Choroidal Abnormalities Detected by Near-Infrared Reflectance Imaging as a New Diagnostic Criterion for Neurofibromatosis 1

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Objective: To investigate in a large sample of consecutive patients with neurofibromatosis type 1 (NF1) the possibility of including the presence of choroidal abnormalities detected by near-infrared reflectance (NIR) as a new diagnostic criterion for NF1.

Design: Cross-sectional evaluation of a diagnostic test.

Participants and Controls: Ninety-five consecutive adult and pediatric patients (190 eyes) with NF1, diagnosed based on the National Institutes of Health (NIH) criteria. Controls included 100 healthy age- and gender-matched control subjects.

Methods: Confocal scanning laser ophthalmoscopy was performed for each subject, investigating the presence and the number of choroidal abnormalities.

Main Outcome Measures: Sensitivity, specificity, and diagnostic accuracy for the different cutoff values of the criterion *choroidal nodules detected by NIR* compared with the NIH criteria.

Results: Choroidal nodules detected by NIR imaging were present in 79 (82%) of 95 of the NF1 patients, including 15 (71%) of the 21 NF1 pediatric patients. Similar abnormalities were present in 7 (7%) of 100 healthy subjects, including 2 (8%) of the 25 healthy pediatric subjects. The highest accuracy was obtained at the cutoff value of 1.5 choroidal nodules detected by NIR imagery. Sensitivity and specificity of the examination at the optimal cutoff point were 83% and 96%, respectively. Diagnostic accuracy was 90% in the overall population and 83% in the pediatric population. Both of these values were in line with the most common NIH diagnostic criteria.

Conclusions: Choroidal abnormalities appearing as bright patchy nodules detected by NIR imaging frequently occurred in NF1 patients. The present study shows that NIR examination to detect choroidal involvement should be considered as a new diagnostic criterion for NF1.

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Neurofibromatosis type 1 (NF1) is an autosomal dominant disorder with an estimated incidence of 1 in every 3000 newborns.[1] A minimum of 2 of the following criteria are required for the diagnosis: 6 or more café au lait spots, axillary or inguinal freckling, 2 or more cutaneous neurofibromas, 1 plexiform neurofibroma, distinctive osseous lesions (e.g., pseudarthrosis, sphenoid wing hypoplasia), optic glioma, 2 or more iris Lisch nodules, and a first-degree relative with NF1.[2]

Choroidal neurofibromatosis, as described in postmortem examinations,[3–8] is characterized by ovoid bodies consisting of proliferating neoplastic Schwann cells that are arranged in concentric rings around axons. There are only a few reports of choroidal abnormalities in clinical studies. Recently in 2 small series, confocal near-infrared reflectance (NIR) imaging provided superior visibility of NF1-related pathologic features in the choroid.[9,10] Choroidal abnormalities, undetectable with conventional ophthalmoscopic examination or in fluorescein angiograms, appeared as bright, patchy nodules. Indocyanine green angiography, at a wavelength similar to that of NIR, also is helpful in recording fundus alterations associated with NF1, but it is an invasive diagnostic tool.[11] The advantage of NIR imagery for recording pathologic features underneath the retinal pigment epithelium may be the result of the good transmission of long-wavelength light through melanin and lipofuscin.[12] The purpose of this study was to investigate, in a large sample of consecutive NF1 patients, the possibility of including the presence of choroidal abnormalities detected by NIR as a potentially new diagnostic criterion for NF1.

Patients and Methods

All patients and subjects (or their parents if they were children) gave their informed consent, and the study was conducted in accordance with the tenets of the Declaration of Helsinki. The protocol of this study was approved by the Institutional Review Board of Fondazione IRCCS Cà Granda Ospedale Maggiore Policlinico.

Between September 2008 and October 2009, 95 white patients (190 eyes) with NF1 based on the stringent National Institutes of Health (NIH) diagnostic criteria were examined.[2] Also, 100 age-,

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Table 1. Demographic and Ocular Characteristics of Patients

	Neurofibromatosis Type 1*	Controls	Neurofibromatosis Type 1 (≤12 yrs)	Controls (≤12 yrs)
Women/men	40/55	48/52	11/10	12/13
Mean age±SD (yrs)	28±16	30±18	7±2	8±2
Range	3–68	4–72	3–12	4–12
Iris color				
Bright	25/95 (26%)	28/100 (28%)	6/21 (29%)	7/25 (28%)
Dark brown	70/95 (74%)	72/100 (72%)	15/21 (71%)	18/25 (72%)
Refractive error				
Myopia	23/95 (24%)	16/100 (16%)	2/21 (9%)	2/25 (8%)
Hypermetropia	10/95 (11%)	10/100 (10%)	4/21 (19%)	5/25 (20%)
Astigmatism	19/95 (20%)	21/100 (21%)	2/21 (11%)	2/25 (8%)

SD = standard deviation.
*Includes adult and pediatric cases.

gender-, and race-matched healthy control subjects were examined. Patients with NF1 were referred to the Eye Clinic of Fondazione IRCCS Cà Granda Ospedale Maggiore Policlinico of Milan from the Medical Genetic Unit and the Pediatric Department. Patients for whom the fundus could not be examined because of media opacity were excluded. Each subject underwent a best-corrected visual acuity evaluation, general ophthalmologic examination, including assessment for iris hamartomas (Lisch nodules), and mydriatic indirect fundus biomicroscopy with a 90-diopter (D) lens.

Near-infrared reflectance at 815 nm, autofluorescence (AF) at 488-nm excitation and a barrier filter of 500 nm, and red-free (RF) 488-nm images of the posterior pole and mid-periphery of the retina were recorded routinely with a confocal scanning laser ophthalmoscope (cSLO; HRAII or Spectralis HRA+OCT, Heidelberg Engineering, Heidelberg, Germany).[13] The same operator (GB) used different camera objectives to provide 30° and 50° or more angle field-of-views. With confocal image acquisition, light from a conjugate plane of interest was detected by the image sensor. This permitted suppression of light from planes anterior and posterior to the plane of interest and resulted in high-contrast fundus images. The image resolution was 768×768 pixels. The Spectralis HRA+OCT was used when available in the authors' clinic after January 2009. The combined cSLO/optical coherence tomography (OCT) system allowed for simultaneous recording with precise alignment of OCT images and topographic images, including NIR, AF, and RF, as previously described in detail.[13] The OCT cross-section was placed precisely over the area of interest to obtain a correlation of the tomographic images to morphologic changes in the retina and choroid.

Using automated eye tracking and image alignment based on cSLO images, the software allowed averaging a variable number of single images in real time (ART [Automatic Real Time] Module; Heidelberg Engineering, Heidelberg, Germany). Choroidal abnormalities defined as bright, patchy nodules by NIR[9,10] were quantified in the entire detectable fundus oculi by means of a 50° lens. To study the topographic distribution of the choroidal abnormalities, the ocular fundus was divided into 5 areas, as suggested by Nakakura et al.[10] Nodules on the border between the regions were assigned to the region containing the greater part of the lesion. All of the images were analyzed in a blind manner by the same investigator (D.V.), who looked for choroidal abnormalities and any other unusual findings. The same investigator and another independent investigator (C.M.) repeated the analysis to obtain data for, respectively, intraobserver and interobserver agreement evaluation.

Data regarding refractive errors, race, and iris color that could affect the amount of melanin in the choroid, and consequently the detection of bright, patchy nodules by NIR, also were noted. Myopia was defined as the spherical equivalent refraction of at least −0.50 D, hyperopia as the spherical equivalent refraction of at least 2.0 D, and astigmatism as the cylinder of at least 1.0 D.

Statistical Analysis

Frequencies of choroidal abnormalities detected by NIR were compared between patients and control subjects by the Fisher exact test. Intraobserver and interobserver agreement over image scores was calculated using the κ statistic, where agreement was defined according to the guidelines proposed by Landis and Koch.[14,15]

The median test was used to verify the association between the presence of choroidal nodules detected by NIR and the number of NIH diagnostic criteria. A receiver operating characteristic curve

Table 2. Frequency of National Institute of Health Diagnostic Criteria

	Neurofibromatosis Type 1*	Controls	Neurofibromatosis Type 1 (≤12 yrs)	Controls (≤12 yrs)
Café au lait macules	95/95 (100%)	6/100 (6%)	21/21 (100%)	2/25 (8%)
Neurofibromas	67/95 (70%)	0/100 (0%)	3/21 (14%)	0/25 (0%)
Freckling	76/95 (80%)	0/100 (0%)	20/21 (95%)	0/25 (0%)
Lisch nodules	68/95 (72%)	0/100 (0%)	9/21 (43%)	0/25 (0%)
Optic glioma	6/95 (6%)	1/100 (1%)	1/21 (5%)	0/25 (0%)
Distinctive osseus lesion	2/95 (2%)	NA	0/21 (0%)	NA
First-degree relative affected	46/95 (48%)	1/100 (1%)	9/21 (43%)	0/25 (0%)

NA = not applicable.
*Includes adult and pediatric cases.

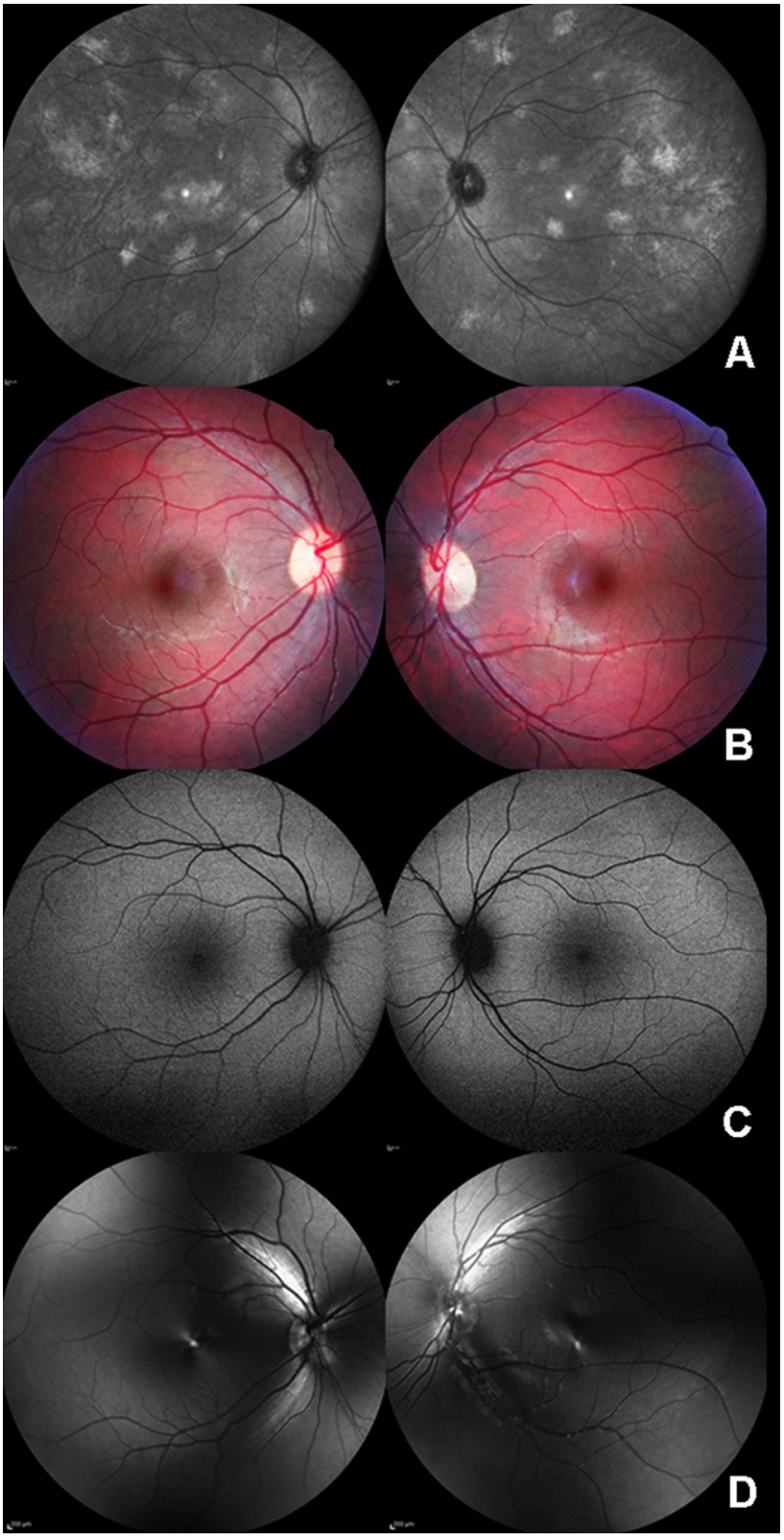

Figure 1. Fundus oculi of a 44-year-old man with neurofibromatosis type 1. **A**, Both eyes have several bright choroidal nodules that were detected by near-infrared reflectance. No abnormalities were recognizable by (**B**) conventional fundus examination, (**C**) autofluorescence, and (**D**) red-free imaging.

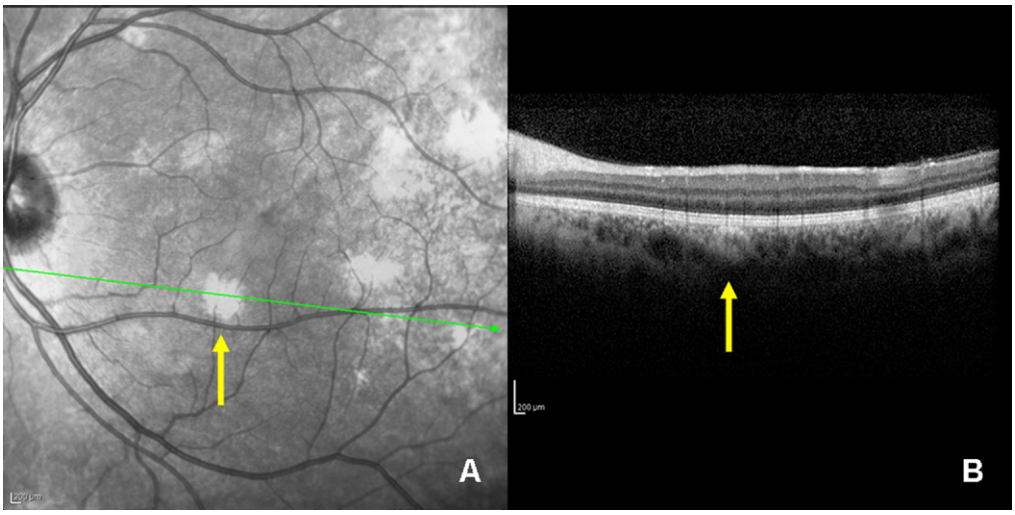

Figure 2. Choroidal nodules detected by ocular coherence tomography (OCT). **A**, In the same subject shown in Figure 1, near-infrared reflectance reflectance of the left eye shows bright choroidal nodules. The OCT cross-sectional image was obtained along the green line. **B**, The corresponding OCT scan shows that these lesions are located within the choroidal tissue.

Table 3. Frequency of Choroidal Nodules

Detection Method	Neurofibromatosis Type 1*	Controls	Neurofibromatosis Type 1 (≤12 yrs)	Controls (≤12 yrs)
AF	0/95 (0%)	0/100 (0%)	0/21 (0%)	0/25 (0%)
RF	5/95 (5%)	4/100 (4%)	2/21 (9%)	2/25 (8%)
NIR†	79/95 (82%)	7/100 (7%)	15/21 (71%)	2/25 (8%)

AF = autofluorescence at 488-nm excitation; NIR = near-infrared reflectance at 815 nm; RF = red-free imaging at 488-nm excitation.
*Includes adult and pediatric cases.
†$P<0.001$ by Fisher exact test for neurofibromatosis type 1 vs. controls and neurofibromatosis type 1 vs. controls 12 years of age or younger. $P<0.001$ for neurofibromatosis type 1 detection of nodules in NIR vs. RF and NIR vs. AF. $P<0.001$ for neurofibromatosis type 1 (≤12 yrs) detection of nodules in NIR vs. RF and NIR vs. AF.

Table 4. Patients Grouped by Number of Choroidal Nodules

Choroidal Nodule Threshold	Neurofibromatosis Type 1	Controls	Neurofibromatosis Type 1 (≤12 yrs)	Controls (≤12 yrs)
0	16/95 (18%)	93/100 (93%)	6/21 (29%)	23/25 (92%)
≥1	79/95 (82%)	7/100 (7%)	15/21 (71%)	2/25 (8%)
≥2	79/95 (82%)	4/100 (4%)	15/21 (71%)	2/25 (8%)
≥3	76/95 (80%)	2/100 (2%)	13/21 (62%)	0/25 (0%)
≥4	76/95 (80%)	2/100 (2%)	13/21 (62%)	0/25 (0%)
≥5	74/95 (78%)	2/100 (2%)	12/21 (57%)	0/25 (0%)
≥6	71/95 (75%)	1/100 (1%)	11/21 (52%)	0/25 (0%)

was constructed to show the variability of sensitivity and specificity for cutoff values for the number of choroidal nodules detected by NIR. The area under the receiver operating characteristic curve was selected as the global index of diagnostic accuracy. The optimal cutoff point was selected based on the highest accuracy.

The accuracy of the new criteria were compared with those of each NIH criterion. The correlations between the number of fundus areas involved, the age, and the number of positive NIH criteria were studied by Spearman linear correlation index. A subgroup analysis was performed on pediatric patients, 12 years old or younger. Data were expressed as mean±standard deviation (range) and as ratio (percentage) for prevalence data. P values of 0.05 or less were considered statistically significant.

Results

There were no significant differences between NF1 and control subjects regarding the prevalence of ocular characteristics such as iris color and refraction (Table 1) or best-corrected visual acuity (data not shown). Lisch nodules were detected by slit-lamp exam-

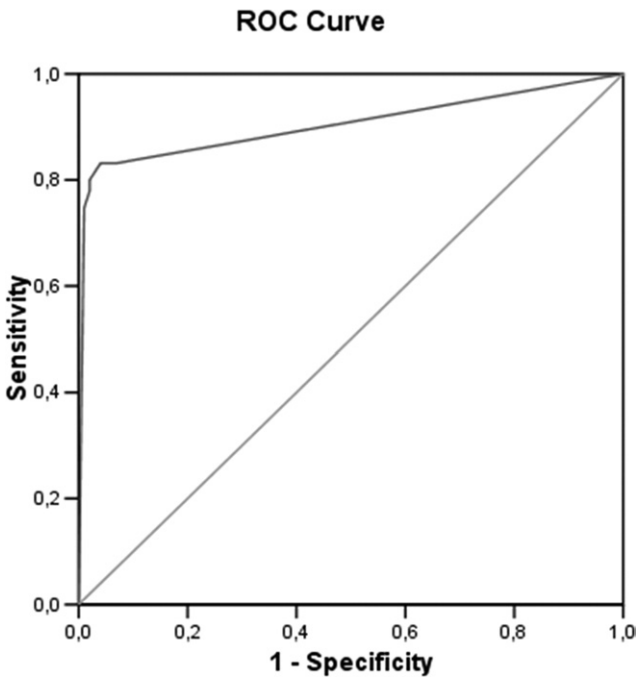

Figure 3. Receiver operating characteristic curve (ROC) at different cutoff numbers of choroidal nodules detected by near-infrared reflectance.

ination in 68 (72%) of 95 NF1 subjects and in 9 (43%) of 21 pediatric NF1 subjects (Table 2).

Bright, patchy choroidal nodules were detected by NIR (Fig 1) in 79 (82%) NF1 patients, including 15 pediatric patients (71%). Conventional fundus examination, including biomicroscopic examination and fundus colour photography, did not show any remarkable changes (Fig 1), except in 8 subjects (3 NF1s and 5 controls) with a choroidal nevus in 1 eye. In OCT examinations, the choroidal abnormalities detected by NIR were observed as irregular hyperreflective foci located under the retinal pigment epithelium (Fig 2).

Twenty of 27 patients without Lisch nodules showed choroidal nodules detected by NIR, and 8 of 16 patients without choroidal nodules detected by NIR showed Lisch nodules. There was no significant correlation between the Lisch nodules and the choroidal nodules ($P>0.05$, Spearman correlation index). Differences in the frequencies of choroidal nodules detected by NIR between the entire NF1 subject group and the control group and between the pediatric NF1 subgroup and controls were significant ($P<0.001$ each, Fisher exact test; Table 3). However, there were no differences between groups in the frequencies of choroidal nodules detected by AF or RF. Within the NF1 and NF1 pediatric groups, NIR detected significantly more nodules than did AF or RF ($P<0.001$; Table 3). There was no significant difference in the proportion of patients with choroidal nodules detected by NIR in relation to the number of NIH diagnostic criteria ($P>0.05$, chi square test).

Table 6. Diagnostic Criteria Accuracy

	Overall Population	Pediatric Population (≤12 yrs)
Café au lait macules	0.98	0.96
Neurofibromas	0.86	0.61
Freckling	0.90	0.98
Lisch nodules	0.86	0.74
Optic glioma	0.54	0.56
Distinctive osseus lesion	0.52	0.54
First degree relative affected	0.74	0.74
Choroidal nodules detected by NIR	0.90	0.83

NIR = near-infrared reflectance at 815 nm.

Using the number of choroidal nodules as threshold values (Table 4), receiver operating characteristic curves were constructed (Fig 3) to determine the sensitivity and specificity of nodules as diagnostic indicators of NF1. The highest accuracy was obtained at the cutoff value of 1.5 choroidal nodules detected by NIR. Sensitivity and specificity of the examination at the optimal cutoff point were 83% and 96%, respectively (Table 5). The area under the receiver operating characteristic curve was 0.904 (95% asymptotic confidence interval, 0.856–0.952). For the general population, the accuracy of NIR detection of choroidal nodules, 0.90, was somewhat less than that of café au lait macules, the same as that of freckling, and slightly more than that of neurofibromas (Table 6). For the pediatric population, the accuracy was 0.83, which was less than that of freckling and café au lait macules, but more than that of Lisch nodules and of having a first-degree relative affected.

Choroidal abnormalities occurred in all of the regions of the fundus, but were more frequent at the posterior pole ($P<0.001$, chi-square test and Fisher exact test for the NF1 overall population and the NF1 pediatric patients, respectively; Table 7). There was a statistically significant correlation between patient age and the number of involved fundus areas (Spearman $r = 0.62$; $P<0.001$).

The number of choroidal nodules detected by NIR and the number of involved areas showed an almost a perfect intraobserver ($\kappa = 0.948$ and $\kappa = 0.970$, respectively) and interobserver ($\kappa = 0.857$ and $\kappa = 0.953$, respectively) agreement.

Discussion

The frequency of each NF1 clinical manifestation observed in this study was similar to that reported previ-

Table 5. Sensitivity and Specificity of Choroidal Abnormality Cutoff Numbers

Cutoff Value	Sensitivity	1–Specificity	Specificity	Sensitivity–(1–Specificity)
0.50	0.832	0.070	0.930	0.762
1.50	**0.832**	**0.040**	**0.960**	**0.792**
3.00	0.800	0.020	0.980	0.780
4.50	0.779	0.020	0.980	0.759
5.50	0.747	0.010	0.990	0.737

Boldface values indicate the highest accuracy.

Table 7. Topographic Distribution of Choroidal Abnormalities

	Neurofibromatosis Type 1	Controls	Neurofibromatosis Type 1 (≤12 yrs)	Controls (≤12 yrs)
Area 1 (posterior pole)	151/158 (96%)	2/7 (29%)	27/30 (90%)	0/2 (0%)
Area 2 (supratemporal)	116/158 (73%)	4/7 (57%)	9/30 (30%)	2/2 (100%)
Area 3 (infratemporal)	110/158 (70%)	4/7 (57%)	4/30 (13%)	2/2 (100%)
Area 4 (supranasal)	103/158 (65%)	2/7 (29%)	5/30 (17%)	0/2 (0%)
Area 5 (infranasal)	86/158 (54%)	3/7 (43%)	5/30 (17%)	0/25 (0%)
No. of fundus areas involved (sum of both eyes)	8.27 (2–10)	2.14 (0–3)	3.00 (2–6)	2.00 (2–2)

ously for adult and children populations.[16,17] Notably, the prevalence of choroidal nodules detected by NIR in the NF1 overall population was 82%, similar to the NIH diagnostic criteria. Additionally, in the NF1 pediatric group, the prevalence of NIR-detected choroidal nodules was 71%, much higher than the frequency of the NIH ophthalmic diagnostic criteria of 43% for iris Lisch nodules detected by slit-lamp examination.

With a cutoff value of 1.5 choroidal nodules observed by NIR imaging, the diagnostic sensitivity was 83% and the specificity was 96%. Thus, with a diagnostic criterion of 2 or more choroidal abnormalities detected by NIR, there is a high diagnostic accuracy both in the overall NF1 population and in the NF1 pediatric population. As described by Yasunari et al[9] and Nakakura et al,[10] choroidal abnormalities were easily detectable by NIR light and indocyanine-green angiograms in 100% of their NF1 patients. However, these same defects were undetectable using conventional ophthalmoscopy and fluorescein angiograms. The data presented by Yasunari et al[9] and Nakakura et al[10] are not in absolute consonance with the current results, possibly because of the small number of subjects included in the 2 series or because of bias in the enrollment of their patients. For instance, refractive errors and conditions related to the amount of melanin in the choroid, such as race and iris color, might have affected the detection of bright, patchy nodules using NIR. These were not reported by them.[9,10]

Regardless of the real prevalence of choroidal involvement, the presence of the bright, patchy regions seen with NIR imaging seems to be highly specific for NF1 patients. Seven control subjects with bright patchy nodules detected by NIR were found. However, 3 of them had only 1 bright nodule in 1 eye as a result of a choroidal nevus. In the other 4 control subjects, the bright nodules were probably the result of atrophic areas, vitreoretinal reflex, or optical reflectance artifacts. Thus, infrared monochromatic light penetrates the retinal pigment epithelium better than visible light, showing deeper tissues. Therefore, the bright, patchy regions noted under infrared light and the absence of such regions under AF and RF laser monochromatic light by cSLO or conventional ophthalmoscopy indicate the choroidal localization of the abnormalities. Additionally, high-resolution OCT, allowing the investigation of the area of interest on a quasihistologic level, confirmed the choroidal localization of these abnormalities.

As described by Nakakura et al,[10] the present study found that most choroidal nodules were located at the posterior pole, especially in pediatric patients. Furthermore, the extent of ocular fundus involvement was variable and increased with age. The topographic distribution of the abnormalities already has been well discussed.[10] The higher presence at the posterior pole is probably the result of the greater choroidal thickness and the higher number of melanocytes and neural cells.[10] In NF1 patients, these findings are amplified by the choroidal hamartomatous thickening.[18]

Recently, the presence of unidentified bright objects on brain magnetic resonance images has been advocated as a new diagnostic criterion in young children with 1 feature of NF1. However, this age group needs general anesthesia for that procedure, thereby reducing the usefulness of the investigation.[19]

In conclusion, NIR imaging allows a noninvasive, quick, reliable, glare-free examination of the choroid to detect choroidal nodules. The present study offers the first reliable data on the prevalence of choroidal abnormalities in the NF1 population. Further epidemiologic information is essential for testing these findings as an additional new diagnostic criterion for NF1. If supported by epidemiologic studies, this information will allow the testing of choroidal abnormalities detected by NIR imaging as an additional diagnostic criterion for NF1, mainly in infants and in patients suspected of having the condition.

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