Efficacy of screening immune system function in at-risk newborns

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REVIEW

Please cite this paper as: Pavlovski C. Efficacy of screening immune system function in at-risk newborns. AMJ 2014, 7, 7, 272–284. http://doi.org/10.21767/AMJ.2014.1980

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ABSTRACT

This paper explores the introduction of a screening test to highlight impaired immune system status for newborn infants and its efficacy as a preventative clinical measure. Moreover, it is suggested that screening of the infantile immune system has the potential to highlight susceptibility to a range of infant and childhood diseases, bestowing an opportunity to introduce early intervention to reduce the incidence of these diseases. Development of the neonatal immune system is an important health issue, implicated in many childhood problems such as allergies, infection, and autoimmunity. The neonate has a limited immune system and ability to combat bacteria. Depleted levels of the tripeptide reduced glutathione (GSH) have been linked to numerous conditions and its intracellular level is acknowledged as an indicator of immune system function. Introduction of an immune system screening programme for infants is formally reviewed and assessed. Several benefits are reported in the treatment of impaired immune systems, a trial screening programme is proposed for at-risk infants to gather further evidence as to its efficacy. Infants at risk of impaired immune system function include cystic fibrosis, premature infants, and low birth weight infants. The interventions include breastfeeding, milk banks, and appropriate formula to support the immune system.

Key Words
Glutathione, immunosuppression, infant, neonatal care, newborn screening

What this study adds:

1. What is known about this subject?
There is related literature on the screening of glutathione synthetase deficiency, a rare disorder of glutathione metabolism.

2. What is the key finding in this case study?
This review assesses the practicality of screening newborns for impaired immune system. Given that immunological mediated diseases in adulthood emerge during postnatal life, such a programme may prove cost-effective long term.

3. What are the implications for future practice?
A programme for detecting compromised immune system for the neonate at birth may be established with the potential to reduce the incidence of health problems.

Background

Newborn screening programmes address a range of disorders,[1] including phenylketonuria, hypothyroidism, and cystic fibrosis. This is conducted by taking a small blood sample from the heel of the newborn. The blood sample is placed upon an absorbent paper, the Guthrie card,[2] and profiles are subsequently analysed using tandem mass spectrometry to determine a range of metabolic disorders. Emerging infectious diseases are increasing,[3] and a test for immune system function at birth may provide an opportunity to highlight susceptibility to disease and bacteria. Such a preventative measure may qualify the need for immune system support during the first critical 12 months of an infant's life.

In this paper, a newborn screening test to detect the single condition of immunosuppression, using whole blood reduced glutathione levels as the marker, is assessed. A number of additional conditions are known to deplete glutathione levels and hence impact the immune system. Where a positive screening result occurs and interventions to restore glutathione levels fail, then further diagnostic analysis is required to determine the

underlying cause. The method and approach to assessing such a new screening test is now discussed.

Method and approach

Analysis has been undertaken regarding the efficacy of introducing an infant screening programme to highlight impaired immune system status and its benefits as a preventative clinical measure. We followed the method and approach for newborn essential fatty acid screening[4] and use the UK National Screening Committee criteria (see Table 1).[5] The UK criteria adds further questions to those proposed by Wilson and Jungner.[6,7] Similar criteria are also outlined by the Human Genetics Society of Australia.[8]

Screening assessment

The following sub-sections are defined by the UK screening criteria (Table 1) and detail the analysis of the proposed screening programme.

Condition assessed

Importance as a health problem

Development of the neonatal immune system is an important health issue, implicated in many childhood problems such as allergies,[9] infection,[10,11] and autoimmunity.[12] Newborn infants have a limited immune system[13] and ability to combat bacteria.[14] Premature and low birth weight infants have impaired immune systems.[15] Kelly and Coutts observed that since the neonatal immunological response to antigens is untried, it is now accepted that adaptation of the immune system is critical with a failure to regulate response leading to recurrent infection, allergies, and inflammatory disorders.[16] The interaction of infant nutrition and the immune system is an important health issue.[11]

History, epidemiology, and disease markers

The natural history of the developing immune system is well understood.[17] In uteri, the foetus receives immune system support via the placenta until birth,[18] and during labour vital immunological increases are passed from mother to baby.[19,20] At birth the initial colostrum is abundant in immune properties,[21] and during lactation infants receive constant immune system support.[13,16,22,23] There are several risk factors for immunosuppression, including cystic fibrosis,[24] dietary behaviour during pregnancy,[25] delivery complications,[12] low birth weight,[15] prematurity,[26] duration of breastfeeding,[27] protein deficiency,[28] and mode of delivery.[29] Moreover, caesarean delivery increases risk of atopic disease,[30] which together with anaesthesia decreases natural killer cell activity potentially increasing risk of infection,[31] and reduces leukocyte and neutrophil counts (lasting two months) with other immune alterations remaining for six months.[32] The development of the infant immune system is a factor in the onset of many conditions such as asthma,[33] diabetes,[34] and atopic disease.[9]

Table 1: UK National Screening Committee Criteria[8]

The condition should:

- be an important health problem;
- have an understood history, with a detectable risk factor, disease marker, or symptomatic stage; and
- have cost-effective primary prevention implemented as far as practical.

The screening test should:

- be simple, safe, precise, and validated;
- have known distribution of test values within target population and cut-off levels agreed;
- be acceptable to the population; and
- have an agreed policy on further diagnostic investigation and choices available.

The treatment should:

- be effective, with evidence of better outcomes from intervention;
- have agreed policies covering the treatment offered; and
- optimise clinical management of patients in healthcare providers prior to participation.

The screening programme should:

- have evidence from RCTs demonstrating effective reduction in mortality or morbidity;
- be clinically, socially, and ethically acceptable to health professionals and the public;
- have benefits that outweigh the physical and psychological harm;
- have opportunity cost balanced with expenditure on medical care (i.e., cost-effective);
- ensure cost-effectiveness by considering all other options for managing the condition;
- have a management/monitoring plan with agreed quality assurance standards;
- have adequate staffing and facilities for testing, diagnosis, and treatment;
- have information available to participants to assist informed choices; and
- anticipate widening of eligibility criteria and increasing sensitivity of testing, with decisions scientifically justifiable.

Note: Unrelated criteria on mutations omitted.

Glutathione status is a highly sensitive indicator of cell functionality and viability,[35] it regulates immunological functions,[36] and is a detectable risk factor to monitor the severity and progress of disease.[37] A tripeptide composed of three amino acids (glutamine, cysteine, and glycine) glutathione is suggested to be the most accurate indicator of overall cell health, playing a central role in the function

of immune cells,[38] with numerous studies[39–46] and reviews[35,36,47–51] confirming the immune system relationship. The immune system functions best when lymphoid tissue is correctly balanced with glutathione.[51] Low glutathione levels impair neutrophil function,[45] and impact effective glutathione peroxidase activity[52] which consequently reduces survival of eosinophils.[53] Macrophage killing of mycobacterium improves significantly with glutathione increases.[44] Lymphocyte activation and differentiation is determined by glutathione,[54] with moderate changes having a significant impact on lymphocytic functions,[51] and lymphocyte response to antigens is determined by their ability to regenerate stores of glutathione.[55] The most widely known immune disorder, AIDS due to HIV, impacts the glutathione homeostasis of immune cells,[56] and the increased biosynthesis of glutathione is acknowledged as supporting the immune system.[43,57,58]

The availability of glutathione has been assessed in both full-term and pre-term infants, with very low birth weight infants possessing an active biosynthesis capability.[59] Deficiencies are due to factors such as availability of precursors,[60,61] bacterial or viral infections,[38] and other diseases. Cysteine is a crucial limiting factor in glutathione synthesis[62] and excessive urinary excretion of cysteine is observed in critically ill neonates.[63] The amino acid cysteine is found in whey protein, which constitutes 60 per cent of human breast milk.[64]

Implementation of cost-effective primary prevention

The most cost-effective primary prevention measure is ensuring appropriate dietary behaviour through breastfeeding, providing the best form of nutritional supplies,[65] and active stimulation of the immune system.[66] Present data indicates that the duration of exclusive breastfeeding is less than the recommended guideline until six months of age,[67] and it is acknowledged that formula is inferior to breast milk.[65] Consequently, more work is required to increase duration and extent of breastfeeding as the primary means of intervention. More work is also needed to determine which formula is most appropriate to support the infant immune system. This may also require new formula designed with immunological components.

Screening test

Simple, safe, and validated screening test

While a complete blood count of immunoglobulin antibodies and lymphocytes have been employed to assess the immune system,[68] Burns et al. remark that many results are inconclusive and conflicting, suggesting that alterations in immune function such as interleukin-2 receptor, antigen, and neopterin are possibly more important than cell numbers.[69] Glutathione is a sensitive indictor of overall cellular health; the proliferation, growth, and differentiation of immune cells is dependent on glutathione.[38] Glutathione enhances the functional activity of natural killer and T-cells.[70] Reductions of glutathione impact CD8+ and, particularly, CD4+ T-cell numbers.[39] Further studies show that glutathione modulates IgE,[71] downregulates interleukin-4 and IL-4 induced IgG while increasing B-cell proliferation,[46] and increases memory and naïve B-cell survival.[72]

The idea of newborn screening for depleted levels of glutathione was originally proposed in 1981 by Garrick et al. where a simple and straightforward procedure was outlined to detect glutathione synthetase deficiency.[73] Further recommendations for screening glutathione as a disease marker have also been suggested for 5-oxoprolinuria,[74] lung inflammation,[75] and to detect retinopathy disorders in premature infants.[76] A number of precise and validated tests exist for both plasma and blood. The assays employ gas/liquid chromatography, mass spectrometry,[77,78,79] and high performance liquid chromatography (HPLC).[80] Pastore et al. comprehensively review these and other methods, suggesting that HPLC is most favourable;[35] although newer approaches using mass spectrometry are emerging.[81]

Distribution of test values and agreed cut-off level

There is discussion suggesting measurement of total glutathione, including the reduced (GSH) and oxidised (GSSG) form, is required as an indicator of disease risk;[35] however, studies confirm that diseased patients have no difference in GSSG level, but rather are deficient in GSH alone.[37] Newborn blood glutathione levels are observed to stabilise after 24 hours,[82] and more than 99 per cent of whole blood GSH is found in erythrocytes.[83] Numerous studies have been conducted to determine blood concentrations of glutathione within the populations for pre-term and term infants.[76,82,84] Due to multiplicity of aetiological factors, [43,76,85-89] whole blood with sub-fraction analysis of erythrocyte GSH may be required. Lands et al. also observe that since erythrocyte turnover of GSH is relatively slow compared with lymphocytes it may not reflect acute changes.[75] As such, a trial will confirm cut-off levels that denote an impaired immune system.

Acceptable to population and diagnostic investigation policies

Newborn metabolic screening is a well-established screening programme with up to 97.8 per cent of parents opting for screening in some Australian states.[90] A further survey confirms that 85–86 per cent of mothers support screening.[91] Although an additional test (as an extension to the Guthrie card) that assesses the neonatal immune

system appears likely to be acceptable to the population, a initial trial programme would seem necessary to confirm acceptability.

Since the screening test is intended to detect the condition of immunosuppression, where interventions fail to restore glutathione levels (and the immune system), then further medical tests may be required. A key challenge for the programme is that the underlying condition(s) that may be impacting the immune system may be wide and varied. This may present obstacles in general acceptability and may also increase parental anxiety. However, since the test is not aimed at detecting a genetic condition, as existing newborn screening tests are, this may make it more acceptable to the public.

Positive test results require follow-up investigations to determine if glutathione levels are progressively restored within the first year of life, and a trial may be used to define the optimal follow-up screening interval. If levels are not restored, then additional prognostic tests are required to determine the aetiology. Several causative factors have been described, including viral infections,[38,89] surgery,[92] obstructive airways disease,[88] HIV,[40,43] inflammation,[93] septic shock,[94] heavy metals,[95] and dietary deficiencies of glutathione precursors and enzyme cofactors.[96,97] Gamma-glutamyl cycle metabolic disorders are also directly associated with depleted levels of glutathione,[85] and if suspected, diagnostic investigation of urinary 5-oxoproline is conducted.[98]

Treatment of the condition

Evidence of effective treatment or intervention

Studies show that intravenous administration of immunoglobulin does not significantly alter outcomes for neonatal infection,[99,100] as such alternative avenues are suggested to be pursued.[100] Breastfeeding is the most effective form of infant nutrition and immunity,[65] with ample scientific evidence indicating that such infants have better health outcomes. Hanson points out that breastfeeding protects against diarrhoea, respiratory tract infections, otitis media, bacteraemia, bacterial meningitis, botulism, urinary tract infections, and necrotizing enterocolitis; with evidence that such defence is retained.[101] Breastfeeding improves vaccine response.[18,101] Clearly the omission of breastfeeding results in a deficiency of several key immunological components, including anti-inflammatories,[23] antibodies,[102] and leukocytes.[103,104]

Immune modulating components that may be added to infant formula include nucleotides,[105] prebiotics,[106] and long-chain polyunsaturated fatty acids (LCPUFAs).[107] Although one study showed no significant difference for severely malnourished infants receiving formula with or without nucleotides added,[108] a randomised controlled trial concluded that antigen response to diphtheria and tetanus were higher than infants fed a control formula,[109] they also found no difference in proportion of lymphocytes, natural killer activity, or cytokine production. In addition, infants fed formula containing LCPUFAs increased the proportion of antigen mature CD4+ cells, while modulating cytokine production.[107]

Human breast milk comprises 60 per cent whey and 40 per cent casein protein; conversely, bovine milk contains 20 per cent and 80 per cent, respectively.[64] Several studies have shown whey dominant infant formula to be in closer proximity with breast milk than casein formula.[110–112] Whey protein is composed of several immune regulating components, including immunoglobulins, alpha-lactalbumin, beta-lactoglobulin, lactoperoxidase, and lactoferrin.[113] Furthermore, the whey proteins beta-lactoglobulin, lactoferrin, serum albumin, and alpha-lactalbumin are generous sources of cysteine.[62] Cysteine is considered an essential amino acid for premature and low birth weight infants.[114] Cysteine and glutamyl-cysteine composition, of these whey proteins, are the significant contributor to increasing intracellular glutathione;[55,61,62,115–117] particularly in plasma[118–120] and blood;[88,121,122] with glutamate cysteine ligase the rate limiting enzyme.[123] While a previous study showed that direct supplementation of GSH may not be effective,[124] a more recent trial indicates that direct supplementation can in fact increase GSH stores in adults,[125] although a similar study for neonates is required. A further study shows that administration of amino acids after birth in preterm infants increases absolute synthesis rates of GSH.[126] Partially hydrolysed whey protein in infant formula is particularly beneficial in atopic conditions such as allergies,[127] eczema,[128,129] asthma,[110] wheeze, and rhinitis.[130] Hydrolysed whey protein has been shown to support the immune response in infants at risk of atopy similar to breast milk,[131] long-term results support the benefits of hydrolysed infant formulas,[132] other studies support these findings.[133] Notwithstanding, infant formula remains considerably different from breast milk.[112,134]

Policies, treatment, and clinical management of patients

Policies from the National Health and Medical Research Council recommend exclusive breastfeeding for the first six months of life, observing that debates on exposure to foods focus upon immune function, acquisition of immuno-tolerance, and intestinal function.[135] Just over 15 per cent of infants are exclusively breastfed until the

recommended six months,[67] with a relationship between breastfeeding and socioeconomic status existing.[67] Several additional factors have been observed, including poverty level, age, and education of parents.[136] While breastfeeding actively stimulates the neonatal immune system and significantly reduces risk of infection,[137] policies addressing infants with impaired immune systems on appropriate infant formula, when breast milk is not available, are yet to be developed. Donor milk banks are often used for low birth weight infants[138] and may be considered, although issues such as donor selection, pasteurisation, and quality measures need consideration.[139] A trial for at-risk infants would help to establish such treatment protocols. The properties of bovine milk can enhance or suppress the immune system and may also be studied further to fully understand the potential benefits.[140]

The screening programme

Evidence in reducing mortality or morbidity

While a trial screening programme would bestow the opportunity to conduct focused randomised controlled trials (RCTs) to gather evidence in reduction in mortality or morbidity, existing RCTs illustrate the benefits of breastfeeding upon the immune system;[105,110] and as an immunological resource is considered to be the most effective means of reducing mortality in children under five.[141] Additional RCTs highlight benefits of hydrolysed whey formula on reducing morbidity,[128-130,142,143] with studies noting relationship to immunogenicity,[131] and RCTs demonstrate that nucleotide supplementation of infant formula is beneficial to the immune system.[144,145]

Clinical, social, and ethical acceptance

Evidence of acceptability of an entirely new screening programme is generally only available once established.[146] Existing surveys highlight overwhelming support for newborn screening in general,[90,91] with general agreement that breastfeeding reduces risk of many diseases.[147] As an extension to the existing Guthrie card system the test may also be more acceptable. Notwithstanding, a trial programme would seem a necessary first step to assess the acceptance.

Weighing the benefits and harm of programme

Several psychological issues must be considered when assessing the benefits of newborn screening, including the additional stress placed upon parents, and false-positive and false-negative readings. Research highlights that a false-positive reading does place more stress upon parents and increases parent-child dysfunction. When compared to clinical detection, an overall reduction of stress on parents is observed.[148] False-positive readings may also be countered with follow-up testing and conclusive diagnosis, while false-negatives lead to the status quo where clinical detection occurs later in life. Proving the benefit of a new screening test is difficult;[149] however, newborn screening, when compared to clinical diagnosis, leads to improved health outcomes for affected children.[148] Instituting a trial screening programme will provide the essential data to more clearly evaluate the benefits versus the harm.

Cost-effective balance of programme

To evaluate cost balance, the screening programme in Australia is used as an example by applying the estimation techniques used for essential fatty acid deficiency (EFAD) infant screening.[4] In 2003, the amortised cost for conducting an individual newborn screening test in Australia was AUD $1.1.[150] With 300,000 births annually,[151] and the incidence of cystic fibrosis at one in 3,000,[152] pre-term and low birth weight at around 8 per cent and 6.5 per cent, respectively,[151] the at-risk population is 43,600 annually; this excludes combined incidences within risk groups. Assuming costs have doubled since 2003, with the initial test conducted at newborn screening with follow-up testing conducted externally,[153] then the cost of the trial is estimated at around AUD $2.5 million. In general, newborn screening with early treatment of conditions, reduces overall healthcare costs since subsequent hospitalisation is reduced.[148] However, the true programme cost may not be known due to the diversity of underlying conditions that may impact the immune system. Hence, a key requirement of the trial would be to more fully understand the cost-effectiveness.

Management plans, staffing, and informing participants

The proposed screening test may be integrated within the existing newborn screening programme, by increasing the established staffing and facilities to accommodate the additional testing, diagnosis, and treatment for the new programme. This will enable immediate reuse of established standards, procedures, and plans for managing and monitoring screening, including the effective dissemination of evidence-based information explaining consequences of testing and treatment. Follow-up diagnostic and treatment policies, however, will require further development for the new screening test.

Eligibility criteria and increasing sensitivity of testing

Public pressure to extend criteria associated with testing requires consideration, particularly when one observes the favourable response to newborn screening.[91] and the testing of new conditions.[154] With advancement in screening technology it is possible to detect a greater

range of disorders.[155] Glutathione deficiency has been linked to occupational exposure that causes oxidative stress,[156] coronary heart disease,[157] retinal dysfunction,[76] pulmonary inflammation,[88] cystic fibrosis,[75,158,159] diabetes,[160] sickle cell anaemia,[161] coeliac disease,[162] and lupus.[163] Glutathione s-transferase is strongly associated with asthma,[164,165] with interventions that restore GSH balance proposed to combat GSH oxidation.[166] Additionally, glutathione deficits are observed in immunodeficiency diseases such as AIDS/HIV,[43,86] and impact the non-homologous end-joining pathway,[167] which is linked to SCID.[168,169] When interventions to restore glutathione levels fail, then further tests may be required to identify the underlying cause to immunosuppression. This may pose challenges due to the wide variety of underlying conditions.

Discussion and Conclusion

This review assesses the efficacy of a new screening test to detect immmunosuppression in newborns using whole blood glutathione as a marker. The new test may be considered less confrontational to the public when compared to existing newborn tests that detect genetic disorders.

Where a positive result is returned by the test and interventions fail to restore glutathione levels, then further diagnostic testing may be required. Given the broad nature of the underlying conditions that may contribute to the depleted levels of glutathione, there may be increased parent anxiety and hence impact the general acceptability to the population. If, however, no test is conducted at birth and a suppressed immune system is not revealed at that time, then any possible underlying condition may manifest further (later in life) to a point where parents may seek medical attention for the infant. As such, the screening test may prove to be useful as a preventative clinical measure to highlight potential problems early. On the other hand, infants may also progressively restore their glutathione levels and immune system status through sound parental practices in nutrition and support.

Other aspects that require further consideration include false-positives and false-negatives. Since a number of conditions may be causing glutathione depletion, a false-positive is likely to add parental anxiety. This can be managed to some degree with follow-up testing and helped with parental education about the programme.

False-negatives, however, will mean the status quo where immunosuppression will go undetected until a condition presents itself later in life. This may be addressed by offering to parents the option to conduct a six-month follow-up test as a way to monitor the infants' developmental progression.

A trial screening programme may be considered for at-risk infants, including those with cystic fibrosis, and pre-term and low birth weight infants. The programme would commence with the first test conducted at newborn screening with follow-up testing at six monthly intervals during the first 12 months. Infants identified within the at-risk group have a greater need for support, and a trial programme targeting this group is likely to yield benefit. Where circumstances reduce the exclusive breastfeeding period, the use of milk banks or selection of an infant formula that supports the immune system becomes a key measure, particularly as the range of infant formula and human milk fortifiers available can lead to confusion.[170] There is considerable understanding in immunology and human milk;[171] comparatively, there is less work on the immune modulating components of bovine milk. Hence a trial for at-risk infants will also provide an opportunity to conduct such studies. Given that immunological mediated diseases in adulthood emerge during postnatal life,[17] such a programme may prove very cost-effective long term.

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Not commissioned. Externally peer reviewed

CONFLICTS OF INTEREST

The authors declare that they have no competing interests.