# Correlations between clinical, imaging and histological findings in a patient with neurofibromatosis type 1 (von Recklinghausen's disease)

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## ABSTRACT

Neurofibromatosis type 1 (NF1) or von Recklinghausen disease is one of the most common genetic diseases, affecting 1/4,000 individuals. It is transmitted by autosomal dominant inheritance and the gene NF1, which is responsible for the disease, is located on the long arm of chromosome 17. NF1 is characterized by varied expressions of the disease, even within the same family.

We present the case of a 22-year-old patient with NF1 admitted in the Department of Neurosurgery for a two months history of diffuse intercostal nevralgias that did not respond to treatment and discuss the histopathological and immunohistochemical features of her cutaneous and spinal neurofibromas.

Our case adds new data to the knowledge of the diverse biological behaviour of NF1, highlighting the fact that this condition is a complex disease even in the same individual. We report here a highly variability among neurofibromas in the same patient from a histopathological point of view. Our data are also important as they demonstrate the fact that the management of a patient with NF1, due to the various and complex manifestations of the disease, requires a multidisciplinary approach, including neurologist, neurosurgeon, ophthalmologist, plastic surgeon, dermatologist, radiologist and pathologist.

## INTRODUCTION

Neurofibromatosis type I (NF1) is a complex neuro-cutaneous disease with dominant autosomal transmission, whose NFI gene is located on Chromosome 17. The definitive diagnosis needs the existence in the same individual of two or more criteria from the following seven main elements: a). six or more café-au-lait spots, larger than 5 mm in puberty and larger than 15 mm after puberty, disseminated on the whole body;

b). axillary and inguinal freckling (Crowe sign); c). two or more cutaneous or spinal nerve neurofibromas of any type or at least 1 plexiform neurofibroma; d). optic nerve glioma; e). two or more Lisch nodules (iris hamartomas); f). characteristic skeletal dysplasia; g). at least one first-degree relative affected by NF1 (parent, sibling, child) (1).

However, NF1 is characterized by varied expressions of the disease, even within the same family.

We present the case of a 22-year-old patient with NF1 fulfilling five main criteria of the disease and discuss the histopathological features of her cutaneous and spinal neurofibromas highlighting the fact that NF1 is a complex disease even in the same individual.

## CASE PRESENTATION

A 22-year-old female patient was referred to Neurosurgery Department, „Prof. Dr. N. Oblu" Emergency Clinical Hospital Iaşi, with a two months history of diffuse intercostal nevralgias that did not respond to treatment.

The patient had a positive family history for von Recklinghausen's disease. She had an uncle (44 years-old), her father's brother, with NF1, who was diagnosed five months ago with plexiform neurofibromas of the spinal nerves (from D12 nerve roots to the sacral roots), the terminal segments showing the appearance of pelvic "masses".

Our patient's past medical history revealed a diagnosis of NF1 that was established when she was 8 years old as she has had already *café-au-lait* spots, disseminated on her limbs and trunk, and cutaneous soft, painless tumours, one of them located in her left retroauricular area.

The tumour was incompletely excised when the patient was 12 years old and the diagnosis of a cutaneous diffuse neurofibroma was histopathologically established, thus confirming the diagnosis of von Recklinghausen's (NF1) disease.

As the tumour slowly increased in size, after 9 years a head Magnetic Resonance Imaging (MRI) was performed, identifying the presence of a lesion that developed in the dermis and distorted the left temporo-occipital region (FIGURE 1).

The tumour was completely excised and the specimen was sent to the Pathology Department where it was processed using the standard histological technique. The immunohistochemical

stainings were performed using anti-S-100 protein antibody, anti-Vimentin antibody, and a two-step staining technique (EnVision+ Dual Link System-HRP, Dako). On histological examination, an intact epidermis covered a relatively ill-defined dermal tumoural proliferation of Schwann cells with hyperchromatic wavy nuclei, few aggregates of pseudomeissnerian corpuscles, associated with fibroblasts and normal appearing nerve bundles. Tumour mass infiltrated the hypoderm, entrapping adipocytes and distorting serous gland acini. Immunopositivity for Vimentin in randomly arranged fibroblasts and intense immunopositivity for S-100 protein in randomly arranged Schwann cells, but also in the cells of pseudomeissnerian corpuscles, established the final diagnosis: cutaneous neurofibroma with pseudomeissnerian corpuscles (FIGURES 2, 3, and 4).
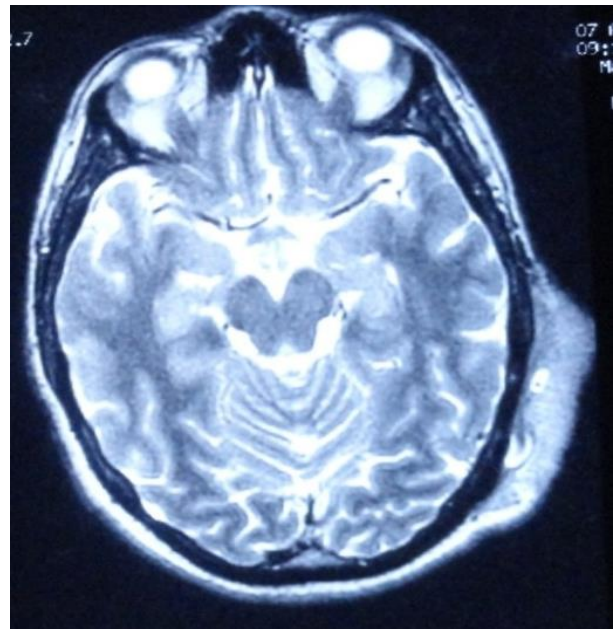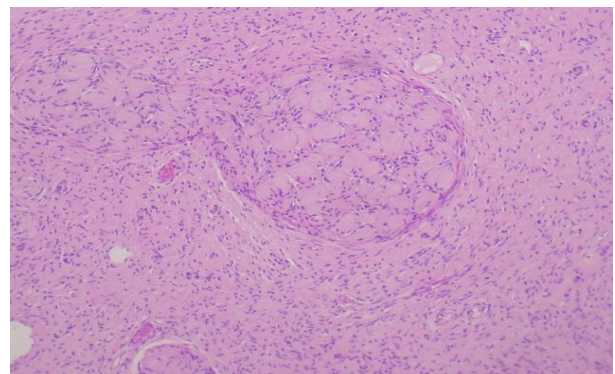


FIGURE 1. MRI T2 SE scan revealed a cutaneous tumour in the left temporo-coccipital region.

Figure 3. Intense immunopositivity for S100 protein in randomly arranged Schwann cells, but also in all cells located in the pseudomeissnerian corpuscles (anti – S100 protein, x 200).

FIGURE 4. Immunopositivity for vimentin in randomly arranged fibroblasts (anti - Vimentin, x 100).

One year later, on admission in our hospital due to diffuse intercostals nevralgias, the patient's physical examination revealed: axillary and inguinal freckling, six *café-au-lait* spots with diameters ranging from 1,5cm to 6 cm, located on the trunk, six cutaneous tumours that were soft, pink, painless, ranging from a few millimeters to 2 centimeters, which were located on the dorsal trunk and right shoulder (FIGURE 5).
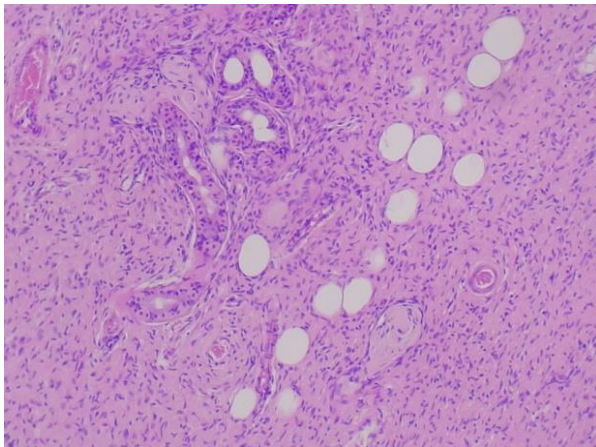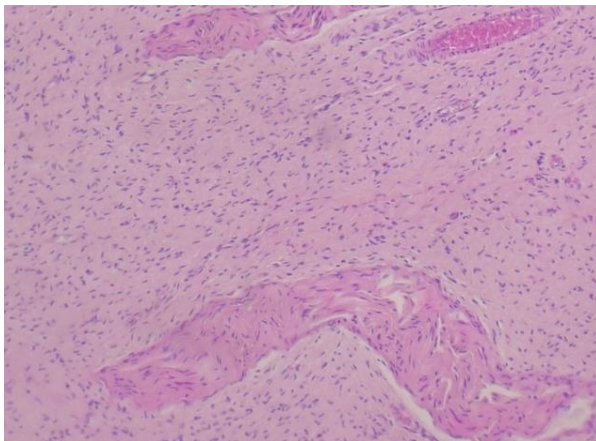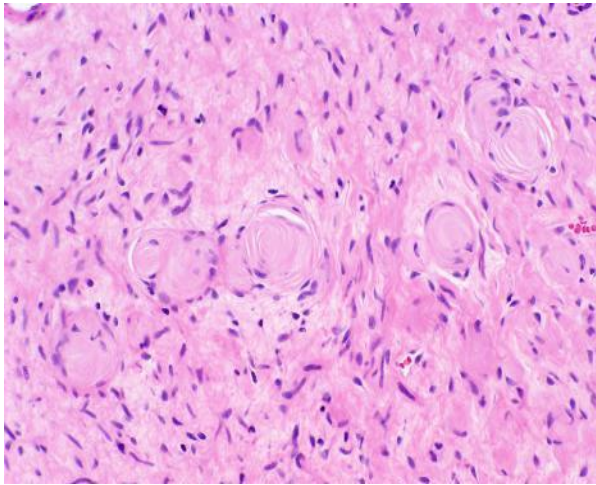
FIGURE 2. Cutaneous neurofibroma: a). Tumoral proliferation of Schwann cells with hyperchromatic wavy nuclei, which were intimately associated with fibroblasts and fine collagen strands. In some areas, there were aggregates of pseudomeissnerian corpuscles (HE, x100); b). tactile-like/pseudomeissnerian corpuscles in tumoral mass (HE, x200). c). Small scattered fascicles of nerve bundles entrapped into the tumour mass (HE, x100); d). tumour mass infiltrated the hypoderm, entrapping adipocytes and distorting serous gland (HE, 1200).
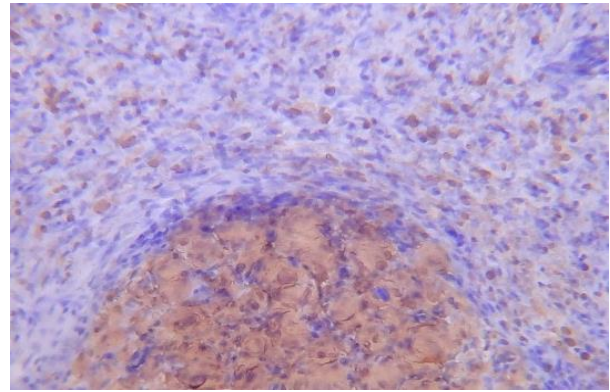
**FIGURE 5.** a). On the dorsal inferior trunk there were several "*café-au-lait*" spots, along with red, soft, depressible tumours. b). On the dorsal superior trunk there were three large (ranging from 2 cm to 6 cm in long diameter) "café-au-lait" spots; c). On her right shoulder area there was a cutaneous soft, pink-colored tumour, with a diameter of 1.5cm.
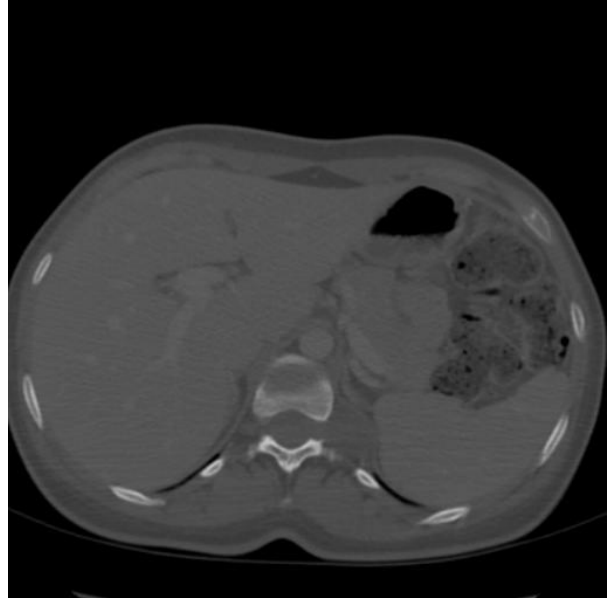






**FIGURE 6.** The contrast enhanced CT exam showed an intraforaminal and para-vertebral tumour formation with widening of the vertebral left foramen at D10-D11, with left paravertebral extension, being in contact with the superior pole of the left kidney. The nodular aspect of the lesion, visible in the sagital plane, is associated with the inclusion of the left D10 and D11 spinal roots into the tumoural mass, as seen in the axial image. a). Axial section in a parenchymal window; b). Axial section in a bone window; c). sagital section.

An abdominal CT scan was performed and revealed the existence of a left paravertebral solid tumour, which was developed intraforaminal and para-vertebral around the dorsal level D11. The tumour extended upward at the D10 level and downward at the D12 level, encompassing left D10 and D11 spinal

roots. The tumoural formation was solid, oval, well defined, with dimensions of 35/16 / 65mm (Figure 6).

The standard laboratory tests values were in the normal range.

Surgical intervention was performed by external approach at D11-D12 lateral level, with microsurgical total ablation of the tumour. The surgical specimen was sent to the Pathology Department of the same hospital for histopathological analysis.

The specimen was fixed in formalin, embedded in paraffin, cut at 5 µm and stained with hematoxylin-eosin (HE). On representative sections, immune-histochemical stainings were performed using anti-S-100 protein antibody, anti-Vimentin antibody, and a two-step staining technique (EnVision+ Dual Link System-HRP, Dako). Histological sections stained with HE showed a non-encapsulated tumour, consisting of neoplastic wavy Schwann cells, entrapped ganglion cells as the tumour extended to associated ganglia, a myxoid stroma, and delicate collagen fibers (Figure 7). S100 protein immune-staining highlighted neoplastic Schwann cell component and ganglia cells of the spinal root that were entrapped into the tumour (Figure 8). Vimentin immunostaining revealed numerous fibroblasts (Figure 9). The final pathological diagnosis was spinal neurofibroma with tumoural infiltration of the associated ganglia.

The ophthalmologic examination revealed myopia in both eyes (best corrected visual acuity in right eye was 1 with -1 D and in the left eye was 1 with – 0,5 D). The ophthalmoscopic examination showed normal appearance of the ocular fundus in both eyes. Direct photomotor reflex was bilaterally present. Intraocular pressure was within normal range: right eye = 12 mmHg and left eye = 13 mmHg. Both eyes presented many small, bright brown, oval, and dome-shaped irian nodules (Lisch nodules) (Figure 10).
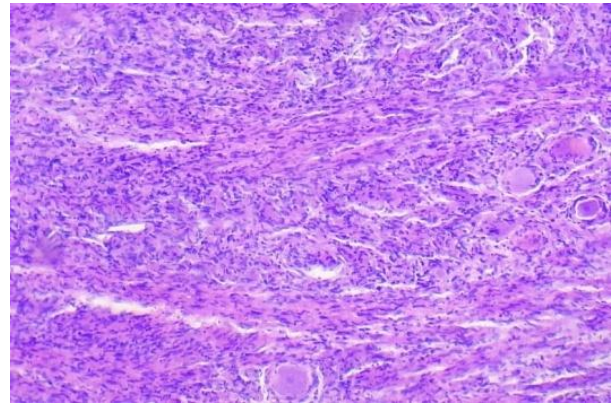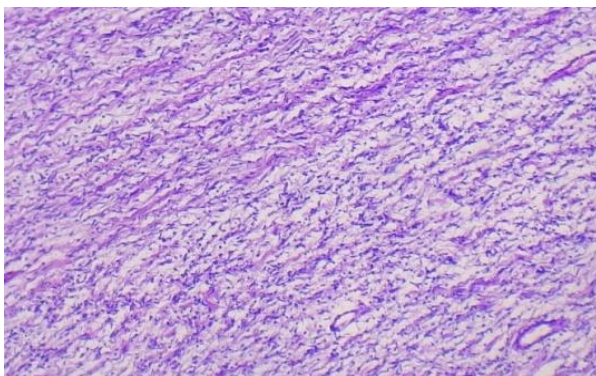


**Figure 7.** Spinal neurofibroma: a). non-encapsulated tumour made up of neoplastic wavy Schwann cells, a myxoid stroma, and delicate collagen (H-E, x100); Numerous ganglia cells entrapped into the tumour mass (HE, x100).
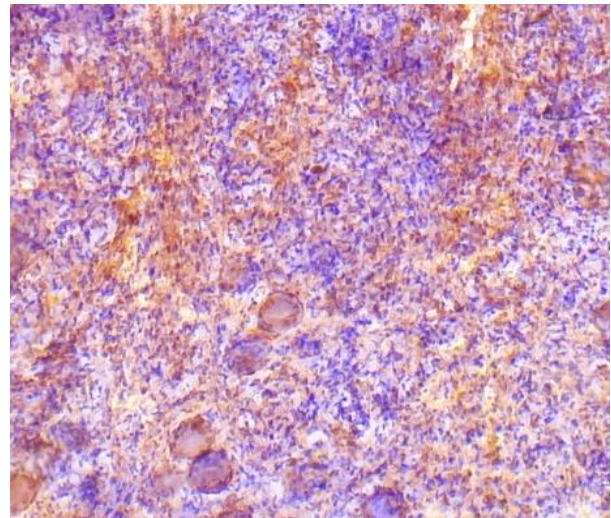


**Figure 8.** Immunopositivity for S100 protein in neoplastic Schwann cells and ganglia cells (anti – S100 protein, x 100).
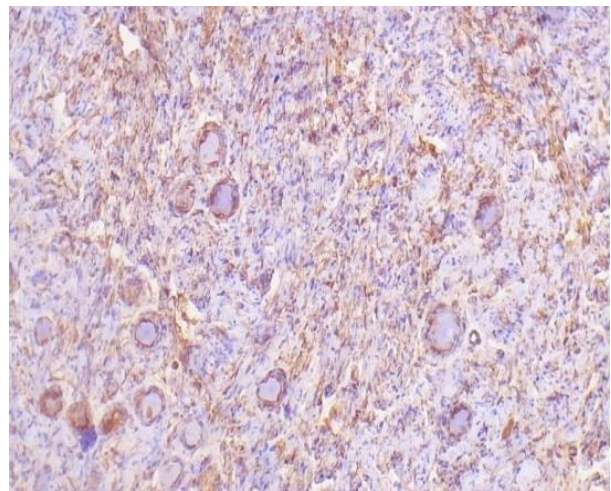


**Figure 9.** Immunopositivity for vimentin in randomly arranged fibroblasts (anti - Vimentin, x 100).
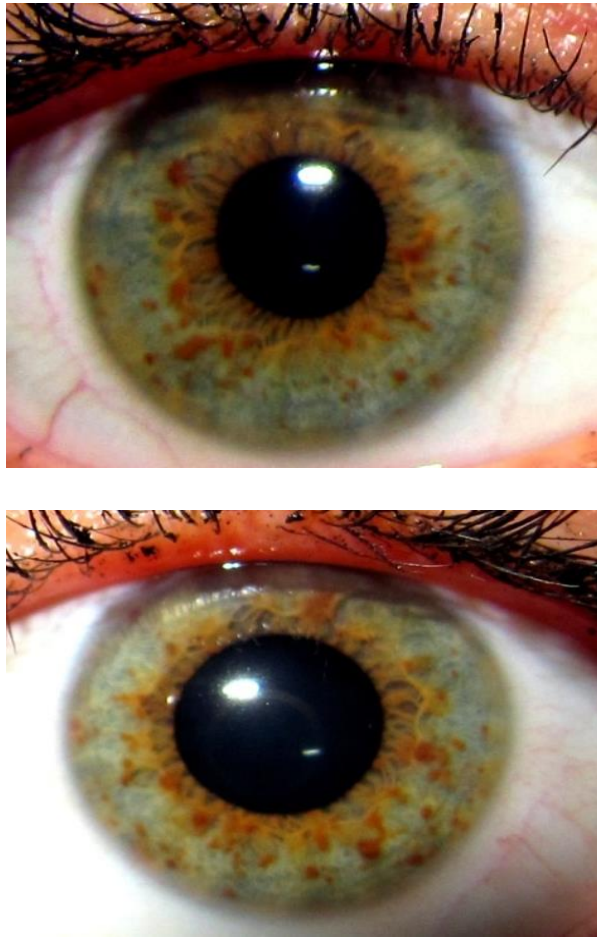
**Figure 10**. Numerous small Lisch nodules at both eyes [a). right eye; b). left eye].

## Discussion

There are suppositions that Ebers Papyrus case #873 can represent the first mention of a case of neurofibromatosis type 1 (NF1) (2). The important landmarks in the history of the disease are the Irish neurosurgeon Robert William Smith, who, in 1849, realized a systematic review of clinical cases (3), and the German pathologist Friedrich Daniel von Recklinghausen (1833-1910), who fully described this condition in 1882. Von Recklinghausen introduced the term "neurofibroma" into clinical practice to define the benign tumours that accompany this disease. He was the first who found that these tumours develop from the peripheral nerve sheath, consisting of a mixture of Schwann cells and fibroblasts (4). In recognition of his discovery, the disease received his name.

However, there are different forms of neurofibromatosis that VM Riccardi, the director of the Baylor NF Program (1978-1990), attempted to classify the disease in 1982 (5). He considered that there were eight types, but later, in 1986, Carey et al. proposed a new classification into only five types, as follows: NF1 - classical, NF2 - acoustic, NF3 - segmental, NF4 - CALM - familial and NF5 - NF-Noonan phenotype (6).

NF1 represents 90% of all cases with NF, being characterized mainly by multiple café-au-lait spots and neurofibromas along peripheral nerves.

Even though NF1 has been recognized as a clinical entity for more than a century, its etiopathogenesis was better understand only in 1990 when the NF1 gene was identified on chromosome 17. NF1 gene is a tumour suppressor gene that encodes a protein named neurofibromin (7), which is a negative regulator of the "rat sarcoma viral oncogene homologue" (RAS) (8).

Approximately 50% of the cases with NF1 disease represent new mutations and the expression of the disease is highly variable, both between and within families (9).

The most important hypothesis regarding the neurofibromas was that these tumours can develop only after both NF1 alleles have been lost in the Schwann cell lineage. When Schwann cells begin to proliferate, they recruit some other cells from their environment (10). As such, neurofibromas are complex tumours, consisting in a mixture of cell types: Schwann cells, fibroblasts, perineurial cells, and mast cells (7, 10).

It was also hypothesized that these different cellular phenotypes can represent divergent cellular differentiation pathways of multipotent precursor cells (11).

It was considered that the development of neurofibromas include the presence of a clonal population of NF1-/- Schwann cells in a microenvironment harbouring other cell types with an NF1+/- genotype. It was also hypothesised that the multipotent NF1+/- cells are the major source of different cell types found in the neurofibromas (11).

Neurofibromas developing in a NF1-affected individual can be classified according to their anatomical location into: cutaneous, subcutaneous, intraneural, and spinal nerve roots tumours (12).

Cutaneous neurofibromas generally appear during preadolescence. They are soft, pink coloured tumours, sessile or dome-shaped, being most numerous on the trunk and limbs. The tumour diameter usually varies between a few millimetres

and approximately 2 centimetres (11). It is considered that these benign tumours developed from small nerve tributaries of the skin (13). Histologically, cutaneous neurofibromas are nonencapsulated, loosely textured dermal mixed tumours, consisting of Schwann cells, neurons, perineurial cells, fibroblasts, and mast cells, but also adipocytes, epithelial cells, and axonal processes (11). All of these cellular elements are embedded in an abundant collagenous extracellular matrix.

Megahed (14) described ten histopathological variants of neurofibroma: classic, cellular, myxoid, hyalinized, epithelioid, plexiform, diffuse, pigmented, granular cell, pacinian. Subsequently, some other variants such as dendritic cell neurofibroma with pseudorosettes (15), lipomatous neurofibroma (16), and angioneurofibroma (17) have been reported. Pseudomeissnerian corpuscles can be seen in cutaneous neurofibromas, but not so frequently.

Using traditional histological staining as well as immunohistochemical staining (anti-S100 protein antibody and anti-Vimentin antibody) can be the proofs that the neoplastic component is made of Schwann cell. Neurofilament protein immune-reactivity (NFP) revealed the occurrence of residual axons within tumour nodules. Some authors highlighted the fact that, using factor XIIIa as an immunohistochemical marker, the pathologist can differentiate cutaneous neurofibromas from neurotized nevi and cutaneous schwannomas (18.) Aggregates of pseudo-meissnerian corpuscles are frequent in diffuse cutaneous neurofibromas (19). As these structures consist entirely of Schwann cells, they can be can be highlighted with anti-S100 protein antibody immunostaining (20).

Our patient presented at least one cutaneous neurofibroma since he was 8 years-old, but the definitive diagnosis of NF1 was realized only after the surgical excision of the tumour (when patient was 12 years-old). Histological and immunohistochemical examinations identified diffuse cutaneous neurofibroma with clusters of organoid structures resembling Meissner corpuscles (pseudo-meissnerian structures), thus being in line with other researchers' findings.

The incidence of spinal involvement in NF1 patients varies among authors. Mautner et al. reported that spinal neurofibromas are found in up to 38% of NF1 patients but very few of them are symptomatic (21).

In a study of patients with NF1 made by Thakkar et al., 6% of patients had intramedullary tumours, 57% had intraforaminal tumours (dumbbell), and 33% had extradural tumours (22).

At the spinal level, patients with NF1 may have either one or more tumours that can develop in one or more spinal nerve roots. Usually, spinal tumours are diffuse or plexiform neurofibromas. Some authors found out that more frequently these spinal tumours are solitary neurofibromas (23), but others reported plexiform neurofibromas, which grow involving multiple nerve fascicles, branches and plexuses (24). Our patient had a solitary spinal neurofibroma, but her uncle had multiple plexiform spinal neurofibromas. Our article prove the fact that neurofibromas developing in a patient with NF1 can have different histological features, each of them displaying microscopical appearances more closely related to the histology of the region in which the tumour occurs, i.e., cutaneous neurofibroma exhibited pseudomeissnerian corpuscle, and spinal neurofibroma revealed Schwann cells arranged in bundles, reminding of spinal nerve histology.

The age at onset of symptoms caused by spinal neurofibromas can vary from 11 to 49 years (mean 32.8 years), but most cases present symptoms at adult ages (22 to 43 years) (25).

Spinal neurofibromas cause neurological symptoms in only 2% of NF1 patients, provoking both sensory and motor deficits due to compression of the spinal cord or nerve root (22). In a recent article, Mauda-Havakuk et al. analyzed the radiological findings of a series of thirty-four patients with NF1 with spinal neurofibromas. They classified spinal involvement into four types according to the anatomic location of the tumours along the spine and to their type of involvement of the spinal canal and foramina: 1. foraminal tumour; 2. "kissing" tumours; 3. paraspinal tumour; 4. intradural tumour (26). These authors reported that most spinal neurofibromas developed in the lumbo-sacral area and fell into group 1 (foraminal).

Our patient was diagnosed as having a spinal foraminal neurofibroma at the age of 22. As her left D10 and D11 spinal roots were entrapped into the tumour mass, the patient presented a two months history of diffuse intercostal nevralgias that did not respond to the treatment, thus imposing the surgical intervention.

Lisch nodules are hamartomas of the iris that are also characteristic for NF1. They have variable dimensions and dome-shaped configuration. Some researchers reported that patients affected by NF1 and older than twenty could have an incidence of Lisch nodules of 100% (27). Lewis et al. found out that 92% of subjects aged 6 years and older had Lisch nodules, but their presence was not correlated to number of café-au-lait spots, number of neurofibromas, or severity of disease (28). Our patient, being in her third decade of life, also presented Lisch nodules in both her eyes.

Due to varied and complex manifestations of the disease, the management of a patient with NF1 requires a multidisciplinary approach. Once the diagnosis is established, clinical monitoring is needed to identify possible complications. As a result, annual assessment is required to reduce morbidity and improve the quality of life. This assessment should include: a) dermatological examination to analyze the progression of the existing neurofibromas and to identify possible new lesions; b). head and spine MRI, chest and abdomen imaging to detect the majority of complications that could be associated with this disease; c). full ophthalmological examination for early detection of optic nerve lesions; d). neurological examination for early detection of paresthesia, radiculopathy, muscle fatigue or muscle atrophy; e). removal of skin tumours for cosmetic or therapeutic purposes; f). education and psychological support for NF1 patient.

## Conclusion

The patient described in this article is a very typical case of NF1, having six "café-au-lait" spots, six neurofibromas, axillary or inguinal freckling, Lisch nodules, and a first degree relative (her father's brother) with NF1.Our case presents a considerable interest because of a complete description of the natural evolution of NF1, highlighting the histological pictures of her cutaneous and spinal neurofibromas. Because the expression of the disease is highly variable, both between and within families, our study data are especially valuable as they also showed a highly variability among neurofibromas in the same patient from a histopathological point of view. These data are also important as they demonstrate the fact that the management of a patient with NF1, due to the various and complex manifestations of the disease, requires a multidisciplinary approach, including neurologist, neurosurgeon, ophthalmologist, plastic surgeon, dermatologist, radiologist and pathologist.

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