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Original article

Proposal to modify the diagnostic criteria for neurofibromatosis type 1

Proposal of modification of the diagnostic approaches of neurofibromatosis type 1

Proposal for modification of two diagnostic criteria for neurofibromatose type 1

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SUMMARY

Of patients with Legius syndrome, 2% meet the diagnostic criteria for neurofibromatosis type 1, as they present café-au-lait macule and Crowe's sign, making the two diseases indistinguishable. The objective of the study wasevaluate the proposalmodification of the diagnostic criteria ofneurofibromatosis type 1A study was carried out in Las Tunasquasi-

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experimental. Using the Delphi variant of the expert method, the proposal to modify the diagnostic criteria for neurofibromatosis type 1 was designed.samplecorresponded to 119 patients who met the criteria for neurofibromatosis type 1A molecular genetic study for neurofibromatosis type 1 was performed on those who only met the criteria of café-au-lait macules and Crowe's sign.When the molecular test was negative, the diagnosis of Legius syndrome was considered.The proportion of patients was studied according to the manifestations,positivity rate,Fisher's exact test and Yates' adjusted correction with$p \leq 0.05$ andTo determine the degree of agreement between current criteria and the modified criteria, Cohen's Kappa index was determined. Most patients who only presented café-au-lait macules associated with Crowe's sign without other manifestations, were negative in the molecular genetic study for neurofibromatosis 1, and were diagnosed with Legius syndrome.In evaluating the effectiveness of the proposed modified criteria it was determinedthat allowmake the diagnosis of neurofibromatosis type 1 with 95% reliability,and the diagnostic error with Legius syndrome is avoided.

Keywords: Genodermatosis; Medical Genetics; Neurofibromatosis; Legius Syndrome.

ABSTRACT

Of the patients with Legius syndrome, 2% complete the diagnostic approaches of neurofibromatosis type since 1 they present brown stain with milk and Crowe's sign, this makes the two diseases indistinguishable. The objective of the study was to evaluate the proposal of modification of the diagnostic approaches of neurofibromatosis type 1. It was carried out in The Tunas, a quasi-experimental study. By means of the varying Delphi of the method of experts, the proposal of modification of the diagnostic approaches of the neurofibromatosis type 1 were designed. The sample corresponded with 119 patients that completed the approaches of the neurofibromatosis type 1. It was carried out molecular genetic study for neurofibromatosis type 1 to those that completed alone with the brown presence of stains with milk and sign of Crowe. When the molecular exam was negative, it

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was considered the diagnosis of Legius syndrome. Proportion was studied of patient according to the manifestations, positivity index, exact test of Fisher and adjusted correction of Yachts withp≤0.05and to determine the agreement degree between current approaches and the modified approaches the Kappa index of Cohen it was determined. Most of the patients that alone presented brown stains with milk associated with the sign of Crowe without other manifestations, they were negative to the molecular genetic study for neurofibromatosis 1, they were diagnosed as Legius syndrome. When evaluating the effectiveness of the proposed modified approaches it was determined that they allow to carry out the diagnosis of neurofibromatosis type 1 with 95% of dependability, avoiding the error diagnoses with the Legius syndrome.

Keywords: Genodermatoses; Medical genetics; Neurofibromatosis;Legius syndrome.

SUMMARY

Two patients with Legius syndrome, 2% preenchem the diagnostic criteria for neurofibromatose type 1, pois apresentam brown macula com leite e sinal de Crowe, o que tornas duas doenças indistinguishable. The objective of the study was to evaluate the proposed modification of two diagnostic criteria for neurofibromatose type 1. A quase-experimental study was carried out in Las Tunas. Using the Delphi variant of the specialist method, two diagnostic criteria for neurofibromatose type 1 were developed in order to modify them. The sample corresponds to 119 patients who met the criteria for neurofibromatose type 1. The molecular genetic study for neurofibromatose type 1 was carried out in groups that only attended to the presence of coffee-and-leite and sinal spots. by Crowe. When the molecular test was negative, a diagnosis of Legius syndrome was considered.The proportion of patients in agreement with the manifestations, positivity index, Fisher exact test and Yates correlation adjusted with p≤0.05 were studied, to determine the degree of agreement between the current criteria and the modified criteria, determined by the Cohen's Kappa index. Most of the patients who presented only brown and milky macules

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associated with Crowe's sinus without other manifestations, were negative for the molecular genetic study for neurofibromatose 1, foram diagnosed as Legius syndrome.To validate the effectiveness of two proposed modified criteria, it was determined that they allow the diagnosis of neurofibromatose type 1 with 95% reliability, and misdiagnosis with Legius syndrome was avoided.

Keywords: Genodermatose; Medical Genetics; Neurofibromatose; Legius syndrome.

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Introduction

Rasopathies are a group of syndromes caused by germline mutations in genes that code for proteins involved in the same metabolic pathway called RAS-MAPK (mitogen activated protein kinase).[1)]Germline mutations cause developmental abnormalities in individuals that, while specific to the affected gene, often overlap clinically. These conditions include Noonan syndrome, LEOPARD syndrome, Costello syndrome, cardio-facio-cutaneous syndrome, neurofibromatosis type 1 (NF1), and Legius syndrome. The most common dermatological findings can be divided into pigmented lesions such ascafé au lait stain (MCCL), lentigines and melanocytic lesions; ectodermal lesions and hyperplastic lesions. (1) To distinguish one rasopathy from another, it is necessary to gather the specific clinical diagnostic elements of each one.

NF1 (OMIM162200, ORPHA636, GARD7866) is transmitted with an autosomal dominant inheritance pattern caused by a mutation in the tumor suppressor gene neurofibrin 1 with 61 exons located in the precentromeric region of the long arm of chromosome 17q11.2

and rarely by microdeletion 17q11 (only 5%) which has the highest rate of spontaneous mutations in the entire genome. The neurofibrin 1 gene encodes a protein called neurofibromin that participates in the control of cell growth and differentiation. (2-4)

It has been observed that it affects one in every 3000 individuals, 50% of cases present neomutations, penetrance is 100% with variable expressivity, (5,6) it has been described in many ethnic groups and affects both sexes equally. (7)In CubaFew population studies have been conducted on the disease. Authors such as Orraca, who performed an epidemiological, clinical, and genetic characterization of NF 1, found that the prevalence was 1:1141 in pediatric patients, a result that was higher than the international rates described for this disease. (8)In Las Tunas, genodermatoses diagnosed in the period 1989-2018 were studied, and a predominance of NF1 was found with 30.17% of cases and a prevalence rate of13.6:100,000 inhabitants. (9,10)This suggests that although the disease is not recorded in the Cuban national statistical yearbook, its presence in the Cuban population has been demonstrated.

The diagnostic criteria were established in 1987; to establish the diagnosis, two or more of the following criteria must be met:(11)

1. Six either further MACCL of 5 mm in prepubertal patients and older people of 15 mm in postpubescent.
2. Two either further neurofibromas either a neurofibroma plexiform.
3. Sign of Crowe (ephelides axillary).
4. Glioma of the nerve optical.
5. Two either further nodules of Lish.
6. Injuries bony typical (Dysplasia of the at sphenoid or weight loss cortical of bones long with either without pseudoarthrosis).
7. Background family pathological disorders (APF) of NF1 in parents either siblings.

Criteria 4 through 6 require specialized diagnostic tests, such as computed tomography (CT) or magnetic resonance imaging (MRI), slit-lamp visualization of retinal hamartomas, and radiography of the lesion site, respectively. The remaining criteria are clinical.

In 2007, Bremsdescribed,In patients diagnosed with NF1-like, a heterozygous mutation in the SPRED1 geneLocated on chromosome 15q14, it involves loss of function of one of the proteins involved in the pathogenic RAS-MAPK pathway, similar to neurofibromin, and therefore shows clinical similarities with NF1, but with less severity. (12-14) It was considered to be another syndrome. To clearly differentiate these two disorders, at the 13th European Meeting on Neurofibromatosis in 2008, this new syndrome was designated "Legius syndrome" (OMIM611431, ORPHA137605, GARD10714). (12)

Legius syndrome is often clinically indistinguishable from NF1 and occurs in approximately 2% of patients who meet the diagnostic criteria for NF1, (14) although no international studies inferring its prevalence have been reported to date.

Several authors have advocated for the need to modify the diagnostic criteria for NF1. In 2021, Legius et al., using the Delphi variant of the expert method, (15) conducted a review in which they proposed that in individuals without APF of NF1, in addition to meeting two criteria, a heterozygous pathogenic NF1 gene variant with a proportion of the mutated allele of more than 50% in apparently normal tissue such as white blood cells should be identified; and in those descended from a parent who meets the diagnostic criteria for NF, there is no need to identify the mutated gene.

In Las Tunas,eastern province of Cuba,As part of a methodology for the care of patients with genodermatosis, the proposal to modify the diagnostic criteria for NF1 was included. (16) The objective of this research was to evaluate the proposal ofmodification of the diagnostic criteria ofNF1.

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Methods

Type of study, universe and sample

A quasi-experimental study was conducted in Las Tunas from 2019 to 2021.Thesamplecorresponded to 119 patients who met the criteria for neurofibromatosis type 1.

It is aapplied the Delphi variant of the expert method,A group of Cuban specialists in dermatology, clinical medical genetics and pediatrics, with a high scientific level and experience in working with patients with NF1, were consulted.The proposal to modify the diagnostic criteria for this disease was designed. (17)

Proposal for modified diagnostic criteria for NF1, unifying the first and third criteria, as follows:

1. Six either further stains coffee with milk of 5 mm in prepubertal patients and older people of 15 mm in postpubescent and/or presence of the Sign of Crowe (ephelides axillary and/or inguinal).
2. Two either further neurofibromas either a neurofibroma plexiform.
3. Glioma of the nerve optical.
4. Two either further nodules of Lish.
5. Injuries bony typical (Dysplasia of the at sphenoid or weight loss cortical of bones long with either without pseudoarthrosis).
6. NF APF in parents or siblings (who meet the modified criteria).

For diagnosis, 2 or more criteria must be met.

In the implementation stage, training courses and workshops were given to the personnel who would participate in the research andproceeded to implement the proposal for modified diagnostic criteria for NF1.

In the evaluation stage

The age at which signs of NF1 have been described to appear was taken into consideration, and it was decided to use 10 years as the reference age for evaluating suspected Legius syndrome.Molecular genetic testing for NF1 was performed on those who only met the criteria for the presence of café-au-lait spots and Crowe's sign.. The result of the molecular genetic study by indirect method using five markers: four microsatellites (IVS27AAAT2.1, IVS38GT53.0, IV27AC28.4 and Mfd15) and a restriction fragment length polymorphism (Rsa I NF1 exon 5), which was performed on the patient and both parents of those patients older than 10 years, who presentedMACCLand/or Crowe's sign, without other clinical elements.

Epidemiological measurement methods were carried out given byproportion of patients according to diagnostic manifestations andthe positivity index (PI) in thosewithMACCLand/or presence of ephelides in the axillary or inguinal region that met the diagnostic criteria ofNF1. The modified diagnostic criteria were considered to be met and therefore a diagnosis of NF1 was made when theMolecular study for NF1 corroborated this. A diagnosis of Legius syndrome was considered when the molecular study for NF1 was negative..

To determine the effectiveness between the proposed criteria and the results found, the inferential statistical measurement method was used.by Fisher's exact test (F) and Yates' adjusted correction (X2Y). It was considered highly significant, and therefore,accepted a relationship between the proposed criteria and the diagnosis of NF1, when $p \leq 0.05$ for 95% confidence interval. The results have been presented in 2 x 2 contingency tables and bar and pie charts.

To determine the degree of agreement between patients diagnosed with NF1 according to current criteria and the modified criteria, Cohen's Kappa index (KC) was determined, which was interpreted as follows (Landis and Koch):

< 0.20: very low agreement.

0.20 -0.40: Poor agreement.

0.40 - 0.60: Moderate agreement.

0.60 - 0.80: Good agreement.

> 0.80: Very good agreement.

With a significance index of P≤ 0.05

H0: no agreement exists.

HA: there is agreement and coincidence.

Ethical considerations: The research protocol was submitted for approval by the Research Ethics Committee and the Scientific Council of the Mártires de Las Tunas Provincial Teaching Pediatric Hospital as the implementing institution, in accordance with the principles of the Declaration of Helsinki. (18) For participation in the study, written informed consent was obtained from the patients and from the parents and guardians of minors, which was given to all patients seen in the consultation. The form explained the importance of participating, the risks, benefits, rights of the participants and the characteristics of the research. The data were coded to protect the identity of the patients.

Results

When studying the clinical criteria, (Chart 1)In the 119 patients studied who met the diagnostic criteria for NF1, the most frequent manifestations were MCCL in all cases, and Lisch nodules and Crowe's sign in 95.76% and 94.29% of patients, respectively. Of these, 4.24% presented only MCCL and Crowe's sign without other manifestations.

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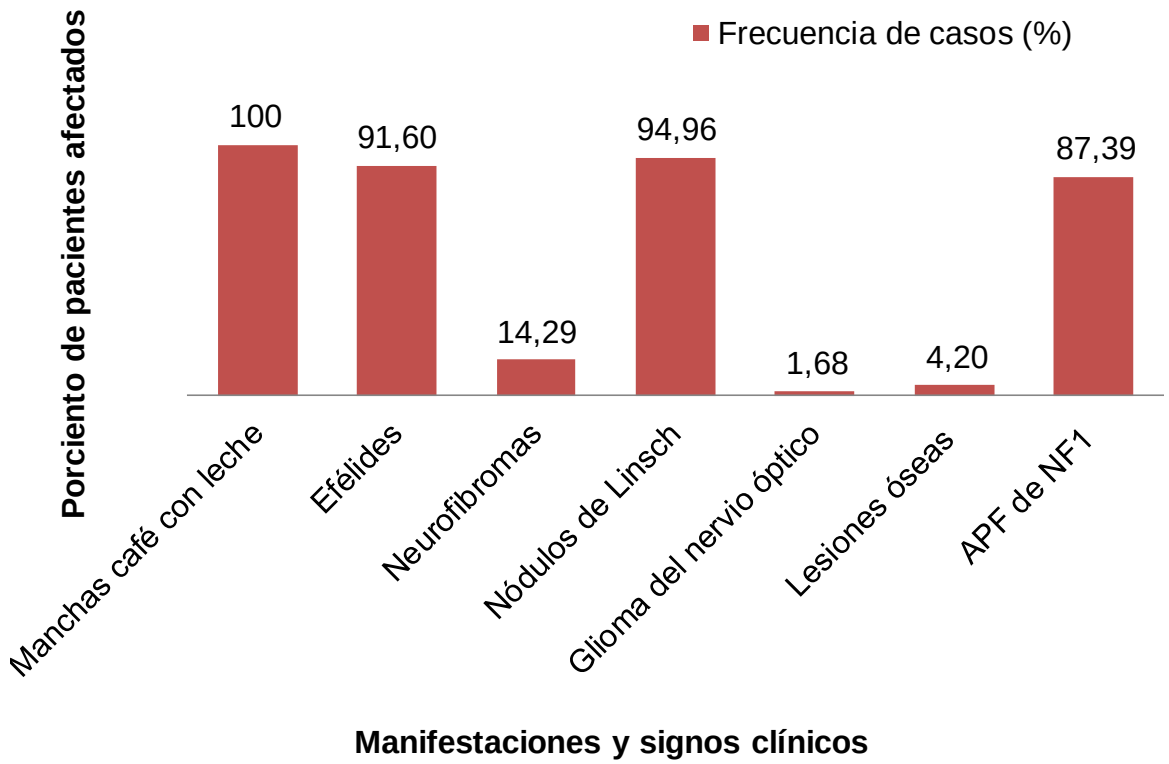

Chart 1. Distribution of clinical criteria in patients with NF1.

Five patients underwent molecular testing (Table 1), with negative results for four, with a PI of 20%. This supports the diagnosis of Legius syndrome in these patients.

Table 1. Evaluation of the molecular diagnosis of NF1.

Cases with MCCL and Crowe's sign	No.	IP
Positive to molecular study	1	20%
Negative to molecular study	4	80%

Some cases that met the diagnostic criteria for NF1 were diagnosed with Legius syndrome (Figure 2). The case that tested positive was interpreted as having low-grade NF1. Legius syndrome was diagnosed in 3.36% of cases that met the diagnostic criteria for NF1.

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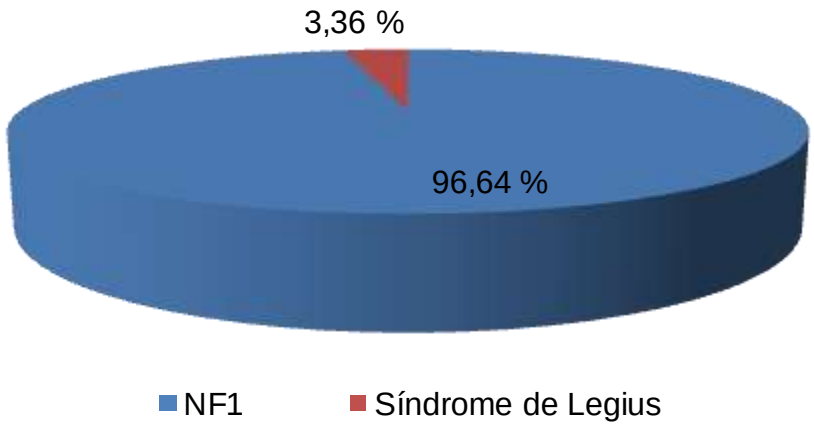

Chart 2. Clinical and molecular diagnosis of patients who meet the diagnostic criteria for NF1.

When evaluating the effectiveness of the modified criteria (Table 2), F=94.37218 was determined with P=0.000001 and X2Y=71.3511 with P=0.000000, so it can be stated that there is effectiveness with 95% statistical significance.

Table 2. Evaluation of thee ffectiveness of the modified NF1 criteria.

	NF with 3 criteria	**Stains and ephelides**	**Total**
They met the proposed criteria	114	0	114
They did not meet the criteria	1	4	5
Total	115	4	119

When evaluating the agreement (Table 3) KC=0.88 was determined.

Table 3. Concordance between both methods for the diagnosis of NF1.

	Current criteria		**Total**
	NF with 3 criteria	**Stains and**	

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			ephelides	
Modified criteria	**NF with 3 criteria**	114	0	114
		95.80	0	
	Stains and ephelides	1	4	5
		0.84	3.36	
Total		115	4	119

Discussion

It was observed that the most outstanding clinical criteria coincide with those studied by Orraca, in which the following predominated:MACCLin 100% of patients, followed by ephelides with 74.4% of cases;(8) in Duat's study, a predominance ofMACCL (99.6%)and the ephelides (93.7%);(16) In the study ofRivera, hyperchromic macules are described in all cases, and ephelides in the axillary and inguinal region as the second most frequent sign in 72% in a group of 5-10 years of age. (7)

In the investigation ofSánchez, the most frequent diagnostic criteria for NF1 in order were: spotsMACCL(100%) described in the first year, ephelides (60.16%) between 3-5 years, first degree relative affected (35.9%), cutaneous neurofibromas (21%) between 8-10 years, Lisch nodules (20.3%) between 6-8 years, optic pathway gliomas (17.18%) between 2-6 years, plexiform neurofibromas (9.3%) between 3-5 years of age, raised that the diagnostic criteria appear progressively with age, which makes clinical diagnosis difficult at early ages. (19)

The severity of clinical manifestations in NF1 varies widely, even within families, and is age-dependent. Diagnostic criteria are quite sensitive and specific in adults, but less so in children under 8 years of age. (6)In NF1 theMACCLThey are the first sign, appear from birth or the first months and usually increase in number during childhood, axillary and inguinal freckles are observed between the second and sixth year of life, neurofibromas can generally appear after puberty, superficial plexiform neurofibroma is usually congeni-

tal. (5) Linsch nodules and optic nerve gliomas have been described between five to six years of age. (6)

García et al. (20) suggest that the presence of café-au-lait spots and ephelides, by themselves, are insufficient criteria for diagnosing NF1.

Patients with Legius syndrome present multipleMACCLand Crowe's sign, dysmorphic features, lipomas in adulthood and learning disorders unrelated to the appearance of neurofibromas, optic gliomas, Lisch nodules or tumor predisposition, (12)that may meet the diagnostic criteria for NF1, which is why it is necessary to propose modifying the diagnostic criteria for NF1 by unifying the criteria for the presence ofMACCLand Crowe's sign, in a single criterion. (21)

Although next-generation sequencing is the most robust molecular technique, it is unattainable for many molecular biology laboratories, but the wide range of molecular techniques allows for the molecular study of genetic diseases, as well as contributing to better genetic counseling for patients and their families. (22)In Cuba there are no molecular studies for the diagnosis of Legius syndrome, so it became necessary to diagnose NF1 through molecular study byindirect method with five markers: four microsatellites (IVS27AAAT2.1, IVS38GT53.0, IV27AC28.4 and Mfd15) and one restriction fragment length polymorphism (Rsa I NF1 exon 5); (8)and thus determine that those patients with a negative molecular study for NF1 had Legius syndrome.

Most patients who only presented café-au-lait macules associated with Crowe's sign without other manifestations, were negative in the molecular genetic study for NF1, and were diagnosed with Legius syndrome (80%).In other investigations, they also diagnosed Legius syndrome by decantation, after performing the genetic study of NF1.

In Duat's study,The direct study was carried out by means of a mutational screening of NF1 cDNA with RNA techniques (cDNA-DHPLC denaturing high performance liquid chromatography) combined with techniques based on MLPA (multiplex ligation-dependent probe amplification) with a sensitivity of 95%, (18) and in the research of SánchezIn a patient with

spots and/or ephelides, a molecular genetic study for NF1 was performed, and only those who were negative for NF1 were further studied for Legius syndrome. (19)

Legius syndrome is clinically indistinguishable from NF1 and occurs in approximately 2% of patients who meet the diagnostic criteria for NF1. (14) However, there are authors who propose higher percentages such as Duat who obtained 3.61%;(18) Sanchez obtained 2.3%;(19) andEvans establishes an even higher prevalence of around 8%. (22)

When concordance is determined KC> 0.80, it is proposed that there is concordance between the modified diagnostic criteria and the current criteria and, therefore, with both methods the diagnosis of NF1 can be made with 95% reliability.

No studies were found where diagnostic criteria were modified to allow comparisons.A small percentage of patients diagnosed with NF1 may have a misdiagnosis and be suffering from Legius syndrome, making it necessary to modify the diagnostic criteria for NF1.

Conclusions

A group of patients with MCCL and Crowe's sign as the only manifestations and negative molecular testing for NF1 were diagnosed with Legius syndrome. The modified criteria allow a diagnosis of NF1 with 95% confidence.avoids diagnostic error with Legius syndrome.

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Conflict of interest

The authors declare no conflicts of interest in the conduct of this study. All photographs were taken by the research team and obtained with informed consent.

Authorship contribution

Conceptualization:Yordania Velázquez Avila;Teresa Petra Rodríguez Torres.

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Methodologies:Yordania Velázquez Avila.

Original draft:Yordania Velázquez Avila;Yasaris López Toranzo.

Writing-reviewing and editing:Yordania Velázquez Avila; Teresa Petra Rodríguez Torres;Lisdany Rodríguez Carbonell; Orendys Feria Rosales;Yasaris López Toranzo.