**Title: Clinical severity in Japanese patients with neurofibromatosis 1 based on DNB classification.**

Short title: Severity and clinical features in NF1

The total number of words 1820, figures 6(1, 2, 3, 4a, 4b and 4c) and tables 2 and references 19.

Yuko EHARA[1], Osamu YAMAMOTO[1], Kenjiro KOSAKI[2], Yuichi YOSHIDA[1]

[1]Division of Dermatology, Department of Medicine of Sensory and Motor Organs, Faculty of Medicine, Tottori University, Yonago, Japan

[2]Center for Medical Genetics, Keio University School of Medicine, Tokyo, Japan

Correspondence to: Yuko Ehara, M.D.

Division of Dermatology, Department of Medicine of Sensory and Motor Organs, Faculty of Medicine, Tottori University, 86 Nishi-cho, Yonago-shi, Tottori 683-8503, Japan

Tel: +81-859-38-6597 Fax: +81-859-38-6599

Email: yukoehara@med.tottori-u.ac.jp

Key words: NF1, clinical severity, DNB classification, diffuse plexiform neurofibromas, quality of life.

**ABSTRACT**

Neurofibromatosis 1 (NF1) is a genetic disease characterized by cutaneous, neurological and osseous complications. Although clinical manifestations of NF1 are variable, there has been no report on evaluation of severity in patients with NF1. To elucidate the grade of severity of NF1, a retrospective study was conducted in 124 NF1 patients at the Department of Dermatology of Tottori University Hospital in 2007-2016. The DNB classification (dermatological, neurological and bone manifestations) in Japan was used for assessment.

Based on our current epidemiological data, there were 55 patients (44.3%) in stage 1, 23 patients (18.6%) in stage 2, 3 patients (2.4%) in stage 3, 1 patient (0.8%) in stage 4 and 42 patients (33.9%) in stage 5. The grade of severity in patients with NF1 tended to be higher with aging. Remarkably, 61.8% of the patients in stage 5 had diffuse plexiform neurofibromas with functional disability. We should pay attention to diffuse plexiform neurofibromas that greatly affect quality of life in patients with NF1.

Key words: NF1, clinical severity, DNB classification, diffuse plexiform neurofibromas, quality of life.

## INTRODUCTION

Neurofibromatosis 1 (NF1, von Recklinghausen disease) is one of the most common autosomal dominant genetic diseases and is characterized by café-au-lait spots, neurofibromas, freckling, optic glioma, Lisch nodules and bone deformity.[1, 2, 3] It is caused by mutation of the *NF1* gene on chromosome 17q11.2.[4] The worldwide prevalence of NF1 is approximately 1 in 3000-4000 individuals with nearly 100% penetrance.[5, 6]

Clinical manifestations of NF1 are variable, and the overall degree of complications is not predictable. As far as we know, however, there is no report on evaluation of the severity index of each symptom in patients with NF1. Therefore, current epidemiological data are necessary. In Japan, the grade of severity is divided into 5 stages based on the DNB classification (dermatological (D1-D4), neurological (N0-N2) and bone manifestations (B0-B2)).[7]

In this study, we evaluated the recent prevalence of each manifestation in Japanese patients with NF1 to reveal the grade of severity of NF1 by DNB classification.

## PATIENTS AND METHODS

### Study population (Patients)

A retrospective study was conducted. We investigated 124 NF1 patients (58 men and 66 women; median age, 31 years; age range, 0-95 years) at the Dermatology Department of Tottori University Hospital over a 9-year period from January 2007 to October 2016.

All of the patients were examined by expert dermatologists, and all met the diagnosis criteria by National Institutes of Health in 1988.[6, 7] The study protocol was approved by the Ethics Committee of Tottori University Hospital (No. 2671).

Demographic and clinical information including information on age, sex and any diagnosed complications was obtained from medical records. We estimated the grade of severity in each patient according to the DNB classification proposed by the Ministry of Health, Labour and Welfare in Japan (Table Ⅰ).[7]

**Manifestations**

**Dermatological manifestations**

Café-au-lait spots are flat, well-circumscribed, light to dark macules that can be present at birth and become conspicuous as the infant grows. [2, 3.]

Neurofibromas can be classified into 3 types (cutaneous, nodular plexiform and diffuse plexiform neurofibromas). Cutaneous neurofibromas are dome-shaped, soft, flesh-colored nodules of 1 to 2 cm in size. They are detectable in most patients at

puberty and increase in size and number with age. [8, 9] Plexiform neurofibroma is thought to be a congenital tumor that often causes disfigurement. [2, 3]

Cutaneous neurofibroma is always benign, whereas plexiform neurofibroma has a potential for malignant transformation into malignant peripheral nerve sheath tumor. [2, 3]

**Neurological manifestations**

Optic pathway glioma is found in 15-20% of children with NF1. Up to half of these patients will develop visual symptoms.[10] It has been reported to be rare among Asian populations.[9] Cerebral glioma also occurs in up to 3% of patients.

**Bone manifestations**

Osseous abnormalities associated with NF1 include scoliosis, sphenoid wing dysplasia and long-bone dysplasia. Scoliosis affects 10-26% of patients with NF1.[11] Sphenoid wing dysplasia is rarely seen (<1% of patients with NF1)[12] and sometimes causes a pulsating exophthalmos or brain herniation.[13] Long-bone dysplasia is rarely seen in infancy (approximately 2%) and can result in pseudoarthrosis.[14]

**Mosaic NF1**

Mosaic NF1 is caused by a somatic mosaicism of the *NF1* mutation and shows typical features of NF1 limited to specific body segments. It has been reported that the prevalence of mosaic NF1 in the general population is 1 in 36,000-40,000 individuals. [15, 16, 17]

**Genetic analysis**

After obtaining informed consent, we performed genetic analysis of 25 NF1 patients by next-generation sequencing as previously described.[18] Briefly, genomic DNA was extracted from peripheral blood according to standard procedures. In-solution hybridization-based enrichment was performed using the SureSelect Target Enrichment system (Agilent Technologies, Santa Clara, CA, USA). When the next-generation sequencing protocol revealed truncating mutations, the variants were validated by direct capillary sequencing. Exon deletions were screened using a multiple ligation-dependent probe amplification method (SALSA P081/082-B2 NF1MLPA assay kit; MRC-Holland).

**RESULTS**

**Patient characteristics**

There were 124 NF1 patients in this study (58 men and 66 women; median age, 31 years). The numbers of patients were large in the under 10 years old group and 30-39 years old group (Fig.1). There was a family history of NF1 in 47.6% of the patients (not shown).

**Dermatological manifestations**

The patients were divided into 4 groups according to the number of cutaneous neurofibromas (Fig. 2). The ratios of patients with <10 cutaneous neurofibromas, <100 neurofibromas, <1000 neurofibromas and ≧1000 neurofibromas were 40.3%, 26.6%, 25.8% and 7.3% respectively (Fig. 2). A large number of cutaneous neurofibromas was seen in elderly patients. Cutaneous neurofibromas tended to increase with aging (Fig. 3).

Nodular plexiform neurofibromas were seen in 14 patients (11.3%). Diffuse plexiform neurofibromas were observed in 26 patients (21%). Malignant peripheral nerve sheath tumor was found in only one patient (0.8%). These results are shown in Table Ⅱ.

**Neurological manifestations**

Optic pathway glioma was seen in 2 patients (1.6%) and cerebral glioma in 3 patients

(2.4%) (Table Ⅱ).

**Bone manifestations**

Osseous abnormalities were seen in 16 patients (12.9%). There were 10 patients with spine deformity (9 patients with scoliosis and 1 patient with atlantoaxial subluxation), 5 patients with deformity of the skull or facial bone and 1 patient with pseudoarthrosis (Table Ⅱ).

**Grade of severity in patients with NF1 based on DNB classification**

Based on the Japanese criteria, the patients were divided into 5 stages (Table Ⅰ). There were 55 patients (44.3%) in stage 1, 23 patients (18.6%) in stage 2, 3 patients (2.4%) in stage 3, 1 patient (0.8%) in stage 4 and 42 patients (33.9%) in stage 5 (Fig. 4a). Twenty-six (61.8%) of the patients in stage 5 had diffuse plexiform neurofibromas with functional disability (Fig. 4b).

The stage of each patient is shown in Fig. 4c. In the 0-9 years old group, most patients were classified into a lower grade. The grade of severity in patients with NF1 tended to be higher with aging.

**Mosaic NF1**

In our study, there are 17 patients (13.7%) with mosaic NF1(9 men and 8 women; median age, 9 years; age range, 2-88 years) (not shown).

**Relationship between type of *NF1* mutation and clinical severity**

NF1 mutation was detected 19 of the 25 patients (9 frameshift, 6 missense, 3 deletion and 1 nonsense). There was no significant relationship between type of *NF1* mutation and clinical severity (not shown).

**DISCUSSION**

It is well known that NF1 has various complications including dermatological, neurological and bone manifestations.[1, 2, 3] Although there has been a few reports on the frequency of complications in NF1,[3, 9] the grade of severity has not been elucidated. Therefore, we investigated the grade of severity in Japanese patients with NF1 by epidemiological data in our institution. This is the first report on clinical severity of NF1 patients by Japanese DNB classification.

In our study, there were diphasic peaks (< 10 years old group and 30-39 years old group) in the number of NF1 patients. It is likely that the younger patients were referred

to our hospital by pediatricians for evaluation of café-au-lait spots. In most patients <10 years old, the grade of severity was low. In contrast, NF1 patients >30 years old presented with cosmetic and social problems due to dermatological manifestations (neurofibromas). We are not sure about the precise reason why the number of patients with age 30-39 is large and that of age 20-29 is small. The patients with age 20-29 may not have enough time to visit hospitals because they are busy for school or business. Or the number of cutaneous neurofibromas is relatively small in those patients, which does not affect daily life and social activity.

We investigated the approximate number of cutaneous neurofibromas. The number of cutaneous neurofibromas tended to increase with advance of age. The ratio of patients with numerous neurofibromas (>1000 number) was not high (7.3%) in our study. This might be because elderly patients with severe dermatological manifestations hesitated to visit hospitals.

Diffuse plexiform neurofibroma was seen in about 20% of the NF1 patients. The frequency was much higher than that in a previous report in Japan.[9] The number of NF1 patients with diffuse plexiform neurofibromas who need medical treatment has been increasing.

In our study, optic pathway glioma was rarely seen in the NF1 patients (1.6%), as was

reported previously for Japanese patients.[9] The frequency was lower than in other countries.[10] The low frequency in our study might be because imaging examination has not been performed for asymptomatic NF1 patients in our department.

Regarding bone manifestations, spine deformity was most frequently seen (8.1%) in our study, in agreement with a previous report.[9]

Based on current epidemiological data, we evaluated the grade of severity in patients with NF1. About 60% of the patients showed mild manifestations (stage 1 or 2), and about 34% of the patients had severe manifestations (stage 5). In stage 5, the most influential factor was diffuse plexiform neurofibromas causing functional disability. Although NF1 patients with mild symptoms might not visit hospitals, one third of the NF1 patients in our study had severe complications affecting daily life. The grade of severity tended to be higher in elderly patients than in younger patients. Meanwhile, there were a few patients with ≧1000 cutaneous neurofibromas. Some patients with numerous cutaneous neurofibromas had another severe complication and were classified into more severe grade (the stage 4 or 5). Therefore, the number of patients in stage 3 was small.

There is a limitation in our study because the clinical manifestation of NF1 patients is highly variable and some complications, e.g., pigmented macules, are not included.

Although the DNB classifications may not be enough for the absolute evaluation of NF1 patients severity, it represent one aspect of NF1 condition.

In conclusion, we found that one third of the NF1 patients had severe manifestations with diffuse plexiform neurofibromas. Several clinical trials are currently being performed worldwide.[19] In order to improve functional disability and impaired quality of life in NF1 patients, we should focus on diffuse plexiform neurofibromas and provide appropriate medical treatment as early as possible.

**ACKNOWLEDGMENTS**

This work was partly supported by Health Labour Sciences Research Grant (Y.Y) from the Ministry of Health, Labour and Welfare and the Platform Project for supporting in Drug Discovery and Life Science Research from the Japan Agency for Medical Research and Development (Y.Y and K.K) and Tottori University Faculty of Medicine Alumni Association (Y.E)

Conflict of interest: none

REFERENCES


1. National Institutes of Health Consensus Development Conference. Neurofibromatosis: Conference statement. *Arch Neurol* 1988; **45**: 575-578.
2. Anderson JL, Gutmann DH. Neurofibromatosis type 1. *Handb Clin Neurol* 2015; **132**: 75-86.
3. Korf BR. Diagnosis and management of neurofibromatosis type 1. Curr Neurol Neurosci Rep 2001; 1: 162-167.
4. Murchuk DA, Saulino AM, Tavakkol R, et al. cDNA cloning of the type 1 neurofibromatosis gene: complete sequence of the NF1 gene product. *Genomics* 1991; **11**: 931-940.
5. Lammert M, Friedman JM, Kluwe L, MautnerVF. Prevalence of neurofibromatosis 1 in German children at elementary school enrollment. *Arch Dermatol* 2005; **141**: 71-74.
6. Johnson KJ, Hussain I, Williams K, Santens R, Mueller NL, Gutmann DH. Development of an international internet-based neurofibromatosis Type 1 patient registy. *Contemp Clin Trials* 2013; **34**: 305-311
7. Japan Intractable Disease Information Center [homepage on the internet]. Japan: Association of Ministry of Health, Labour and Welfare Online Resources, Inc.; c2000-01 [Cited 2017 February 21]. Available from:

http://www.nanbyou.or.jp/entry/3992

8. Yoshida Y. 71 Neurofibromatosis. In: Kelly, A. P., Taylor, S. C., editors. Taylor and Kelly's Dermatology for skin of Color 2nd eds. McGrawHill Press; 2016: p. 499-504.
9. Niimura M. Neurofibromatosis in Japan, In: Ishibashi Y, Hori Y (eds): Tuberous sclerosis and neurofibromatosis epidemiology, pathophysiology, biology and management. Excerpta Medica, Amsterdam, the Netherlands: Elsevier Science Publishers; 1990: 22-31.
10. Fisher MJ, Loguidicie M, Gutmann DH, et al. Visual outcomes in children with neurofibromatosis type 1-associated optic pathway glioma following chemotherapy: a multicenter retrospective analysis. *Neuro Oncol* 2012; **14**: 790-797.
11. Dulai S, Briody J, Schindeler A, North KN, Cowell CT, Little DG. Decreased bone mineraldensity in neurofibromatosis type 1: result from a pediatric cohort. *J Pediatr Orthop* 2007; **27**: 472-475.
12. Alwan S, Tredwell SJ, Friedman JM. Is osseous dysplasia a primary feature of neurofibromatosis 1 (NF1)? *Clin Genet* 2005; **67**: 378-390.
13. Friedman JM. Neurofibromatosis 1: clinical manifestations and diagnosis criteria. *J Child Neurol* 2002; **17**: 548-554.

14. Friedman JM. Birch PH. Type 1 neurofibromatosis: a descriptive analysis of the disorder in 1,728 patients. *Am J Med Genet* 1997; **70**: 138-143.

15. Ruggieri M, Huson SM. The clinical and diagnostic implications of mosaicism in the neurofibromatosis. *Neurology* 2001; **56**: 1433-1443.

16. Tanito K, Ota A, Kamide R, Nakagawa H, Niimura M. Clinical features of 58 patients Japanese patients with mosaic neurofibromatosis 1. *J Dermatol* 2014; **41**: 724-728.

17. Garcia-Romero MT, Parkin P, Lara-Corrales I. Mosaic Neurofibromatosis Type 1: A Systematic Review. *Pediatr Dermatol* 2016; **33**: 9-17.

18. Maruoka R, Takenouchi T, Torii C, et al. The use of next-generation sequencing in molecular diagnosis of neurofibromatosis type 1: a validation study. *Genet Test Mol Biomarkers* 2014; **18**: 722-735.

19. Clinical Trials.gov [homepage on the internet]. U.S.A .: A Service of the U.S. National Institutes of Health Online Resources, Inc.; c2000-01 [Cited 2017 February 21].Available from: http://www.clinicaltrials.gov/

**Figure Legends**

**Figure 1)** Gender and age of the 124 patients in the study population.

**Figure 2)** The number of cutaneous neurofibromas. The patients were divided into 4 groups according to the number of cutaneous neurofibromas.

**Figure 3)** The number of cutaneous neurofibromas in each age.

**Figure 4) (a)** Ratios of NF1 patients in each stage. **(b)** Ratios of manifestations in stage 5. We defined severe plexiform neurofibromas as D4a and malignant peripheral nerve sheath tumor as D4b in dermatological manifestations. **(c)** Stages of patients according to age.

Table I. Grade of severity of NF1 by Japanese DNB classification.

| Stage | DNB classification | | | life function and social activity |
|---|---|---|---|---|
| | D | N | B | |
| 1 | D1 | N0 | B0 | no problem in daily life and social activity |
| 2 | D1 | N0–1 | B0–1 | mild problem in daily life and social activity |
| | D2 | N0–1 | B0–1 | |
| 3 | D3 | N0 | B0 | mild problem in daily life and severe problem in social activity |
| 4 | D3 | N1 | B0–1 | moderate problem in daily life and severe problem in social activity |
| | D3 | N0–1 | B1 | |
| 5 | D4 | AnyN | AnyB | severe problem in daily life due to physical abnormality |
| | AnyD | N2 | AnyB | |
| | AnyD | AnyN | B2 | |

**Dermatological manifestations**

| | |
|---|---|
| D1 | pigmented macules and a few neurofibromas |
| D2 | pigmented macules and many neurofibromas |
| D3 | numerous neurofibromas ( over 1000 number, > 1 cm in size ) |
| D4 | severe plexiform neurofibromas or malignant peripheral nerve sheath tumor |

**Neurological manifestations**

| | |
|---|---|
| N0 | no neurological symptom |
| N1 | neurological symptoms (e.g., paralysis or pain etc) or/and abnormal neurological findings |
| N2 | severe or progressive neurological symptoms |

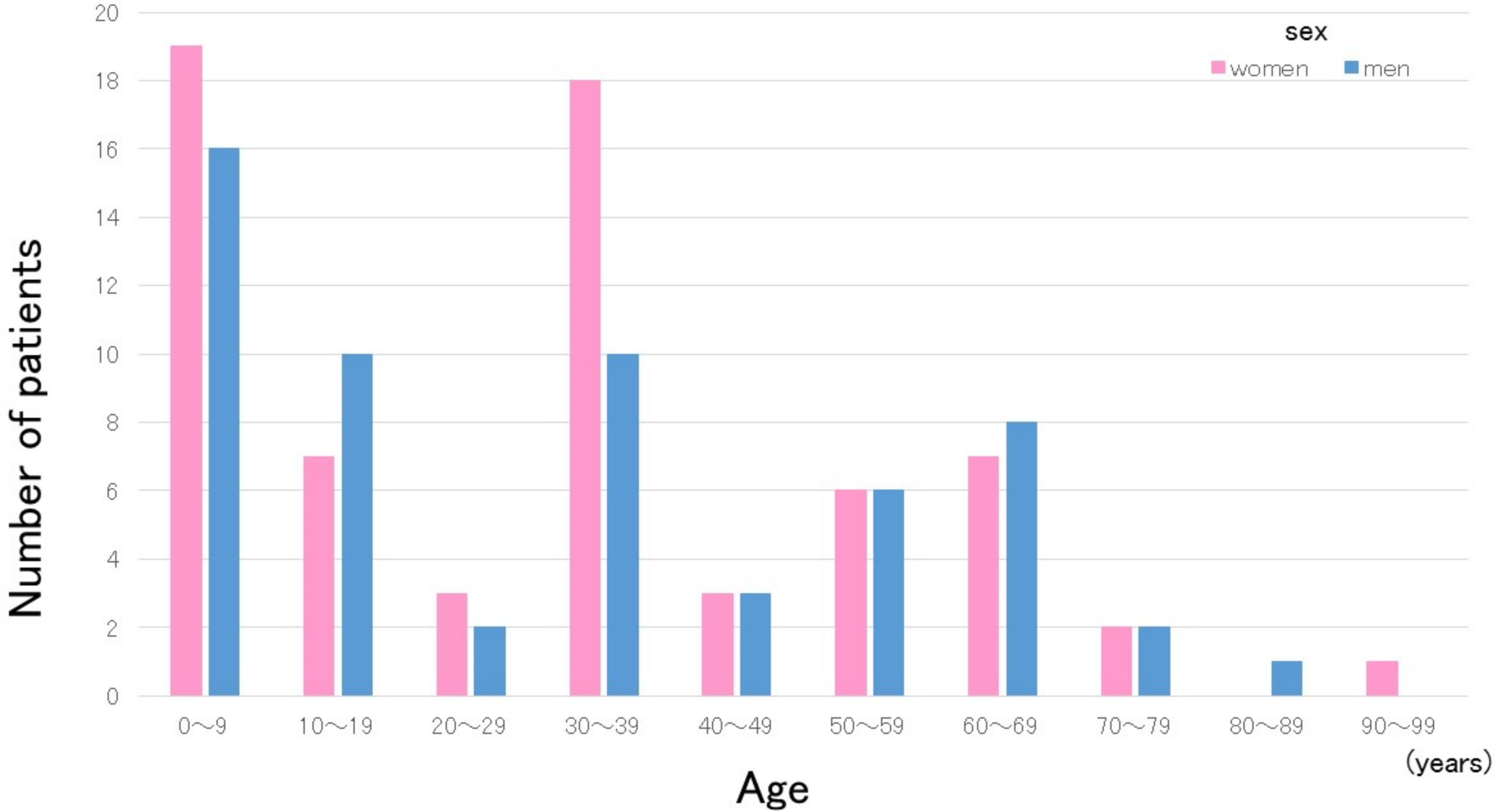
**Bone manifestations**

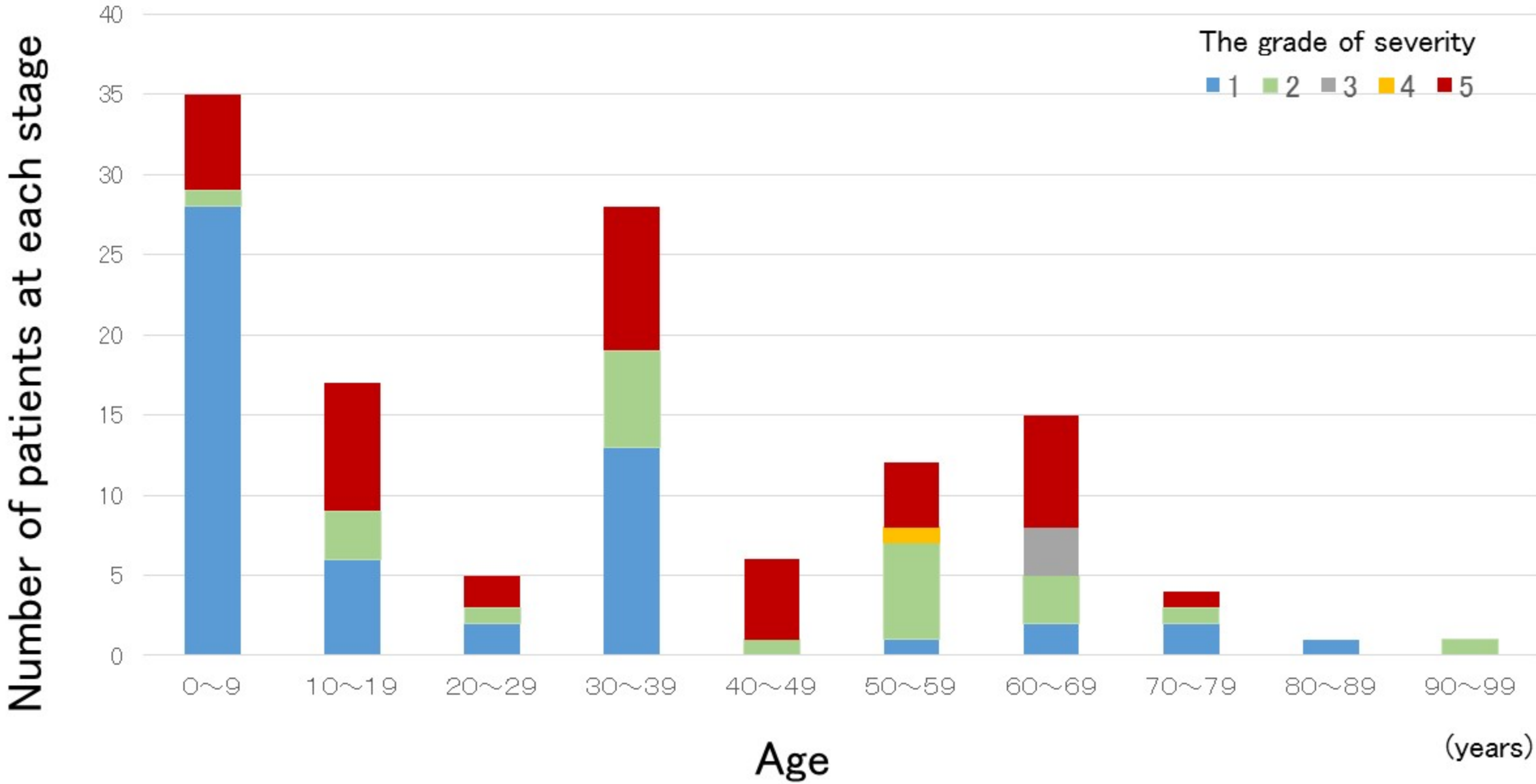
| | |
|---|---|
| B0 | no bone lesion |
| B1 | mild or moderate bone lesion ( deformity in spine or extremities that does not need treatment) |
| B2 | severe bone lesion ( dystrophic type or spine deformity that needs surgical treatment (e.g., scoliosis or kyphosis), severe bone deformity in extremities (e.g., pseudoarthrosis, fracture) or defect of the skull or facial bone |

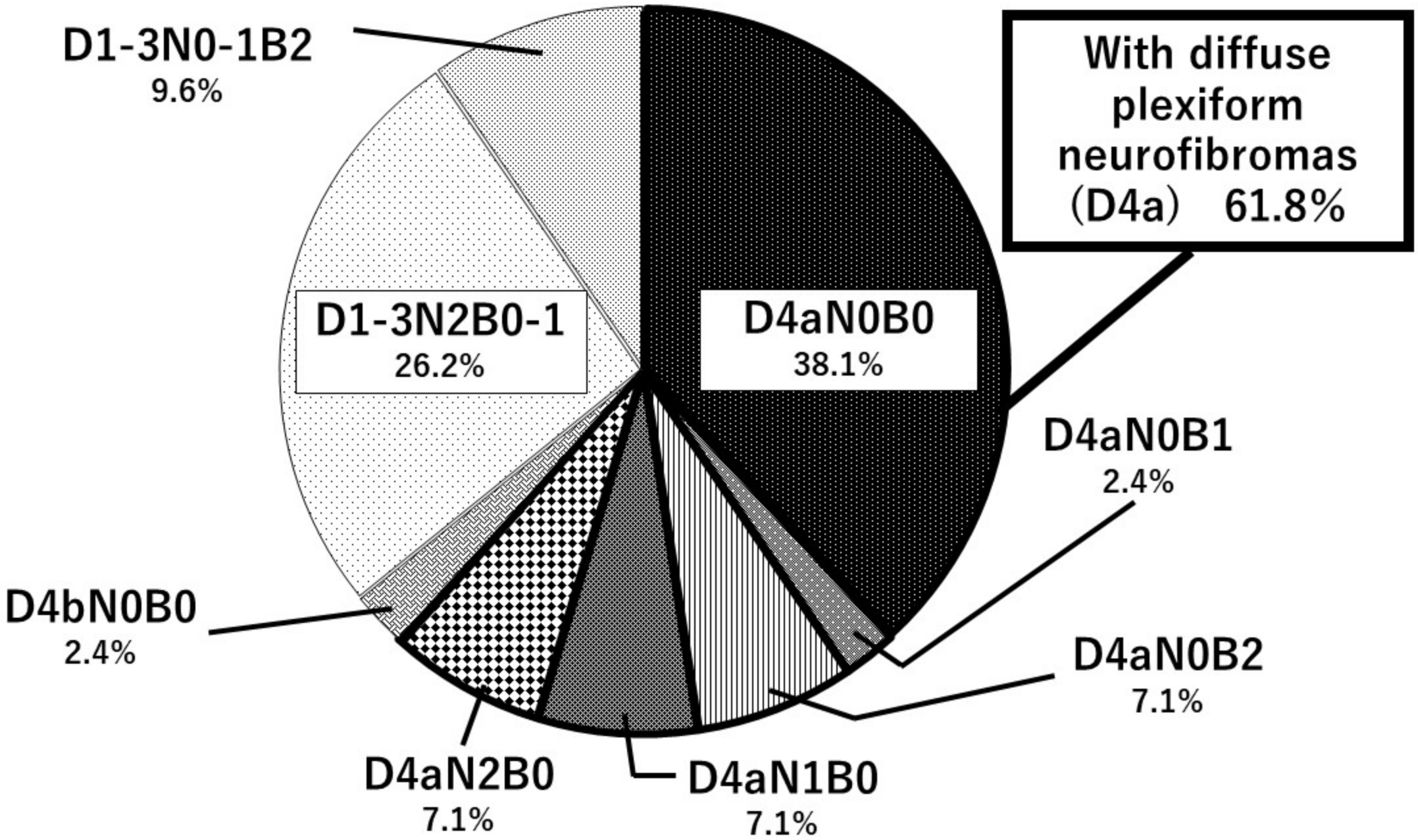
Table II. Epidemiology of dermatological, neurological and bone manifestations in NF1.

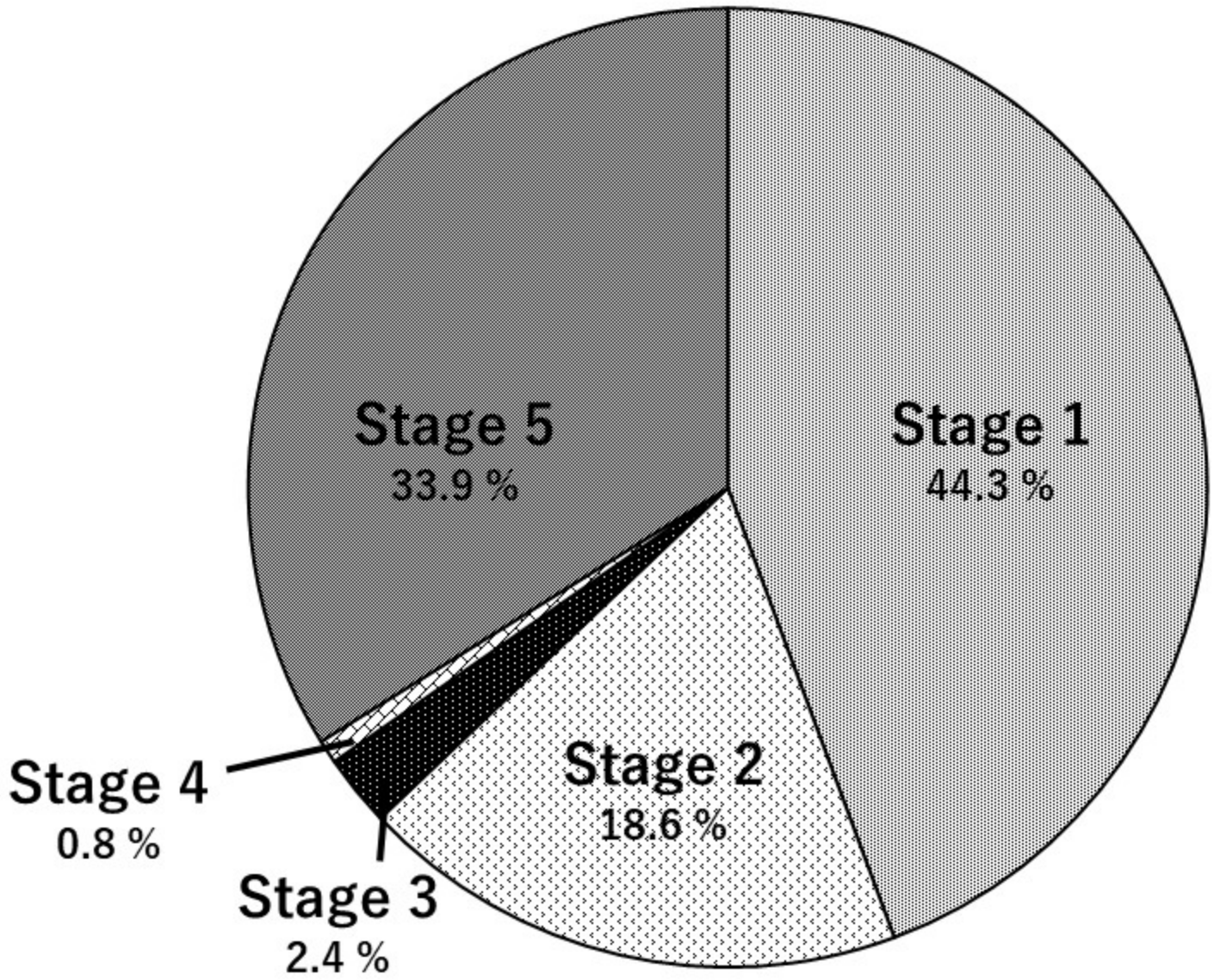
| manifestations | | | the frequency in our study | the frequency in previous Japanese report[9] |
|---|---|---|---|---|
| dermatological manifestations | cutaneous neurofibromas | ＜10 | 40.3 % | 95 % |
| | | ＜100 | 26.6 % | |
| | | ＜1000 | 25.8 % | |
| | | ≧1000 | 7.3 % | |
| | diffuse plexiform neurofibromas | | 21 % | 10 % |
| | nodular plexiform neurofibromas | | 11.3 % | 20 % |
| | malignant peripheral nerve sheath tumor (MPNST) | | 0.8 % | 2 % |
| neurological manifestations | optic nerve glioma | | 1.6 % | 1 % |
| | cerebral glioma | | 2.4 % | Not shown |
| bone manifestations | spine deformity | | 8.1 % | 10 % |
| | deformity of the skull or facial bone | | 4.0 % | 5 % |
| | pesudoarthrosis | | 0.8 % | 3 % |

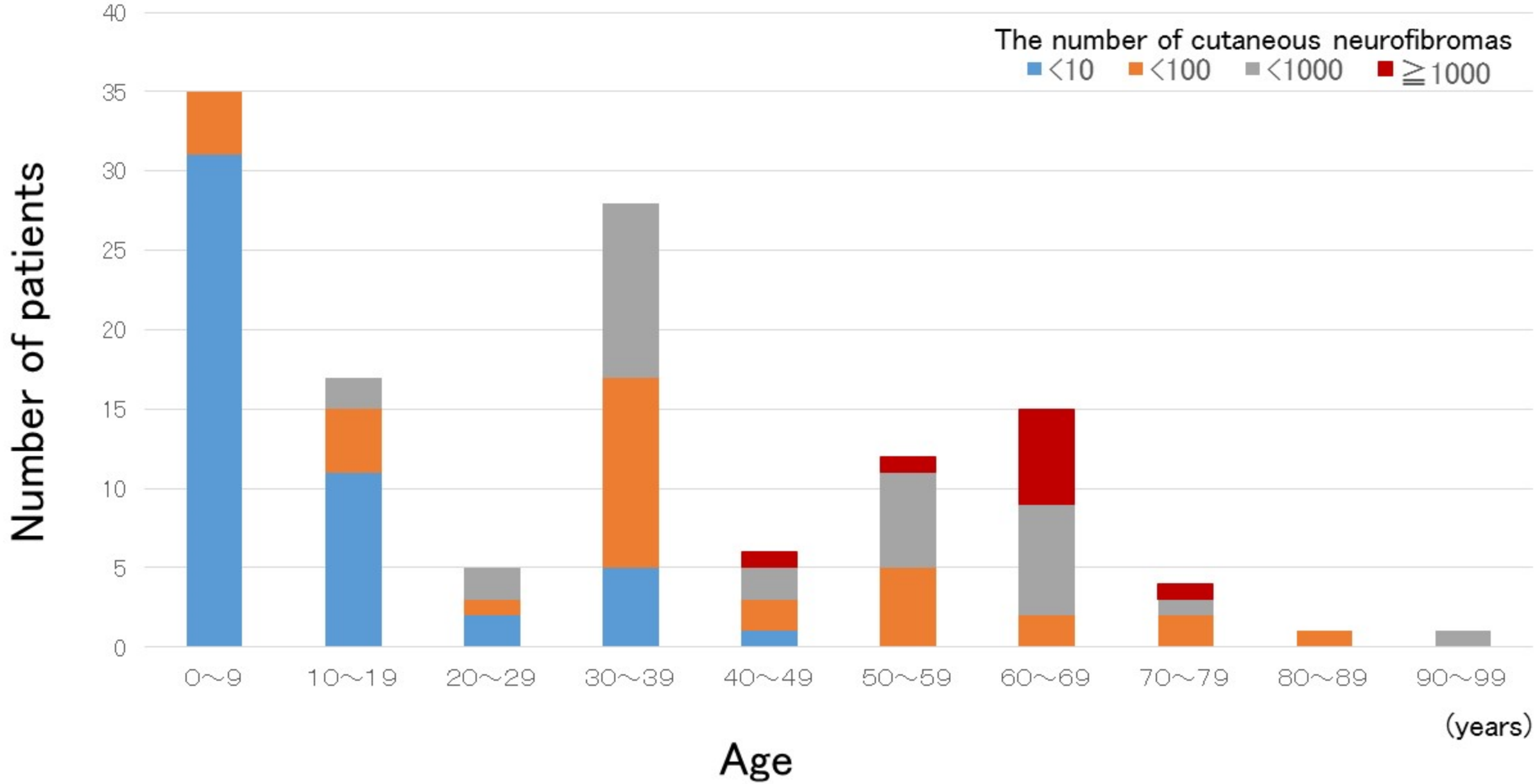
9) Niimura M et al. Neurofibromatosis in Japan: epidemiology, pathophysiology, biology and management, *Excerpta Medica, Amsterdam* 1990 : 22–31.

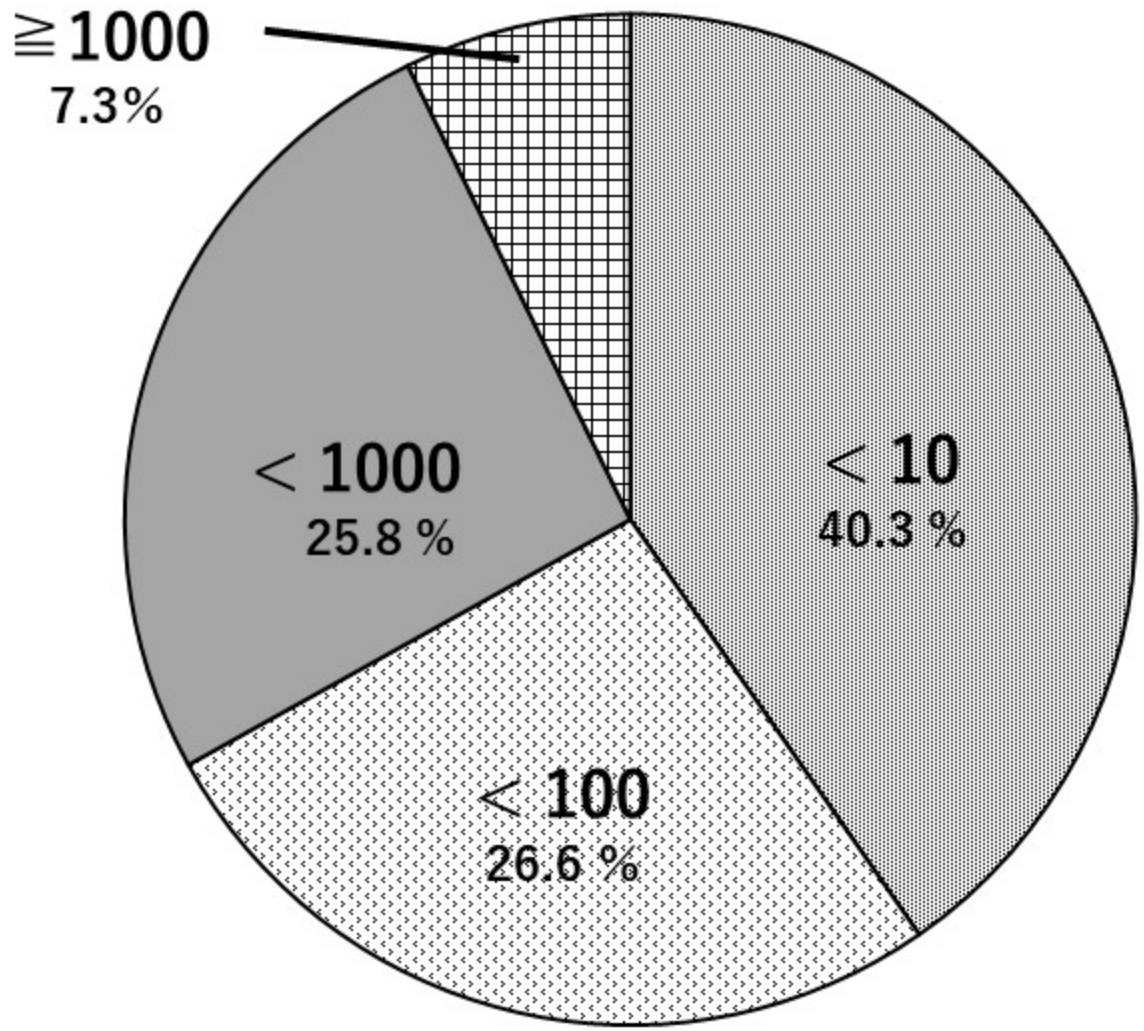

sex
women
men
Number of patients
20
18
16
14
12
10
8
6
4
2
0
0～9
10～19
20～29
30～39
40～49
50～59
60～69
70～79
80～89
90～99
(years)
Age


Number of patients at each stage
40
35
30
25
20
15
10
5
0
The grade of severity
1
2
3
4
5
0～9
10～19
20～29
30～39
40～49
50～59
60～69
70～79
80～89
90～99
Age
(years)


D1-3N0-1B2
9.6%
With diffuse plexiform neurofibromas (D4a) 61.8%
D1-3N2B0-1
26.2%
D4aN0B0
38.1%
D4aN0B1
2.4%
D4bN0B0
2.4%
D4aN0B2
7.1%
D4aN2B0
7.1%
D4aN1B0
7.1%


Stage 5
33.9 %
Stage 1
44.3 %
Stage 4
0.8 %
Stage 2
18.6 %
Stage 3
2.4 %


The number of cutaneous neurofibromas
<10
<100
<1000
≧1000
Number of patients
40
35
30
25
20
15
10
5
0
0～9
10～19
20～29
30～39
40～49
50～59
60～69
70～79
80～89
90～99
(years)
Age


≧1000
7.3%
＜1000
25.8 %
＜10
40.3 %
＜100
26.6 %