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REVIEW ARTICLE

Newborn Screening – Indian Perspective with Issues & Challenges

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Background:

Newborn Screening (NBS) began in early 1960's by a pioneering work of Dr. Robert Guthrie, USA with discovery of detecting Phenylketonuria (PKU) from dried blood spots (DBS) on filter paper, by a simple test as bacterial inhibition assay (Guthrie, 1961). His research work led to the now well-known 'Guthrie' card procedure/ test which is nothing but blood absorbed from the baby's heel prick onto the special thick filter paper to screen PKU in the newborn.

The credit of the development of screening tests goes to Bickel and his co-workers, when they successfully made dietary control on phenylketonuria in 1954 and made a remarkable breakthrough in the management of PKU and innovations in detection techniques. The importance of early diagnosis of phenylketonuria was realised when it was observed that individuals with phenylketonuria had improvement in their clinical status. However, very soon the development of robust immunoassays for thyroxine and thyroid stimulating hormone (TSH) in the 1970s became feasible to add congenital hypothyroidism (CH) to the NBS panel. In 1963, Massachusetts in United States began universal mandatory screening for phenylketonuria and rapidly other states started establishing newborn screening programs by adding more disorders to the panel.

Newborn screening popularly now known as neonatal screening is testing newborn babies for serious developmental, genetic and metabolic disorders so that important action can be taken during the critical time before symptoms such as mental and/ or motor retardation, physical disabilities or death occurs (Dave and Das, 2010). It is the process of testing newborn babies after 48 hours of birth for treatable genetic, endocrine, metabolic, and hematologic diseases before the development of symptoms because in the newborn period, inborn errors of metabolism (IEM) can be asymptomatic and easily be misdiagnosed as sepsis or birth asphyxia. The delay in diagnosis or undiagnosed IEMs can lead to severe mental deterioration and even death. The prompt detection therefore requires vigilance and the early & pre-symptomatic measurement of biochemical markers of IEMs.

Newborn Screening is considered by many countries as a modern public healthcare program that identifies inborn errors of metabolism (IEM) affecting a child's long-term health and survival. The program is aimed at pre-symptomatic detection of possible risk of neonates & infants with life threatening genetic diseases, facilitating

proper diagnosis and intervention of their clinical conditions. By preventing morbidity or mortality of children, it ensures proper growth and development of children.

How the NBS Disorder is selected ? :

In 1968, Wilson and Jungner published their World Health Organization (WHO) report entitled "Principles and practice of screening for disease" which remains till date a significant contribution toward public health and population study literature. The WHO issued guidelines and criteria for selecting disorders in a particular nation/ geographic area which also have important ethical and legal implications.

The ten Wilson-Jungner criteria for selection of newborn screening disorder and appraising the validity of a screening program (Wilson and Jungner, 1968) are as follows:

1. The condition being screened for should be an important health problem.
2. The natural history of the condition should be well understood.
3. There should be a detectable early stage.
4. Treatment at an early stage should be of more benefit than at a later stage.
5. A suitable test should be devised for the early stage.
6. The test should be acceptable.
7. Intervals for repeating the test should be determined.
8. Adequate health service provision should be made for the extra clinical workload resulting from screening.
9. The risks, both physical and psychological, should be less than the benefits.
10. The costs should be balanced against the benefits.

American Association of Paediatrics Newborn Screening Task Force in 1999, recommended that "Maternal and Child Health Bureau of Health Resource and Services Administration" should engage in a national process involving government, professionals, and consumers to advance the recommendations of this Task Force and assist in the development and implementation of nationally recognized Newborn screening system standards and policies; outlined a process of standardization, of outcomes and guidelines for State Newborn Screening Programs, defined responsibilities for collecting and evaluating outcome data, recommended uniform panel of conditions to include in State Newborn Screening Programs (AAP, 2000). American College of Medical Genetics, Newborn Screening Expert Group also provided guidelines towards a uniform screening panel and system for newborn screening (ACMG, 2006).

There are some disorders whose screening offers clear and direct benefits to the newborn, and others in which the benefits of screening are not that obvious. The screening of few diseases that are not treatable are also included with a primary objective of getting important information for future genetic counselling of the family or prenatal diagnosis with preventive approach. All of these issues have led to significant discrepancies in the criteria used to establish the diseases to be included in screening (Castineras et al, 2019).

What is a Newborn Screening Test ? :

NBS is a simple blood or urine screening test conducted on apparently healthy babies soon after birth & is not designed to be diagnostic. Therefore, abnormal newborn screen results prompts the initiation of further confirmatory diagnostic testing, neonate evaluation, and consideration of treatment while waiting for the diagnostic test results. Hence, newborn screening is always considered as a program rather than a simple laboratory test as it involves early detection, treatment and management of the newborn who may be affected with IEMs (Dave, 2016). These disorders may individually be rare but their collective incidence is 1 in 1500–2000. Their early & pre-symptomatic detection is significant as timely intervention, treatment and therapy

by the referring doctor can lead to the reduced morbidity, mortality and associated disabilities in affected infants, thus giving baby the best chance of healthy life. Any presumptive positive result of the NBS test requires confirmation, preferably with an independent sample and test method. The prompt detection therefore requires vigilance and measurement of biochemical markers with appropriate technology.

Advances in NBS Technology:

From the first generation of PKU screening using ferric chloride reactions in neonatal diapers to Guthrie and Susi's bacterial inhibition assay, the next significant milestone in newborn screening methodology was the advent of tandem mass spectrometry (TMS) using dried blood spot (Chaceetal,1993). Other techniques include spectrophotometry, fluorometry, and immunoassays.

The rapid and multi component techniques of tandem mass spectrometry (LC/MS-MS) screens about 46 metabolic conditions simultaneously from a single blood spot. Since its first application by Tanaka in 1966 to discover isovaleric acidemia, the GC/MS urinary metabolic screening has been used worldwide to diagnose number of IEMs because of its high accuracy, sensitivity and power of analyzing multiple compounds simultaneously. Matsumoto and his research team developed a rapid, practical, non-invasive and simultaneous urinary metabolite analysis in Japan for Newborn Screening (Matsumoto & Kuhara, 1996). The author of this Review is the first to introduce the same GC/MS technology in 1998 in India for High-Risk Screening of IEMs using urine filter paper, when concept of NBS was not initiated/accepted due to various health constraint factors.

The High-risk screening differs from NBS in that the metabolic screening is conducted on critically ill sick / NICU babies. Over a period of time the hospital/Lab based data offers the incidence of common IEMs in that area or referral population. Based on the last 20 years' of our experience in high-risk neonates and children, the most common 13 IEMs are identified which are satisfying the Wilson & Jungner guidelines to select NBS disorders (Dave, 2022) in Indian population. Though individually rare, the collective incidence of IEMs in more than 7300 high-risk babies was found to be 1: 30 - 1220 (Table-1). Out of total 22% metabolic abnormality (1633 of total 7330 cases), these 13 common IEMs constituted 12.4%. It is evident that State or private hospitals can focus on these 13 metabolic conditions while considering newborn screening service. In general population, the collective incidence of IEMs is reported to be around 1:1500-2500.

Recently, screening methodologies have subsequently expanded to include DNA-based testing strategies. Targeted genetic testing has been included in newborn screening algorithms for cystic fibrosis, where an elevated immunoreactive trypsinogen measurement is followed by screening for a panel of CFTR (cystic fibrosis transmembrane conductance regulator) gene mutations. Similarly, a targeted genetic testing strategy has also been described for screening the newborns for familial conditions (e.g. familial hemophagocytic lymphohistiocytosis (FHLH) due to UNC13D inversion mutations) (King and Hammarstrom, 2017). The advent of next generation sequencing (NGS) has opened up the new era of Newborn Genomic Screening which is currently at the research level, viz. NIH Genome Screening project.

Newborn Screening (NBS) Program :

Screening programs are often run by the state or national governing bodies such as public health departments. It is done for all neonates born in their jurisdiction for a defined panel of treatable disorders. The number of diseases screened for is set by each jurisdiction, and can vary greatly.

The high-risk screening data from these areas often indicates the priority NBS disorders.

While typically using blood taken from a heel-prick, more recent newborn screening expansion has included bedside testing to detect conditions

such as hearing loss (Nelson et al, 2008) and congenital heart disease (Thangaratinam et al, 2012 ; Therrell et al, 2015). A population-based dried blood-spot screening (NDBS) received the general acceptance in early 1960s as an essential preventive public health activity. The nickname of NBS test was a 'PKU test' in general population. Since then many feedbacks from the medical & social scientists helped in how to implement NBS as a universal screening program.

The importance & outcome of population-based NBS programs has been well illustrated at Boston Children's hospital emphasising the long-term outcome of expanded newborn screening (Landau et al, 2017). Various NBS panels based on the number of disorders to be tested are available, such as NBS-2 (CH & CAH), NBS-3 (CH, CAH & G6PD), NBS-5 (NBS-3+ Galectosemia & PKU), NBS-7(NBS-5+Biotinidase Deficiency & Cystic Fibrosis).

The expanded neonatal screening using Tandem MS technology includes additional 46 conditions involving, amino, organic & fatty acid disorders. The selection of the panel depends on the existing epidemiological data or prevalence in that region and also affordability of the parents, if the NBS tests are not free or covered under national program. The informed consent of the parents is a must to undergo any NBS tests and generally remains the responsibility of the hospital.

What is NBS Referral Laboratory? :

The NBS tests are ideally conducted by the 'Referral NBS Laboratory' exclusively devoted to various NBS Panels using ELISA, Immunofluroscent Assays, or Mass Spectrometry methods having capacity of 100-1000 tests per day. The ideal NBS Laboratory at State level also conducts public awareness, education, training to medical /paramedical professionals, regional data analysis & publications. These laboratories observe strict quality management rules & NBS laboratory accreditation programs. The pre-analytical, analytical & post analytical systems must be followed for the integrity of work flow, sample collection, time & condition of collected sample, transport temperature & conditions, quality of collected sample etc. by a well certified & accredited NBS laboratory.

Being a screening test, the high false positive rate is accepted with a goal of not escaping a single positive case from the program. The less than 0.3% false positive rate & positive predictive value of > 20 is considered as an ideal target by the NBS Laboratory (Jalan & Kudalkar, 2021). The confirmatory diagnostic test in presumptive positive cases with further advice to the patient's family & coordination with the clinician for appropriate dietary & therapeutic intervention should be immediately provided by the same NBS laboratory. This is to avoid the delay in intervention &treatment.

The screened positive newborns, once confirmed with diagnostic tests are often referred to the tertiary care hospital or the expert metabolic paediatrician /neonatologist for treatment. The genetic counseling to the parents is necessary here to explain the nature of NBS condition, recurrence risk with future preventive prenatal diagnosis, importance of therapy & possibility of lifelong care in some cases (Dave, 2022).

Worldwide NBS Scenario:

Newborn Screening is considered as a modern science program, having firm roots in international countries like US, UK, Europe, Australia, Japan, etc., and is slowly entering Indian healthcare system and other developing countries like sub-Saharan Africa, South Africa and some parts of Asia.

The conditions included in newborn screening programs around the world vary greatly, based on the legal requirements for screening programs, prevalence of certain diseases within a population, political pressure, and the availability of resources for both testing and follow-up of identified patients. From a relatively simple blood or urine screening test, originally used for detecting a single congenital condition (viz. CH or CAH) to a more comprehensive and complex

mass-spectrometry screening system that can detect over 46 different disorders in one single test is used in population (Therrell, et.al., 2015). Tandem Mass Spectrometry (TMS) is widespread accepted method in developed countries, referred as expanded neonatal screening test, covering many preventable amino, organic & fatty acid disorders.

The American College of Medical Genetics published the document newborn screening toward a universal screening panel and system in 2006 with the aim of establishing a uniform screening programme across its states which consists of 29 core diseases as primary targets for screening and 25 diseases as secondary targets depending on the benefits of the disease detection (ACMG, 2006).This led to the establishment of the 'Recommended Uniform Screening Panel' (RUSP), including a large group of diseases. Until 2019, RUSP includes 35 primary targets and more than 26 secondary targets and is also considered a reference for the purpose of debate and evaluation in other countries. (RUSP, 2018).

Similarly, newborn screening for lysosomal storage diseases is also considered with an ethical and policy analysis (Ross, 2012).The screening for hemoblobinopathies, with special emphasis on sickle cell disease has also gained the priority in certain countries with high prevalence to reduce the national burden.

In Canada, newborn screening includes a considerable number of metabolic disorders, although fewer compared to the US. Some countries in Central and South America have high-quality, well-established NBS programmes, especially Costa Rica and Uruguay, where all newborns are screened by means of MS/MS. However, most screening programmes in South America include a limited number of diseases in addition to PKU, and few regions use MS/MS (Queiruga, et. al., 2011). Egypt has an established NBS programme, using MS/MS in some part of the population, and other North African countries are aiming at projects for the establishment of routine newborn screening (Shawky, et. al., 2012). The situation in Sub-Saharan Africa and South Africa is quite different, with very few reports on NBS programs.

In Europe in the past 50 years, a screening for PKU with addition of screening for biotinidase deficiency and classic galactosemia is established through the European Commission funded project to analyse newborn screening policies and practices with a goal of setting the foundations to develop guidelines (Burgard, et. al., 2012). There are still differences between different countries, as in France where only routine screening of PKU is done. The situation is quite different in Southeast Europe, as screening is not done there to detect any metabolic disorders (Groselj, et. al., 2014). In Italy, a law was passed in 2016 to do routine NBS programme consisting of 40 conditions (Castineraset al, 2019). The Middle East, Qatar or Saudi Arabia screen all newborns for a broad range of metabolic disorders; others screen only 2 diseases e.g. United Arab Emirates and Kuwait, and a third group continues to not have any form of screening program.

In many Asian developing countries, NBS is now implemented with few parameters reflecting their economy and the public health systems but many other countries with fewer resources have not instituted any NBS programmes till last decade (Padilla & Therrell, 2007). Being the developed countries, all newborns are screened in Australia and Japan for a substantial number of metabolic diseases with MS/MS (Wilcken, et. al., 2009 and Yamaguchi, 2008). In China, screening already covers 80% of newborns and includes PKU, and testing by MS/MS in some regions (Shawky, et. al., 2012).

NBS- Indian Perspective:

For the first time in 1984, screening for Congnital Hypothyroidism (CH) in 12,407 newborns was reported by M. Desai & group from Wadia Children Hospital, Mumbai using fetal cord blood with the incidence of 1:2804 (Desai et al,1987). Later, NBS was carried out for

aminoacid disorders using conventional method in Karnataka in 1987 (Ramadevi & Rao, 1988). A pilot newborn screening program using dried blood spots from heel prick was initiated by CDFD at Hyderabad in 1988 & 12,500 newborns were screened for aminoacidopathies, Congenital hypothyroidism (CH), Congenital Adrenal Hyperplasia (CAH), Glucose-6- phosphate dehydrogenase deficiency(G-6-PD),etc. The CH (1 in 1700) followed by CAH (1 in 2575) emerged as most common disorders (Ramadevi & Naushad, 2004).

There are 3 public screening programs with varying degrees of complexity (panels, geographical areas covered, and births screened per year) that have been running for more than 5 years. In 2007, the union territory (UT) of Chandigarh in India started a program to study the prevalence of three disorders (CH, CAH, and G6PD deficiency), concentrated in four urban government hospitals with about 15,000 births per year (Kaur, et al., 2010). Chandigarh's NBS program is the pioneering public NBS program in India and continues to this day with the addition of other government hospitals.

The Goa NBS Program (2008 to 2013) was initiated based on the desire of the state government to improve neonatal care in a public hospital & screened (~48,000) about 50% of the births in Goa in the five-year period (Mookken, 2020). The six disorders (CH, CAH, G6PD, galactosemia (GALT), biotinidase deficiency, and cystic fibrosis) were initially screened at NSQAP Neogen laboratory, Bengaluru followed by about 46 conditions using MS/MS method. The Goa program is the best example of a public–private partnership (PPP) model that was financially beneficial to the state government. Kerala screens more than 1,40,000 births per year in over 90 government hospitals (Maya, 2015). The program screens for CH, CAH, G6PD, and GALT for 25% of all births in Kerala per year in four laboratories spread across the state. None of the state screening laboratories participate in NSQAP. The 5 conditions were screened by a tertiary care Govt. hospital in Bengaluru, South India for 47,623 babies in 3 years (Year 2016 to 2018) giving G6PD as the most common IEM (1:414) followed by CH (1:2735) & CAH (1:4102) using the infrastructure of private Navigene Genetic Lab. Mumbai (Kommalur et al, 2020). The Galectosemia & PKU were found to be rare with incidence of 1:20513 & 1:41027 respectively, though in a smaller cohort study.

In India, we have certain geographic / tribal belts with high incidence of Sickle cell disease & Thalassemia, emphasizing the screening for Hemoglobinopathies which is also currently undertaken by National Health Mission (NHM) in various States .

As India does not have population-based genetic epidemiology studies, the exact burden & incidence of NBS disorders is not known. We simply followed the Western data. The incidence of PKU is also low in our country compared to the other countries. In 2008, ICMR had launched a pilot multi-centre NBS program to screen 100,000 babies for only 2 disorders (CH & CAH) in five cities – Mumbai, Delhi, Chennai, Hyderabad & Kolkata indicating the feasibility of NBS in India (ICMR, 2014). The collective incidence of CH was found to be 1: 1172, while Southern India showed higher incidence rate as 1: 727, possibly contributing to consanguinity & endogamous marriages. In Bangalore-based study, the incidence of CH was estimated to be 1:1042 in 19,800 babies screened (Kishore et al 2014). The incidence of CAH from Indian reports varies from 1:2600 to 1:16000 livebirths which is relatively higher than Western countries (Maiti & Chatterjee, 2011).

In 2011, National Neonatology Forum (NNF) recommended 3 NBS conditions- CH,CAH & G6PD – as the basic screening panel to implement NBS in India. In the affordable patients, it can be extended NBS panel of 46 conditions using MS/MS test. The West Bengal in 2009 & Gujrat in 2011 have approved a launch of Govt. NBS programs but these are yet to be implemented.

Issues & Challenges in Universal Implementation of NBS in India-

Currently in India, the consensus is that all babies need to be screened, but there is no coherent national strategy for implementing a universal screening program nor guidance on which disorders should be included in the screening panel. The ICMR study was a pilot study conducted on very small cohort considering high annual birth rate (about 27 million per year), though it is well accepted that NBS is the need of the hour in India (Verma & Bijarnia, 2015).

Today, there are numerous NBS laboratories, public and private, in India offering NBS tests.

Some of them offer comprehensive NBS panels, resembling the Recommended Universal Screening Panel (RUSP) in the USA (https://www.hrsa.gov/advisory-committees/heritabledisorders/rusp/index.html). Many, but not all of them participate in the Newborn Screening Quality Assurance Program (NSQAP) offered by the Centers for Disease Control and Prevention (CDC), US. (https://www.cdc.gov/labstandards/nsqap.html)

The biggest difficulties to start NBS program in

India are as follows:

- The cost of case finding (including diagnosis) is not economically balanced to possible expenditure on medical care as a whole.
- Facilities for diagnosis and treatment is not easily available at the screening site/referral NBS laboratory. In short, NBS is not under one umbrella of health services.

All the above mentioned programs use panels of disorders that are well understood by physicians in India and easily treatable. Disorders screened by MS/MS (fatty acid, organic acid and amino acid disorders) are not often the part of the screening panels due to resource constraints (significant capital costs, few experts, lack of treatment facilities, and high cost of diets).

With annual birth rate of about 27 million babies, the hearing defects (4:1000) & congenital heart defects (5:1000) are other NBS conditions requiring serious attention besides IEM screening. The high-incidence rate of consanguinity, endogamous marriages, racial & religious genetic diversity & tribal populations in selected geographical areas contribute significantly to the national burden of NBS conditions.

In our experience, it is not the technology that is preventing Indian babies from getting screened, be it newborn or high-risk screening. But lack of awareness about NBS screening and knowledge about the latest technologies among the healthcare providers, as well as our different national health priorities are the main contributing factors for the delay in implementing NBS program. As in the case of introducing iodinated salt or compulsory polio vaccination, the support & advocacy by the Govt. of India is a prime factor at the population level. Government support will go a long way in establishing national level newborn screening program in collaboration with private laboratories as a countrywide network system. Nevertheless, it is also the moral responsibility of those professionals caring for the neonates to inform & educate the parents about the newborn screening & explain them its long-term benefits & cost-effective approach of prevention of disabilities. The primary care physicians & paramedical staff like nurses, midwives need to be educated. The NBS programs will not only help in improving our IMR & NMR rates but will produce important genetic epidemiological data which is currently lacking in India.

Important questions need to be answered while implementing the NBS process in India, such as

1) What barriers does a primary care physician face in coordinating a medical evaluation and communicating with the family in an infant with a positive screening result? 2) What obstacles do families confront in the time after a newborn screening result returns positive? 3) How can coordination of follow-up care be optimized in a confirmed newborn screen result? These fundamental questions must be addressed to

optimize collaboration between primary care and specialty care physicians by public-private partnership, and to ensure the continued success of newborn screening in the 21st century.

CONCLUSION:

In brief, there is a need of a holistic comprehensive newborn screening program and not just the provision of laboratory testing services. The shortcomings of the previous program need to be addressed, and more emphasis to be placed on follow-up activities, access to experts and availability of diets. Benefits of NBS do not end only with saving life of the diagnosed case but they extend up to prenatal period of diagnosis and family genetic counseling. It is also true that the fruits of genomic science should not remain a luxury available only to the developed nations. The next technological advances like mass spectrometry, microarrays & next generation sequencing are on the horizon and fast entering into clinical practice. Yet, it should be noted that technology is only one facet of a well-functioning newborn screening program, which must have both excellent detection and follow-up services. The ethical, social & legal implications should not be overlooked. The challenge finally in India is the ultimate coverage of 100 % screening of neonates & infants which can only be achieved with a political will & financial commitment considering our socioeconomic infrastructure.

Table 1- High-Risk Screening of 7330 Babies Abnormal=1633 (22 %) & Normal= 5697 (78%)

Incidence of 13 IEMs -1: 30- 1220

Table 1: Selection of NBS Disorders based on Incidence of IEMs High- Risk Screening Data by GC/MS Comprehensive Test (2005-2022)

Sr. No	Inborn Error Of Metabolism	2005 N= 2040 Abn = 176 - 8.6%	2015 N= 3341 Abn= 291- 8.7%	2018 N= 5880 Abn = 568 -9.6%	2020 N= 6510 Abn=717 – 11%	2022 N= 7330 Abn=906 -12.4 %
1.	Methylmalonic Acidemia (MMA)	1: 55 (37)	1: 64 (52)	1: 34 (172)	1: 30 (214)	1:28 (265)
2.	Tyrosinemia / Hepatic Dys	1: 78 (26)	1: 88 (38)	1: 96 (61)	1: 72 (91)	1: 50 (146)
3.	Hyperglycinemia	1: 146 (14)	1: 119 (28)	1:189 (31)	1: 171 (38)	1: 179 (41)
4.	Glutaric Aciduria 1	1: 102 (20)	1: 90 (38)	1:95 (62)	1: 90 (73)	1: 93 (79)
5.	Galactosemia	1: 136 (15)	1:176 (19)	1:120 (49)	1: 130 (50)	1: 113 (65)

6.	Maple Syrup Urine Disease(MSUD)	1: 156 (13)	1: 239 (14)	1: 128 (46)	1: 99 (66)	1: 99 (74)
7.	Propionic Acidemia (PA)	1: 170 (12)	1: 176 (19)	1: 155 (38)	1: 105 (62)	1: 106 (69)
8.	Urea Cycle Disorder (UCD)	1: 170 (12)	1: 134 (25)	1: 168 (35)	1: 186 (35)	1: 188 (39)
9.	Fructose-1, 6-Diphosphatase Def. (FDPD)	1: 136 (15)	1: 134 (25)	1:235 (25)	1: 217 (30)	1: 159 (46)
10.	Multiple Carboxylase Def. (MCD)	1:510 (4)	1: 257 (13)	1:309 (19)	1: 260 (25)	1: 271 (27)
11.	Isovaleric Acidemia (IVA)	1: 680 (3)	1: 835 (4)	1:534 (11)	1: 591 (11)	1: 488 (15)
12.	Beta-Ketothiolase deficiency	- -	1: 304 (11)	1: 420 (14)	1: 383 (17)	1: 215 (34)
13.	Ornithine Trans-Carbamylase Def. (OTC)	1: 408 (5)	1: 668 (5)	1: 1176 (5)	1:1302 (5)	1: 1221 (6)

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