RESULTS OF A BIOMEDICAL APPLICATION IN NEWBORN SCREENING PROGRAMS

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Abstract: The recent advances in tandem mass spectrometers technology had led to its introduction in several neonatal screening laboratories. The effective screening of newborns using dried-blood-spot specimens collected at birth, combined with follow-up diagnostic studies and treatment, helps prevent mental retardation and premature death. The introduction of MS/MS in the Portuguese national neonatal screening lab has led to the development of a new biomedical application that can help technicians handle the large amount of data involved and can assist in the implementation of a reliable quality control procedure.
The developed application allowed the screening of almost 21.000 newborns between September 2004 and May 2005.

Introduction

In the last decades the advances in Tandem Mass Spectrometry (MS/MS) technology introduce it in neonatal screening laboratories [1, 2]. This screening is intended to detect inborn disorders that can result in early mortality or lifelong disability. Significant challenges still remain since it implies a significant financial effort, a change in the way of thinking the diagnosis of inborn errors of metabolism and the need to manage and process a massive amount of data.

Tandem mass spectrometers (MS/MS) allow the rapid analysis of individual compounds in complex mixtures and are composed by two mass spectrometers joined by a collision cell [3-5].

The introduction of MS/MS in the Portuguese national neonatal screening lab (with over 110.000 samples/year) has led to the development of a new application, NeoScreen [6], that can help technicians to handle the large amount of data involved (with more than 80 parameters/sample) and can assist in the implementation of a reliable quality control procedure.
The introduction of this technology also brought new challenges, since the analysis made by using the spectrometers produced large amounts of data. It is necessary to have an information system able of managing and storing all the data as well as to be able to create algorithms that are able to detect the newborns that may be bearer from a specific disease.

Newborn screening and diseases

The metabolic diseases are caused by innate errors of the metabolism and are studied by a specific area of the genetics, the biochemistry genetics. For some diseases there is already some treatment, depending on the disease, the ability of the doctor and the child family. Unfortunately, for the majority, there is no efficient therapeutics. In these cases, the treatment is limited to a clinical assistance which will give the child a better life standard.

The deification of each disease is performed, not by its clinical characteristics, but by the lab investigation which allows the diagnosis through the identification of the biochemical alterations which are present. The subgroups are multiple and the numbers of disorders known are about two hundred [7]. The clinics are varied and most of the diseases are of rare incidence, which brings difficulties to the diagnosis.

The diseases screened through the MS/MS technique are grouped in categories (Table1), according to the markers which define them and the metabolic processes in which the respective markers are involved [8]. For instance, the *Amino Acid Metabolism* is related with markers coming from amino acids measurements.

The *Organic Acid Metabolism* comprises the metabolic deficiencies related to the interaction of the amino acids with the fat acids and the carbo-hidrats. This group of diseases is characterized by a huge concentration of organic acids in the blood and urine, being also present in various intermediate processes of the metabolism [7].

The group of the *Fatty Acid Metabolism* is characterized by a large concentration of the acilcarnites of the long chain, due to problems related to their oxidation, inside the mitochondria.

The *Congenital hypothyroidism* (CH) is a disease which diagnostic does not use the MS/MS technology. Its origin is hereditary and rare, causing a diminution of the thyroid hormone (T4) present in the blood [9].

In Table 2 we can observe the number of cases and frequencies detected in Portugal between 2002 and 2004 [10, 11].

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Table 1: Name and group of disease that belong to the national screening

Amino acid metabolism
Phenylketonuria (PKU)
Maple syrup urine disease (MSUD)
Citrullinemia (CIT)
Organic Acid Metabolism
Argininosuccinate acidemia(ASA)
Propionic Acidemia (PA)
Methylmalonic acidemias (MAA)
Isovaleric Acidemia (IVA)
3-Hydroxy-3-Methylglutaryl-CoA Lyase acidemia (3-HMG)
Glutaric Acidemia Type I (GA-I)
Fatty Acid Metabolism
Mediun-chain acyl-CoA dehydrogenase (MCAD) deficiency
Very long-chain acyl-CoA dehydrogenase (VLCAD) deficiency
Very chain 3-hydroxy acyl-CoA dehydrogenase (LCHAD) deficiency
Carnitine- palmitoyl-transferase type I (CPT I) e Carnitine-palmitoyl-transferase type II (CPT II) deficiency

Table 2: Number of case detected in Portugal

Year	Newborn study	Disease	No of cases	Frequency
2002	113.897	CH	37	1 / 3078
		PKU	16	1 / 7118
2003	112.557	CH	41	1/2745
		PKU	9	1/12506
2004	108.564	CH	43	1/2524
		PKU	11	1/9869

The diseases screening is performed through a simple algorithm, which is illustrated in Figure 1. The markers of each newborn lying outside the percentile limits are flagged by the algorithm. Using these markers, an attempt is made to match against each of the diseases being tested for. This is performed by recovering the markers associated with each disease and attempting to match these with the flagged markers for the newborn. Finally, all probable diseases are identified and listed against the particular newborn being screened.

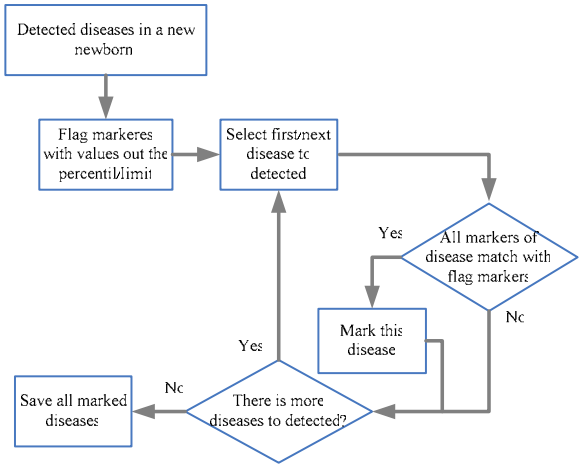

Figure 1: Diseases screening algorithm.

NeoScreen results in Portugal and Spain

The NeoScreen is being used in two laboratories. The *Instituto de Genética Médica* (IGM), which is responsible for all the newborn screening done in Portugal and the *Hospital Universitário de Santiago de Compostela* (HCUS), that performs such screening for the Galicia Spanish region. The IGM has screened a total of 20.581 newborns, in the period comprised between September 2004 and April 2005. The HCUS, in Spain, have submitted 21.235 newborns to the NeoScreen, in the period between January 2004 and April 2005.

With this new application it was possible to enlarge the number of diseases screened by the national program, and this opens the possibility of avoiding coma and death situations or even proceed to an in time genetic advice.

Markers control

The evolution of the markers is performed through the analysis of control charts as is shown in Figure 2. The application allows the automatic analysis of patterns defined as abnormal. The user can, afterwards, confirm the anomalies through the control charts consultation. It also has the possibility of eliminating some values which are not relevant, allowing to reduce the deviation factors introduced in the average calculation.

The evolution of the markers values assumes an important role in the quality control. Some of the abnormal patterns that the software can identify in the daily marker values have been highlighted in Figure 2 and are explained below.

The set (1) illustrate a situation in which the marker values are below the mean for five or more consecutive days. The points in set (2) lie between two and three standard deviations from the mean. Set (3) contains five marker values that lie above the mean and show a monotonic increase. Finally set (4) contains maker

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values that lie beyond three standard deviations from the mean.

These patterns, considered abnormal, may indicate that some procedure in the preparation of the sample failed or that some problem in the calibration of the equipment may be occurring. The application generates automatically an alert when some of these anomalies (as in picture) are detected.

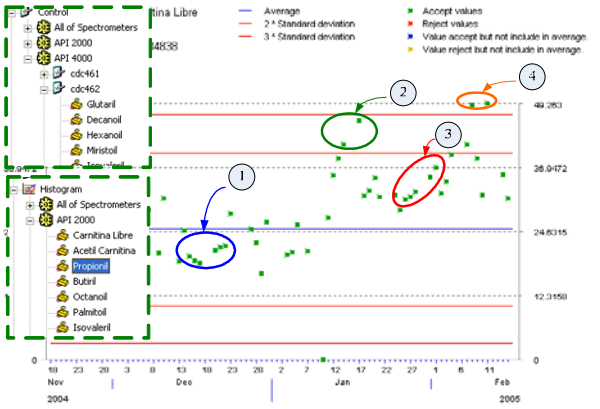

Figure 2: Charts of control supplied by NeoScreen

Results

Besides the immediate social impact of newborn screening, and so of NeoScreen's goal, the use of this software allows yet to redefine continuously, with more accuracy we hope, the levels of markers cut-offs, i.e. the levels, below or above which the sample results are considered suspects.

Table 3 shows the results of such measures in the two laboratories. As one may see, the data to some markers are different among themselves, being more visible in the amino acids. One of the possible explanations is centered in the categories of the individuals who are selected for the calculation of the daily averages of the markers. Even so, the two laboratories are using the same categories for the calculation of the daily averages, being the suspect, normal, high values without disease and low values without disease [12].

One can conclude another important fact. The deviations are always higher in HCUS when compared with those of the IGM, excepting the Decenoyl-carnitine (C10:1).

Table 3: Average and standard deviations gotten for the markers in the two centers in analysis. Values are in µmol/L

Amino acids/Carnitines (symbols)	IGM	HCUS
Phenylalanine (Phe)	57,47 ± 8,23	61,47 ± 16,91
Alanine (Ala)	364,01 ± 70,55	429,69 ± 147,18
Ornitine (Orn)	141,52 ± 60,99	110,21 ± 87,92
Tyrosine (Tyr)	141,76 ± 44,4	106,65 ± 38,04
Citruline (Cit)	134,58 ± 25,1	155,91 ± 40,99
Valine (Val)	9,88 ± 1,63	14,8 ± 4,52
Free carnitine (C0)	24,140 ± 3,371	24,941 ± 7,803
Acetyl-carnitine (C2)	19,750 ± 3,605	21,416 ± 7,265
Propionyl-carnitine (C3)	1,577 ± 0,269	2,015 ± 0,699
Isovaleryl-carnitine (C5)	0,262 ± 0,03	0,203 ± 0,102
Hexanoyl-carnitine (C6)	0,033 ± 0,01	0,084 ± 0,036
Hidroxy-isovaleryl-carnitine (C5-OH)	0,142 ± 0,024	0,151 ± 0,040
Octanyl-carnitine (C8)	0,057 ± 0,011	0,071 ± 0,026
Decanoyl-carnitine (C10)	0,095 ± 0,014	0,123 ± 0,041
Decenoyl-carnitine (C10:1)	0,299 ± 0,245	0,076 ± 0,025
Myristoyl-carnitine (C14)	0,201 ± 0,026	0,222 ± 0,063
Myristoleyl-carnitine (C14:1)	0,089 ± 0,015	0,158 ± 0,061
Hydroxypalmitoyl-carnitine (C16-OH)	0,027 ± 0,006	0,020 ± 0,009
Octadecenoyl-carnitine (C18:1)	1,36 ± 0,253	1,436 ± 0,414
Hydroxyoleyl-carnitine (C18:1-OH)	0,026 ± 0,005	0,024 ± 0,012

The values of Table 4 correspond to the cutoffs used by the various laboratories which took place in the CDC program [13, 14]. The column 'N' corresponds to the number of participants which contributed with values to the respective cutoffs. The column with the maximum and minimum value contains superior, maximum and minimum cutoffs, given to the markers by the different laboratories. As one can verify, the high level cutoffs do suffer a big variation in relation to the average, which highlights a significative difference in the criteria used by the two labs in the definition of the superior cutoffs.

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Table 4: The cutoffs used by the others laboratories

Amino acids/Carnitines (symbols)	N	Mean	Min/Max
Phenylalanine (Phe)	52	158	61-364
Methionine (Met)	44	75	34-134
Tyrosine (Tyr)	47	326	83-751
Valine (Val)	40	325	111-829
Citruline (Cit)	42	74	11-251
Propionyl-carnitine (C3)	48	6,02	1,27-14,30
Butyryl-carnitine (C4)	46	1,39	0,41-5,21
Isovaleryl-carnitine (C5)	47	0,87	0,27-3,30
Hexanoyl-carnitine (C6)	45	0,56	0,18-2,00
Octanyl-carnitine (C8)	51	0,48	0,15-1,00
Decanoyl-carnitine (C10)	46	0,5	0,20-2,00
Myristoyl-carnitine (C14)	44	0,85	0,30-1,80
Hexadecanoyl-carnitine (C16)	48	8,24	3,84-14,50
Glutaryl-carnitine (C5-DC)	45	0,28	0,04-0,66

The data in Table 5 correspond to the values calculated with the percentiles from 95 to 99.5, for the two laboratories through the NeoScreen. The percentis 95 e 99,5 correspond to the superior cutoffs, and are the values which distinguish the suspicious and highly suspicious individual [12].

As one may see, the obtained results are all found in the Max/Min column of Table 4, expecting the *Methionine* for the percentile to 99.5 from the HCUS laboratory. The acilcarnitines *Butyryl-carnitine* and *Octanyl-carnitine* are found closely to the minimum limit of the superior cutoffs.

Table 5: Values calculated for the NeoScreen thought the percentiles. Values are in µmol/L.

Amino acids/Carnitines (symbols)	IGM		HCUS	
	95	99,5	95	99,5
Phenylalanine (Phe)	86,51	125,7	85,51	126,28
Methionine (Met)	29,56	39,93	114,88	252,55
Tyrosine (Tyr)	284,11	504,55	178,4	242,59
Valine (Val)	250,76	358,98	228,26	296,42
Citruline (Cit)	17,09	36,78	20,37	28,83
Propionyl-carnitine (C3)	4,82	5,342	3,42	4,68
Butyryl-carnitine (C4)	0,47	0,7	0,45	0,63
Isovaleryl-carnitine (C5)	0,44	2,12	0,3	0,7
Hexanoyl-carnitine (C6)	0,08	0,16	0,14	0,2
Octanyl-carnitine (C8)	0,12	0,24	0,16	0,23
Decanoyl-carnitine (C10)	0,18	0,35	0,21	0,3

The IGM has already detected four confirmed cases of new diseases through the NeoScreen application. Those were:

- 2 MCAD – this pathology belongs to the group of the organic acidurias and is due to the high concentration of the acilcarinites C6, C8, C10, C10:1 present in the blood;
- 1 MSUD – this pathology belongs to the group of the amino-acidopatias and is due to the high concentration of the amino acids Leu/Ile e Val;
- 1 3HMG – this pathology belongs to the group of the organic acidurias and is due to a high concentration of the acilcanitina C5-OH.

As one can see in Figure 3, between January and March 2005 the importance of this new technology became evident since four more new diseases were able to be detected with the help of the NeoScreen. Thanks to the new screening techniques along with the NeoScreem decision support systems, clinicians were able to improve the life standard of four children in a short period of time, from January to March 2005.

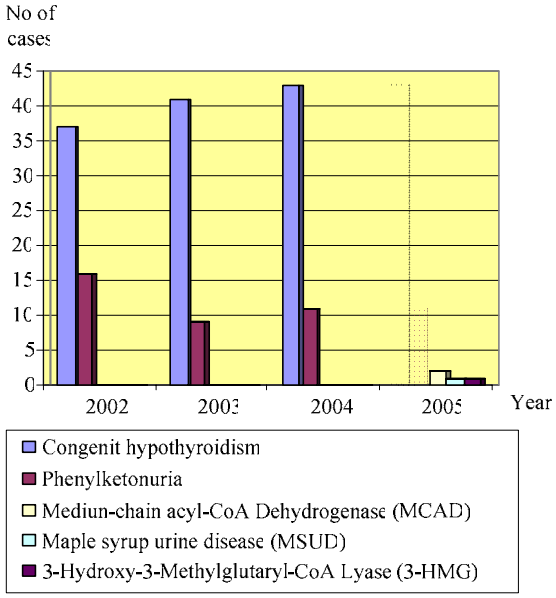

Figure 3: Number of diseases detected in the Portuguese national screening program.

Conclusions

The introduction of MS/MS in the neonatal screening laboratories has provided great benefits to the population, allowing the diagnosis of diseases much before the onset of the disease.

Since the MS/MS technique produces large quantities of information it requires computational support to efficiently process and store the information. Tools that implement tracking of the markers and detection of anomalous situations are also essential. NeoScreen offers just these tools freeing the user's time to more important tasks such as the screening and the analysis of the data of the newborns.

With the NeoScreen the screening labs are able of a permanent management of the information generated by

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the MS/MS, reducing the time and the analysis error, and being able to raise largely the number of screenings performed daily as well as their quality.

The results obtained from the calculations of NeoScreen for the cutoff of markers, are within the limits set by the CDC [14], qualifying NeoScreen as a decision support system for the newborn screening.

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