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EVALUATION OF THE NEONATAL SCREENING IN THE IDENTIFICATION OF HEMOGLOBIN S: BIOLOGICAL COLLECTION

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ABSTRACT

Background: The aim of this manuscript was to evaluate the neonatal screening in the identification of hemoglobin S specifically the biological collection in the state of Mato Grosso do Sul, Brazil, in the period from 2011 to 2015.
Methodology: This is a descriptive study that analyzed information about the National Neonatal Screening Program for hemoglobinopathies contained in the database of the Research Institute, Study and Diagnostics of the Association of Parents and Friends of the Exceptional in Mato Grosso do Sul, Brazil. The variables studied were the year of cancellation of the sample; total number of screenings; number of samples canceled due to failures in the collection of the screening; reasons for cancellation of samples; number and results of new samples resulting from cancellation. For the interpretation of the studied variables, we used the descriptive statistical analysis.
Results: The failures identified in the neonatal screening are related to the collection technique, sample with more than 30 days of harvest and those in duplicate. Of the total of the canceled samples 55% were collected again.
Conclusion: The neonatal screening process requires careful monitoring due to the complexity of sickle cell disease. Permanent education actions are suggested to health professionals, as well as research that identifies the determinants for the National Neonatal Screening Program in Mato Grosso do Sul to reach the 100% coverage ratio.

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INTRODUCTION

The National Neonatal Screening Program (PNTN) was established by the Ministry of Health with decree No. 822, dated June 6, 2001. This establishes screening actions in live births from 0 to 28 days, with the aim of identifying early, treat and monitor the disorders and illnesses of positive people (Brasil, 2016). The Brazilian Ministry of Health articulated the Program with the state and municipal health secretariats, the Federal District and the Special Indigenous Sanitary Districts (DSEI). Its implementation took place in four phases due to the different levels of organization of the assistance networks in the states and in the Federal District, variation of PNTN coverage and diversity of population characteristics in Brazil

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(Brasil, 2001). Screening for sickle cell disease and other hemoglobinopathies were included in a second phase. The distribution of hemoglobin S in the Brazilian population is heterogeneous, with an incidence of 1: 1000 live births. The prevalence of sickle cell trait is higher in the north and northeast regions between 6 and 10%, and in the South and Southeast regions between 2 and 3% (Cançado, 2007). Neonatal screening for sickle cell disease before clinical symptoms appear is critical to initiating preventive care and counseling appropriate to parents. It also allows health education actions regarding the genetic condition of the family and risk of recurrence in future pregnancies (Brazil, 2016).

Neonates' blood collections are performed by nursing professionals at accredited clinics located in basic health units, maternities, hospitals or indigenous communities. Therefore, the permanent education of these professionals is essential. (Silva, 2003). The organization of the collection flow of the blood sample includes training of the professional who will collect and choose the means of transportation of the samples to the laboratory that will carry out the analyzes. The ideal period for the collection of the first sample is between the 3rd and 5th day of life of the newborn. (BRASIL, 2016). The neonatal screening activity needs to be monitored in a systematic way to reduce the false results that involve the difficulties to achieve the goal set by the PNTN in Brazil (Prado, 2014). In Mato Grosso do Sul with PNTN monitoring since its implantation (Holsbach *et al*., 2008), some factors were identified in the collection phase (Torres; et al., 2017; IVO *et al*., 2014), which may be responsible for loss or delay in the presumptive diagnosis of some patients. In view of the above, this study intends to evaluate the neonatal screening in the identification of hemoglobin S, specifically the biological collection in the state of Mato Grosso do Sul, from 2011 to 2015.

METHODS

This is a descriptive study that analyzed information about the Neonatal Screening Program for hemoglobinopathies contained in the database of the Research Institute, Study and Diagnostics of the Association of Parents and Friends of the Exceptional (IPED-APAE). This study considered 182,392 children screened of a total of 231,739 live births in the state of Mato Grosso do Sul, Brazil. The examinations were obtained from the 1,585 collection points registered in 79 municipalities of the state in the period from 2011 to 2015. For the organization of the reference year of the examinations, the period from December 16 of the previous year to December 15 of the reference year was considered. This methodology was adopted by IPED-APAE so that all the collections of exams received until the first fortnight had the results released and invoiced in the same month. In order to identify the types of failures of neonatal biological screening, as well as to estimate the frequency of exams that occurred due to collection failures, an analysis was carried out of the information of 372 samples canceled after being classified in the reception sector of laboratory. Parameters investigated in this reseach were the year of cancellation, total number of screening, number of samples canceled, reason for cancellation of samples, number of new collections after cancellation and results of new collections. The cancellations of the neonatal screening samples were classified according to what was recommended in ref. (BRAZIL, 2016).

We considered the technical failure to collect the sample (inadequate samples, saturated samples, wet samples, wrong material for examination, samples with insufficient blood quantity, sample without biological material, unidentified or unidentified sample), sample taken in duplicate and samples with more than 30 days of collection (denaturation of hemoglobin). A search was performed on the IPED-APAE database, with the following steps: search by name of the child who had the screening sample canceled and confirmation of the date of birth of the same. In the absence of location by the child's name, the search for the mother's name was performed and the date of the child's birth confirmed. If there was a divergence in the child's birth date, the patient's residential address was checked. The process of neonatal screening is performed in accordance with the technical criteria established by the technical manual called Neonatal Biological Screening (Brazil, 2016).

Neonatal screening begins with the collection of blood on filter paper (Scheleicher & Schuel 903 model) after filling in the blood collection card provided by the state reference laboratory IPED-APAE. Such sample collections are performed by nursing professionals or technicians trained for this purpose. It will perform the puncture on the heel side of the newborns in the basic health units (UBS), Family Health Strategy units (ESF), maternity hospitals and hospitals. After the collection and drying of blood, the sample is packed in the proper envelope for biological material, with postage paid and posted to the reference laboratory by the Post Office. Municipalities can also send the samples directly to the reference laboratory, who will be responsible for the analyzes and classifications regarding the quality of the blood sample, filling the data on the collection card and the age of the child at the time of collection. Samples are rejected and canceled when they are considered inadequate (saturated, wet and wrong material for examination), samples with insufficient blood quantity, sample taken in duplicate, samples with more than 30 days of collection (denaturation of hemoglobin) and sample without material biological. When the cancellation of samples occurs, new ones are requested to the responsible for the collection point of origin of the same. The Active Search sector requests the new sample through a letter containing the justification of the cancellation and the photograph of the canceled sample.

The screening of hemoglobinopathies is performed by the VARIANTnbs system through the High Performance Liquid Chromatography (HPLC) method. This screening identifies in the filter paper in blood disk eluates the presence and concentration of normal hemoglobins F and A and variant hemoglobins S, D, C and E. After processing the sample, the laboratory may also be asked to collect new samples as a result of insufficient or inadequate serenity, non-eluted samples, altered and hemolyzed samples. The request flow is the same as for canceled samples in the Material Receipt section. The reports containing the results are available in three forms according to the collection points: online report available on the reference laboratory page, printed report sent by mail to the units and report sent via email. In cases of results without changes, the reports will be released to the patient as a normal result. For patients with sickle cell trait, the results are given together with informational material on hemoglobinopathies. For children with altered outcome for sickle cell disease, is requested to the local of collection (health posts or hospitals) through the Active Search section of IPED-APAE, the retriever of the whole blood test with EDTA to confirm the

result of the neonatal screening. This research was approved by the Committee of Ethics in Research with Human Beings of the Federal University of Mato Grosso do Sul (CEP / UFMS), through the Consubstantiated Opinion 1,469,166. For the analysis of the data obtained in this manuscript was constructed a spreadsheet.

RESULTS

In this study, were found 223 (59.9%) of the 372 samples canceled due to failure in the collection technique , followed by 106 (28.5%) samples with more than 30 days of collection and 43 (11.6%) because of duplicate collection (Table 1).

The year 2015 had the lowest indicator of new sample collection, since of 31 requested samples, 17 (54.84%) requests were left uncollected and 14 (45.16%) were collected with a normal result.

DISCUSSION

For the identification of changes in the newborn the blood samples should be collected correctly. Therefore, it is essential to identify and analyze failures in the collection, in order to promote continuous and ongoing education actions for professionals who perform blood collection on filter paper (Silva et al., 2003).

Table 1. Number of neonatal screening samples canceled due to failure in the collection according to the year and reason for cancellation, Mato Grosso do Sul - from 2011 to 2015

Year	Sample > 30 days of harvesting	Sample taken in duplicate	Sample collection technique failed	Total
2011	45	5	50	100
2012	20	12	58	90
2013	10	4	37	51
2014	6	9	72	87
2015	25	13	6	44
Total	106	43	223	372

Reference: Institute of Research, Teaching and Diagnostics of the Association of Parents and Friends of the Exceptional (IPED-APAE).

Table 2 - Percentage of canceled neonatal screening samples, according to total screening by year and reasons for cancellation of samples, Mato Grosso do Sul, from 2011 to 2015

Year	Total screening	Sample> 30 days of harvest (%)	Sample taken in duplicate (%)	Technical failure in sample collection (%)	Total (%)
2011	36.363	0,12	0,01	0,14	0,28
2012	35.750	0,06	0,03	0,16	0,25
2013	35.816	0,03	0,01	0,10	0,14
2014	36.351	0,02	0,02	0,20	0,24
2015	38.118	0,07	0,03	0,02	0,12

Reference: Institute of Research, Teaching and Diagnostics of the Association of Parents and Friends of the Exceptional (IPED-APAE).

Table 3. Number and percentage of new neonatal screening samples according to the year and collection results, Mato Grosso do Sul - from 2011 to 2015

Year	New sample with normal result		New sample with changed result - FAS		New sample without collection		Total requests	
	Nº.	%	Nº.	%	Nº.	%	Nº.	%
2011	48	50,53	1	1,05	46	48,42	95	28,87
2012	45	57,69	1	1,28	32	41,03	78	23,71
2013	24	51,06	1	2,13	22	46,81	47	14,29
2014	46	58,98	1	1,28	31	39,74	78	23,71
2015	14	45,16	-	-	17	54,84	31	9,42
Total	177	53,80	4	1,22	148	44,98	329	100,00

Reference: Institute of Research, Teaching and Diagnostics of the Association of Parents and Friends of the Exceptional (IPED-APAE).

When analyzing annually the number of canceled samples and the reason for cancellation, highlights the year of 2011 with a total of 100 samples canceled. In 2015, there was a reduction in the number of canceled samples that totaled 44. In the information related to the percentage of canceled samples in relation to total neonatal screening, we highlight the year of 2011 in which there was a higher percentage of return, accounting for 0.28% of total screening. On the other hand, in 2015 there was a reduction of the percentage to 0.12% (Table 2). Table 3 shows the data on the requests and collections of new samples and their results after the cancellation of the first sample. Of the 78 new samples requested, we highlight the year 2014 with the highest proportion of new samples collected in which 46 (58.98%) were identified, with a normal result, one with an altered result for FAS and 31 (39.74%) requests without collection.

The monitoring of the indicators of canceled and summoned samples again, as well as the average time interval between the collection and sending of samples to the laboratory are necessary for the management of the biological neonatal screening activity (Brazil, 2016). Regarding the types of failures found in the collection of neonatal biological screening in the period from 2011 to 2015, out of 182,398 samples from UBS, ESF, hospitals and maternities, only 372 (0.2%) samples were canceled due to failure to collect. On the other hand, 5 59.9% had a cancellation reason for failure in the technique, followed by 28.5% with more than 30 days of harvesting and 11.6% for duplication. Samples that were collected in duplicate did not imply in the request for a new collection. However, these samples characterized a failure in the process because they indicate that children were submitted to the test without checking for the existence of previous collection.

Considering the cancellation of a sample with more than 30 days of harvesting, there are failures in the process of sending samples to the laboratory, which entails a need for a new collection. The storage time and storage conditions of the sample (hemoglobin denaturation) interfere in the laboratory diagnosis of hemoglobinopathies (Ferraz, Murâo, 2007). Such failures in the collection technique (inadequate samples, saturated samples, wet samples, wrong material for examination, insufficient blood samples, sample without biological material, unidentified or unidentified sample) totaled 59.9% of the cancellation reasons in this study. In studies carried out in Brazilian municipality of Sobral, CE, in the year 2012, the neonatal screening tests analyzed showed important observations regarding inadequate samples for analysis, such as insufficient, dilute, dry and precocious samples (MENEZES *et al*., 2014). In the year of 2002 in Paraná, the percentage of 1.25% of the 2,787 tests carried out required new samples due to malpractice and technical errors in collections. The justifications for the highest indexes were insufficient blood and stored for a long time (aged) (SILVA; ZAGONEL; LACERDA, 2003).

In the state of Rio de Janeiro from 2005 to 2007, of the total of 1,721 tests performed in the main referral service in this state, only 0.4% of the samples were considered invalid, and the inadequate ones predominated. This estudy led to the call for new collections and delay in diagnosis, as well as, overload in the Active Search system and decrease of its efficiency (Bloter; Camacho; Cruz, 2011). In fact, these failures may be related to the lack of technical-scientific knowledge, devaluation of the examination by the professional who performed the collection, and exchanges of professionals trained by other non-qualified professionals (Silva; Zagonel; Lacerda, 2003; Menezes *et al*., 2014). A study developed in Tocantins revealed that the constant exchange of employees at the collection points implied the difficulty of training and consequent inadequate sample collection (Mendes; Santos; Bringel, 2013). Samples on filter paper with blood stain less than 8 millimeters, samples in which blood did not completely penetrate the filter paper, and samples with evidence of compression should be rejected because of the risk of producing false-negative results (George; Moat, 2016). Research conducted in other countries such as the UK has shown that the blood volume in the filter paper can substantially affect the measured concentrations of the analyzed analytes in the neonatal screening program and may affect the false positive and negative (Lawson; Bernstone; Hall, 2015).

On the other hand, the results obtained and presented in this manuscript when analyzing the canceled samples as well as those called for new collection, it was verified that 55% of requested samples were collected again between 2011 and 2015. However in this period analyzed, there was an increase in the proportion of first valid samples In a study carried out in the state of Rio de Janeiro (Brazil), from 2005 to 2007, the percentage of valid samples ranged from 99.6% to 99.9%. In the same study, the proportion of attendance for new collection by invalid samples ranged from 71.1% to 81.4% (Bloter; Camacho; Cruz, 2011). If new samples are required, the person responsible for the collection station should carry out an active search, orientation to the family, collection and despatch to the laboratory as soon as possible. It is fundamental that each collection point has special attention to the Active Search of the reconvocated cases.

Among them, positive cases that require urgent care and guidance may be found (Brazil, 2016). The need to repeat the collection due to inadequate sample generates resistance of the relatives and increases the time for the diagnosis and the beginning of the treatment (Mendes; Santos; Bringel, 2013). The professionals at the collection points should be aware of the risk of non-adherence to collections and new collections, as well as considering cultural issues. In addition to considering the difficulty of access to a considerable proportion of Brazilians residing in the border countries Bolivia and Paraguay, but using the basic health services in Brazil (Gazola *et al*., 2011). The limiting factors of this study are related to the need for nominal research in the database for the construction of spreadsheet necessary for statistical treatment. There is also no information in the IPED-APAE database on reasons for non-attendance of children to calls for new collections due to cancellation.

Conclusion

The relevance of the neonatal screening program for the identification of hemoglobin S in live births in the state of Mato Grosso do Sul in Brazil reveals the need for systematic monitoring of screening steps due to the complexity of sickle cell disease. The failures identified in this study are related to the technique of collection, sample with more than 30 days of collected and those in duplicity. From the total of the samples canceled by failures in the collection, it was determined that 55% were collected again. It is important to highlight that the loss of a positive case represents the impossibility of performing the appropriate diagnosis and treatment. The identification and monitoring of failure indicators in neonatal screening are important for the management of the process, since they allow interventions that are suitable for continuous improvement. Results of this study suggest ongoing education actions for health professionals, which lead them to recognize themselves as process actors, essential for standardizing technical procedures.

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