a simple manner. Nowadays, there is an increasing tendency to include legal, ethical and 'consumerist' considerations (informed choice? informed consent?). Acceptance is a consideration in ensuring the credibility and viability of a programme (McCabe et al 2002); false-positive screening results may place families at risk of increased stress (Waisbren et al 2003).
7. *The natural history of the condition, including development from latent to declared disease, should be adequately understood.* The inclusion of DNA analysis in CF screening strategy introduced difficulties in the interpretation of screening results because some genotypes are associated with relatively mild form of CF.

Insufficient knowledge of the natural course of some diseases has been highlighted. In the case of medium-chain acyl-CoA dehydrogenase deficiency (MCADD), the mutation 985A > G concerns only 80% of clinically diagnosed patients, but the incidence of MCADD identified by MS/MS is higher (3 times) than the estimates from clinically symptomatic cases (Liebl et al 2003).

8. *There should be an agreed policy on whom to treat as patients*. This principle raises again the question of a precise definition of the screened condition, but recent experience has also pointed to the extreme variability of the phenotypic expression of a given disease (e.g. CF and severe or mild mutation). Many recommendations or guidelines for the treatment of patients have been established by professional groups and published in the medical literature, but a consensus is often difficult to obtain.
9. *The cost of case-finding (including diagnosis and treatment of patients diagnosed) should be economically balanced in relation to possible expenditure on medical care as a whole*. Health economics issues cannot be ignored. Cost–benefit evaluations have to be strongly encouraged, to help public health authorities, insurers, etc. to decide whether to support a programme but also to provide indicators for adapting existing programmes. Such analyses have to consider all the components from testing to treatment. For example, both false-negatives and false-positives are likely to impose significant costs and may substantially affect the net benefits attributable to a screening intervention. However, such economic evaluations are rare and are always difficult to interpret (Lee et al 2003; Pandor et al 2004; Pollitt et al 1997; Simpson et al 2005); the economic benefit depends on the funding system for health care. Theoretically, all newborns should benefit from the programme and all patients should be cared for free of charge.
10. *Case-finding should be a continuous process and not 'a once and for all' project.* Even if most current programmes are in fact permanent services, the establishment of 'pilot' programmes is to be recommended when it is not evident whether a screening process will or will not fulfil the criteria (real prevalence, performance of the test, etc.). The virtue of a 'pilot' programme is it that it can be stopped! Conversely, permanence of the service should imply frequent evaluation of the programme (coverage, turnaround time, evaluation of costs, ...).

Consequently, a concept of screening system has progressively emerged that integrates education, screening, follow-up, diagnosis, management and evaluation (Therrell 2001). Since more and more developing

countries are considering implementing systematic neonatal screening programmes, the feasibility has to be checked carefully: definition of priorities, financial resources, public health organization, acceptability (which can depend on cultural or religious backgrounds),...

The future of neonatal screening

The new technologies (tandem mass spectrometry, DNA chips, ...) may soon modify the present picture of neonatal screening, but might also lead to some temptations. Screening for at-risk conditions (e.g. diabetes), epidemiological studies (e.g. HIV) and carrier screening are examples. Newborn screening programmes are expanding to include conditions that may not meet all of the traditional screening criteria. As a result, these technologies will move our screening actions from the field of preventive medicine ('standard screening') to that of predictive medicine ('screening for susceptibility to complex disorders').

Nevertheless, it remains important to emphasize that a screening programme might have a 'direct' benefit to the baby from early diagnosis. The scoring system which has been used by the American Academy of Genetics to classify the diseases which are eligible for a screening is a good illustration of the difficulty of defining priorities (American College of Medical Genetics 2006).

Ethical considerations

Ethical debates will be more and more important (parental consent/dissent, disclosure of carrier status, storage of samples in biobanks, information systems for data collection ...). Informing parents will be one of the challenges of 'expanded' programmes and the principle of a 'free access' (equity) to such programmes cannot be ignored. However, it is important to be aware of possible effects on families of screening for conditions which can be perceived as discriminatory.

In some countries, the inclusion of DNA-based testing techniques in the CF screening algorithm required procedures to be brought into line with laws on bioethics, initially established to protect individual rights to privacy (Farriaux et al 2003). One of the main consequence is the need to obtain a written consent to perform the test, with a risk that the parents refuse the test! In addition, genetic testing 'inadvertently' identifies newborn infants who, although not affected by the condition, carry a gene for it (Dhondt 2006). The discovery of a carrier status can lead to testing of parents and family members, with a risk of discovering that the putative father is not the biological father ('non-paternity'). The information of carrier status can raise fears of stigmatization (misuse by insurers, employers, ...), fears which are evident in many 'ethnic minorities' (labelling a person or a family as having 'undesirable' characteristics). In addition it has been shown that, after information about heterozygosity, parents had residual anxiety about the current health of their carrier child (Lewis et al 2006).

Information of professionals, the public and families

The entry into the new millennium coincides with the evolution of the medical world. In the past, it was accepted that only the doctor held the medical knowledge and patients totally relied on the doctor. These days access to medical information is a right. Neonatal screening is a medical act in the context of preventive medicine and consequently has to be explained. However, neonatal screening combines two opposite principles: the need to test *all* newborns to detect a very *small number* of patients. How to explain that without being alarmist and how to convince all of the population to accept the test? This quandary demands that information has to be provided to all the 'partners': the public, the parents, the parents of affected children, the patients, the professionals and the policy formers and decision makers. With the increasing number of diseases being covered by screening programmes, the difficulty of providing appropriate information will also increase.

Nowadays the public is eager for medical information, especially on subjects like genetics that are prominent in the media. In addition, commercial marketing, media campaigns (such as money-raising TV programmes) and consumer groups can influence policy makers (Therrell 2001) without consideration of any negative impact and can introduce confusion and misunderstanding.

The parents (or future parents) are of course the main group to whom neonatal screening has to be explained, but the different socio-cultural backgrounds can make it difficult to choose the most appropriate content of the information. The relevant questions are: Why to inform? How to inform? When to inform? It is also necessary to assist parents whose children suffer from one of the diseases screened for. It is especially important to answer the first questions posed once the diagnosis has been announced, so as to prevent desperate searching through books, dictionaries or

the Internet, or seeking information from friends or from their general doctor, who in most cases knows little or nothing about such rare diseases.

Because screening concerns newborns, the problem of informing the patients has been overlooked for many years. By now, though, screened subjects are adults and developing proper information for them is crucial.

In order to help the members of the maternity-ward staff who intervene in the screening process to carry out this difficult task of informing parents, there need to be designed practical guides aimed at them and dealing with the various stages of screening.

Family doctors and paediatricians also need to be informed about screening programmes since they may be the first link between the screening centre and parents. The survey conducted by Gennaccaro and colleagues (2005) in Massachusetts showed that paediatricians are ill-prepared to talk about screening results with families.

Physicians specialized in paediatric endocrinology or inborn errors of metabolism should also be periodically approached by 'screeners'. It is necessary to develop opportunities to meet together on an international and 'regional' basis.

Conclusion

These questions and many others require international exchanges to provide each of us with a degree of expertise that can help in the decision to modify or expand a programme. In the 1960s, screening programmes for PKU were implemented without real proof by evidence; the world has changed since and 'evidence-based' criteria are a prerequisite before setting up any new procedure.

However, many questions and problems remain unsolved, and are sometimes deliberately ignored because of lack of clear distinction between research and a public health service.

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