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Abstract:

Venous malformations are histopathologically benign, but can greatly impair patients' quality of life. Sclerotherapy is known to be effective to improve symptoms without scar, but surgical resection of residual lesions is sometimes necessary due to inadequate reduction. However, there is no consensus on what criteria should be used to consider switching to surgical treatment, and individualized decisions must be made for each case.

To investigate the factors that contribute to the lack of efficacy of sclerotherapy in reducing lesions and how to predict this, we performed a retrospective clinical imaging and histopathological study of six cases of labial vein malformations treated with sclerotherapy and three cases without sclerotherapy. Clinical image investigations are based on magnetic resonance imaging (MRI) before and after sclerotherapy. We found a significant decrease in the percentage of cystic component in the total lesion of venous malformations after sclerotherapy. Histopathological investigations are based on resected VMs with or without sclerotherapy. Elastica van Gieson stains suggested significant increase in fibrotic tissue inside VMs treated with sclerotherapy compared to those without.

In conclusion, MRI signal changes inside the venous malformations after sclerotherapy was observed, and it may reflect fibrosis of the tissue. These changes in the venous

malformations after sclerotherapy may reduce the effect of sclerotherapy on tissue reduction should be considered.

Keywords: venous malformations, sclerotherapy, volumetry, histopathology, clinical image

Introduction

Venous malformations (VMs) are abnormalities in the morphology of blood vessels that are present since birth, and although histopathologically a benign disease, they can cause morphological abnormalities, pain, and bleeding that can greatly impair a patient's quality of life.¹ Sclerotherapy is less invasive than surgery and is often the first choice when conservative treatment fails to improve symptoms and may be useful in cases where surgical scarring is a problem or total removal is challenging.² While sclerotherapy alone can be successful in some cases, surgical resection of residual lesions is sometimes necessary due to inadequate reduction.³ However, there is no consensus on what criteria should be used to consider switching to surgical treatment, and individualized decisions must be made for each case.

To investigate the factors that contribute to the lack of efficacy of sclerotherapy in reducing lesions and how to predict this, we studied venous malformations of the lips,

which are easy to evaluate in size, from a clinical imaging and histopathological point of view.

Materials and Methods

We searched medical records for venous malformations of the lips treated between February 2003 and March 2021 at the Department of Plastic and Reconstructive Surgery, Kobe University Hospital, and selected patients who underwent resection of the same lesion due to insufficient regression of the lesion after sclerotherapy (combined sclerotherapy group). Clinical information such as histopathology, magnetic resonance (MR) imaging (MRI) before and after sclerotherapy, number of sclerotherapy sessions, type of sclerosing agent, and time between the last sclerotherapy session and surgical resection were collected, and cases lacking any of these details were excluded from the study. The group that underwent only surgical resection without sclerotherapy (surgical resection alone group) was selected for histopathological comparison.

[Histopathological review]

A histopathological comparison between the surgical treatment alone and combined sclerotherapy groups was performed. The section with the largest area was selected as the excised pathology specimen. If there were sections of a similar area, the section that best reflected the histological findings of the VM was selected. One hematoxylin and

eosin stain and one Elastica van Gieson (EVG) stain were prepared for each section.

These histopathology images were captured using a BZ-X700(A) microscope (Keyence, Osaka, Japan) and processed with Fiji®.⁴ After background subtraction processing, the area of the entire lesion, including the cystic area (total area) and the area of the section excluding the cystic area (non-cystic area), were measured (Figure 1). Based on these measurements, the non-cystic area ratio (non-cystic area/total area) was calculated as the ratio of the non-cystic area to the entire lesion. Based on the EVG-stained images, color deconvolution was performed using three colors (red, purple, and yellow), which are the results of staining mainly for collagen fibers, elastic fibers, and muscle fibers, and the area of tissue stained with each color (red-stained area, purple-stained area, and yellow-stained area) was calculated by analyzing the particle function (Figure 2).

The thresholds for area measurements were automatically set using the IJIsodata algorithm. Based on these, the ratio of the area stained with each color to the area of the entire cross-section, excluding the cystic area (red-stained area/non-cystic area, purple-stained area/non-cystic area, yellow-stained area/non-cystic area) was calculated and compared between the sclerotherapy and surgical treatment alone groups.

[Clinical imaging study]

A clinical imaging study was performed on the MR images taken before sclerotherapy and before surgical treatment in the combined sclerotherapy group (Figure 3). Patients

who underwent treatment other than sclerotherapy between pre- and post-sclerotherapy MRI were excluded. The total volume of the lesion was measured as the total volume based on each T2 short-term inversion recover the horizontal cross-sectional image. The growth region function was used to extract high-signal regions from the lesion and measure their volume as the cystic lesion volume. Based on the measured results, the ratio of the high-signal area to the total volume was calculated as the cyst-to-total ratio (cystic lesion volume/total volume), and changes were observed before and after sclerotherapy.

EZR[®] was used for statistical analysis, and the study was conducted using a t-test.

Results

Nine cases were studied: six in the sclerotherapy group and three in the surgical treatment alone group (Supplemental Tables 1 and 2).

In the combined sclerotherapy group, there were one male and five female patients, all of whom were primarily concerned with cosmetic problems: four were infiltrative, and two were focal. In addition to sclerotherapy, three patients underwent partial excision of the lesions, one patient underwent cryotherapy, and one patient underwent dye laser irradiation. A mean of 5.3 sclerotherapy sessions were performed on excised lip VMs,

and polidocanol, absolute ethanol, and monoethanolamine oleate were used as the sclerosing agents. Resection after sclerotherapy was partial in all cases.

In the surgical treatment alone group, there were two male patients and one female patient, all of whom presented with cosmetic problems, one case was of the infiltrative type, and two cases were of the focal type. The patient with the infiltrative type underwent partial resection of a lesion outside the lip area. For VMs of the lips, one patient with the infiltrative type underwent partial resection of the lip lesion, and two patients with the focal type underwent total resection.

Evaluation by histopathology

Six patients in the sclerotherapy group and three patients in the surgical treatment alone group were included in the study (Supplemental Table 3). The mean percentage of non-cystic areas per pathology section was 85% in the sclerotherapy group and 80% in the surgical treatment group, with no significant difference ($p=0.467$). The percentage of red-stained areas (red-stained area/non-cystic area) on EVG staining was significantly higher in the combined sclerotherapy group ($p=0.003$). The percentage of purple- and yellow-stained areas (purple-stained area/non-cystic area, yellow-stained area/non-cystic area) was not significantly different between the two groups ($p=0.702$ and $p=0.083$).

Evaluation by clinical imaging (MRI)

Of the six patients in the combined sclerotherapy group, two patients who underwent partial resection of the lesion between MRI scans were excluded from the study, and four patients were included in the study (Supplemental Table 4). The mean number of sclerotherapy sessions between MRI scans was 1.5 (median, 2.5). The mean volume of the lesions after sclerotherapy was 114.4% (median, 102.8%) of that before sclerotherapy, with no significant difference ($p=0.595$). The difference tended to be smaller, although not statistically significant ($p=0.207$).

Discussion

Sclerotherapy for venous malformations uses sclerosing agents such as absolute ethanol, polidocanol, sodium tetradecyl sulfate, bleomycin, and pingyangmycin.⁵

Although the detailed mechanisms differ among these agents, they are known to cause tissue fibrosis by damaging the vascular endothelium, forming thrombi, and exciting an inflammatory response in the surrounding connective tissue.⁶⁻⁸ The choice of sclerosing agents is at the discretion of the facility and physician, with no firm evidence.⁹

It has been suggested that, lesions that are cystic or localized on clinical imaging and contrast medium tend to respond better to treatment.⁹⁻¹³ However, it is common for lesions to regrow or cause recurrent pain after sclerotherapy, and multiple sessions of sclerotherapy are often necessary. Additionally, repeated sclerotherapy sessions may

result in lesion failure to regress, necessitating surgical resection of the remaining lesion.^{3,8} These cases are often associated with the need for sclerotherapy. In these cases, it is possible that the sclerotherapy may have altered the lesion in some way. To avoid ineffective sclerotherapy, it is desirable to know the histological changes that may have occurred and how to predict them preoperatively. However, this has not been adequately studied, and there is no consensus on the stage at which sclerotherapy should be replaced with surgical resection. Therefore, empirical decisions must be made on a case-by-case basis.

We have focused our study on VMs of the lips, where the morphology and size of the lesion are clinically easier to assess by both physicians and patients, and have examined the pathological and clinical imaging changes in VMs due to sclerotherapy. We believe that the size of lip lesions over time is easier to assess than at other sites, both subjectively and objectively.

Our study suggests that collagen fibers were increased in oral VMs after sclerotherapy compared with VMs without sclerotherapy on histopathological images with EVG staining. In addition, the clinical images showed no significant change in lesion size and a trend towards a decrease in the percentage of high-signal areas relative to the entire lesion. This suggests that replacement of fibrous tissue occurs in the VM after sclerotherapy, which may be reflected by a decrease in high-signal areas on clinical

images. We believe that the decrease in the amount of cystic tissue that could be treated and the stiffness of the fibrotic tissue, which makes it challenging for the tissue to shrink, may contribute to the refractoriness of the treatment.

Sclerotherapy does not allow for the collection of histopathological specimens, making it challenging to confirm tissue fibrosis histologically and develop a future treatment plan. However, if the percentage of high-signal areas on MRI decreases in VMs after sclerotherapy, it is necessary to consider the possibility that tissue fibrosis is progressing, and sclerotherapy may be less effective in reducing the lesion size.

This study has several limitations. Limitations are as follows: the number of patients studied was small; the size of the VM was not evaluated grossly; the MR imaging conditions were not standardized, and high-signal areas had to be extracted subjectively; the analysis based on EVG staining of pathological tissue may not accurately reflect the amount of collagen, elastic, and muscle fibers in the tissue; the sclerosing agents used were not standardized; the study was performed retrospectively. Further studies are required in the future.

MRI signal changes inside the VM after sclerotherapy may reflect fibrosis of the tissue, and the possibility that these signal changes in the VM after sclerotherapy may reduce the effect of sclerotherapy on tissue reduction should be considered.

Conflicts of Interest and Source of Funding

This study was funded by Grant-in-Aid for Scientific Research(C). The authors have no COI to disclose.

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Figure 1. Example of histopathological analysis based on HE stained slides.

- . A HE-stained slide of the VM from patient 6. Tissue fibrosis was observed.
- a. Mask of HE-stained area including small cysts within the slide. This area was calculated as total area.
- b. Mask of HE-stained area without small cysts within the slide. This area was calculated as non-cystic area.

Figure 2. Example of histopathological analysis based on EVG stained slides.

- . An EVG-stained slide of the VM from patient 6. Red, purple and yellow stained areas were extracted from this image by imageJ®.
- a. Mask of red stained lesion from the EVG-stained slide, which is supposed to reflect the amount of collagen fiber.
- b. Mask of purple stained lesion from the EVG-stained slide, which is supposed to reflect the amount of elastic fiber.
- c. Mask of yellow stained lesion from the EVG-stained slide, which is supposed to reflect the amount of muscle fiber.

Figure 3. Clinical Picture and example of clinical image investigation based on patient 6 MRI.

. Clinical Picture of patient 6 before surgery. She had undergone sclerotherapy 2 times, resulted in limited regression. Elastic hard mass was left at the upper lip.

a. STIR MRI axial image of the upper lip. VM was pointed manually as shown in the axial image. Surrounded area was considered as VM and VM's total volume was calculated based on all the axial slices.

b. Extracted image of VM from the STIR MRI. High intensity lesion was extracted as a cystic area which is described as light green lesion.

c. 3D image of total lesion of the VM based on MRI.

d. 3D image of cystic lesion of the VM based on MRI.

Supplemental Table 1. List of sclerotherapy group patients.

6 patients are included in the sclerotherapy group. 4 of them had infiltrative type and the others had focal type. They all complained about

cosmetic deformities. Sclerosing agents were variable among patients. All of them underwent partial resection after sclerotherapy.

Supplemental Table 2. List of surgical treatment alone group patients

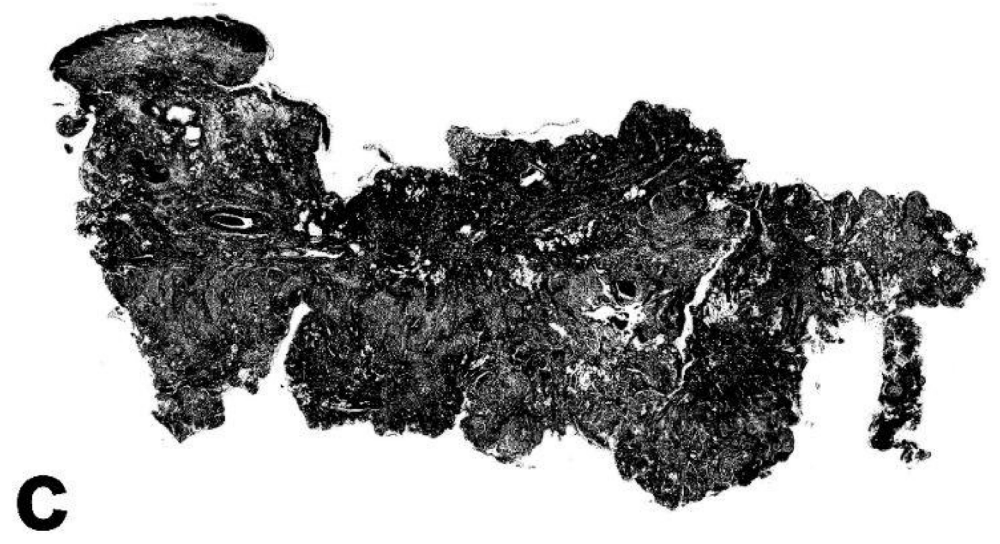
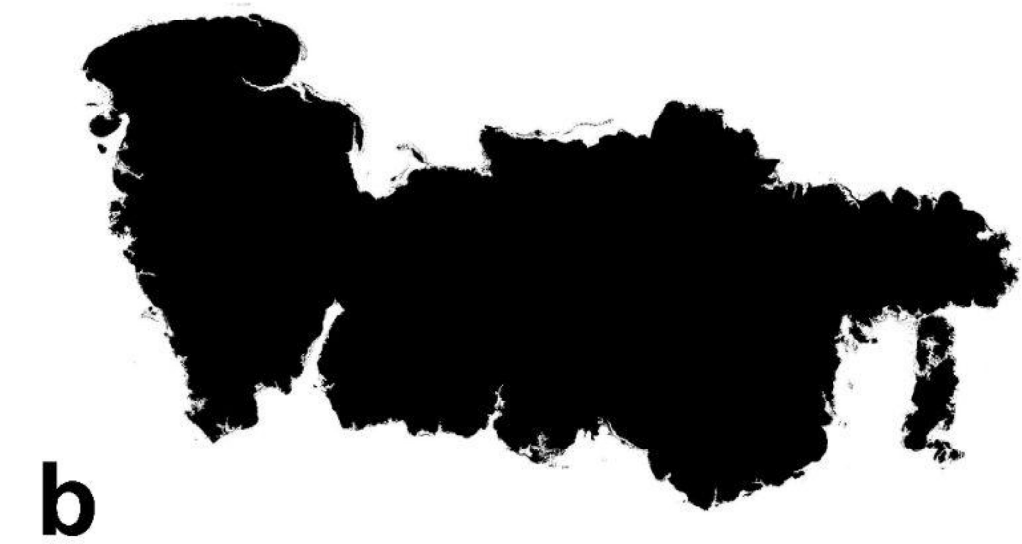
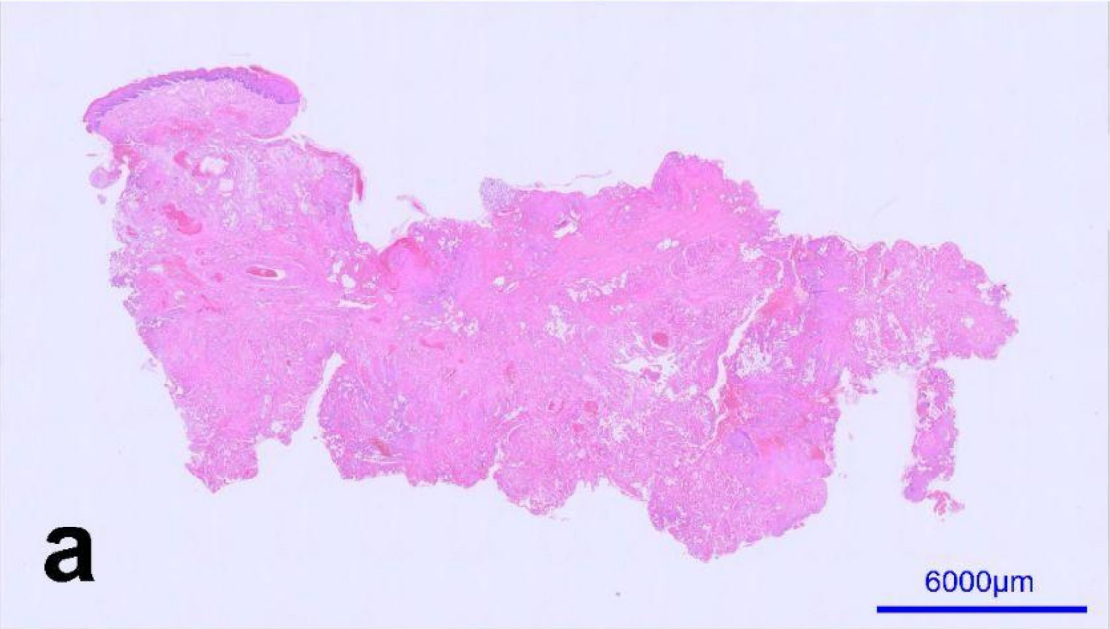
3 patients are included in the surgery group. Only one patient had an infiltrative type VM. They all complained about cosmetic deformities. Two of them underwent total resection, whereas one patient underwent partial resection.

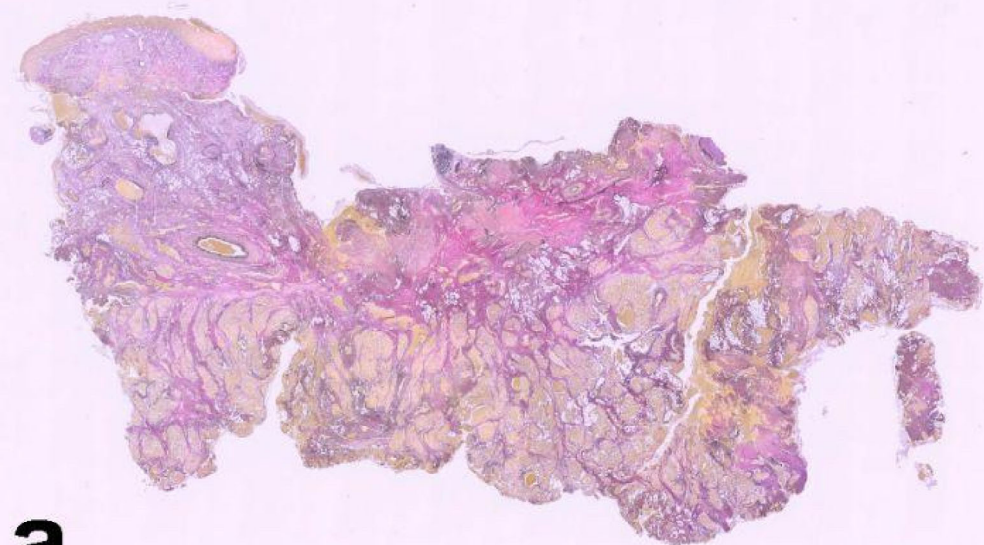
Supplemental Table 3. Results of histopathological evaluation.

Red-stained area percentage was significantly higher in the sclerotherapy and surgery group, when compared by the surgery group, which indicates the increase of fibrosis($p=0.003$). Purple-stained and yellow-stained areas were not significantly different between two groups.($p=0.702,0.083$). Non cystic area percentage showed no significant difference either($p=0.467$).

Supplemental Table 4. Results of MRI evaluation before and after sclerotherapy.

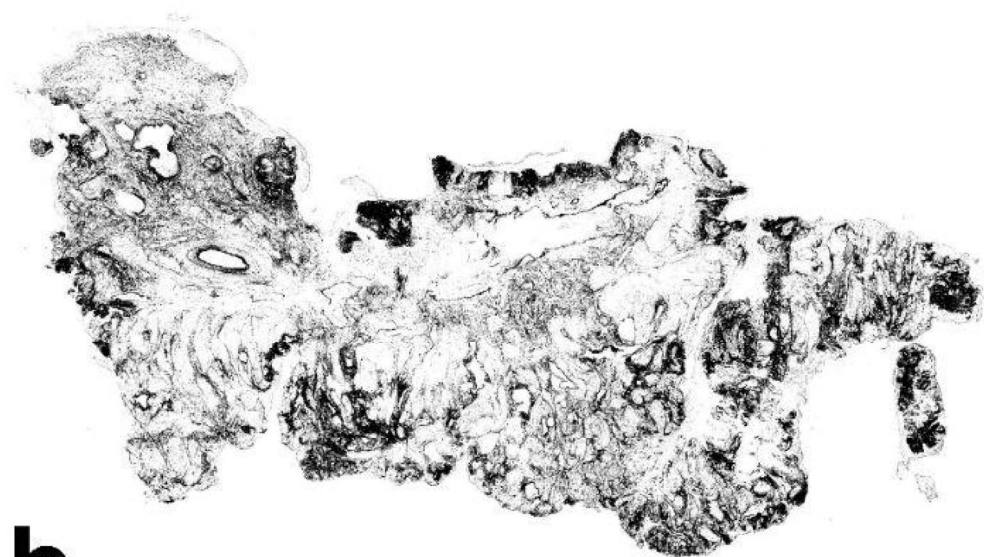
The total volume of VMs based on MRI volumetry was not significantly different when compared before and after sclerotherapy($p=0.595$). Cyst-to-total volume ratio, which was calculated based on T2-STIR MRI, was not significantly different either($p=0.207$).



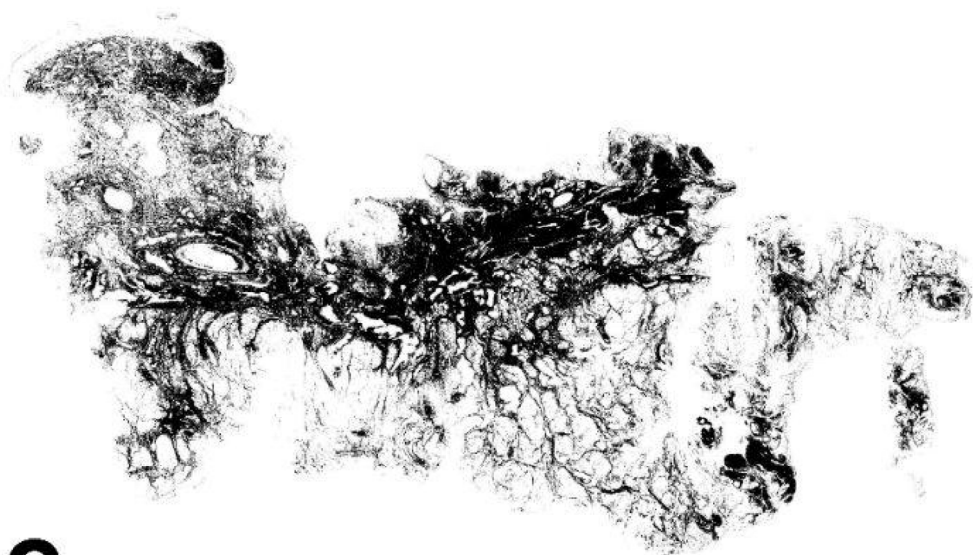


a

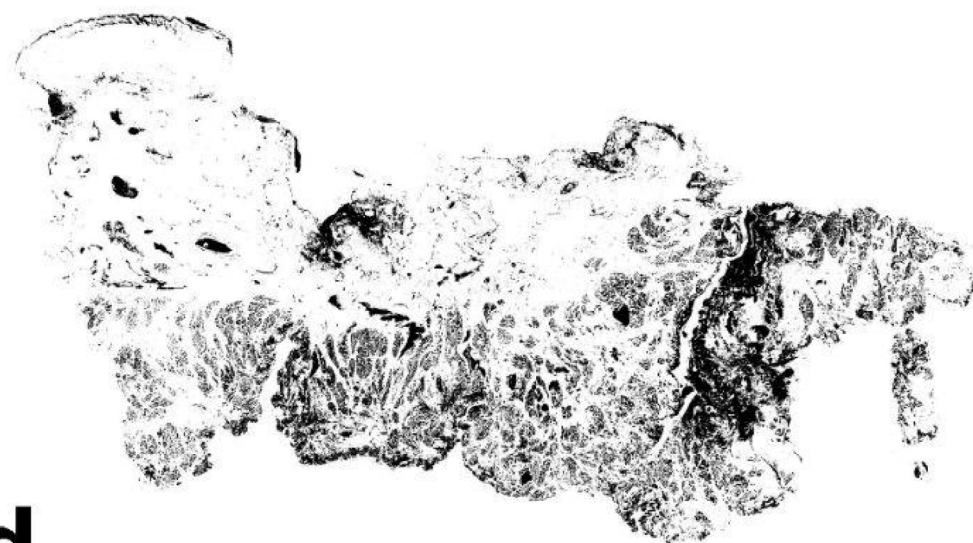
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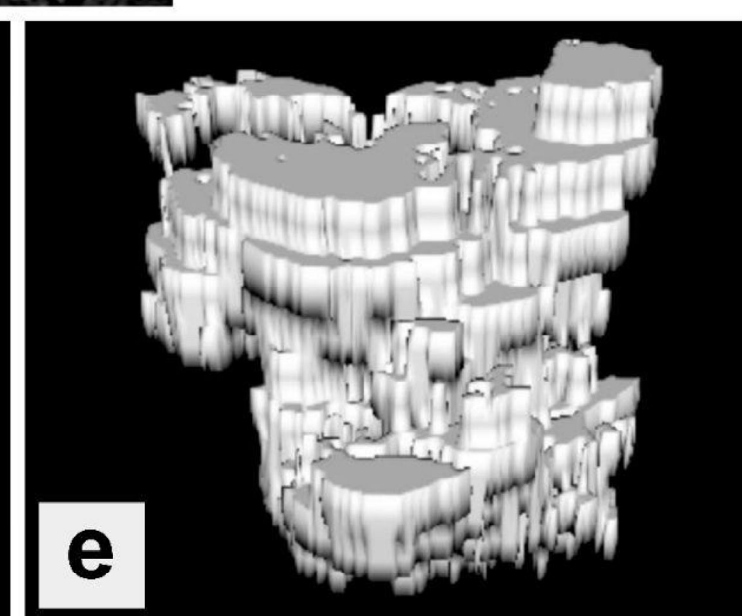
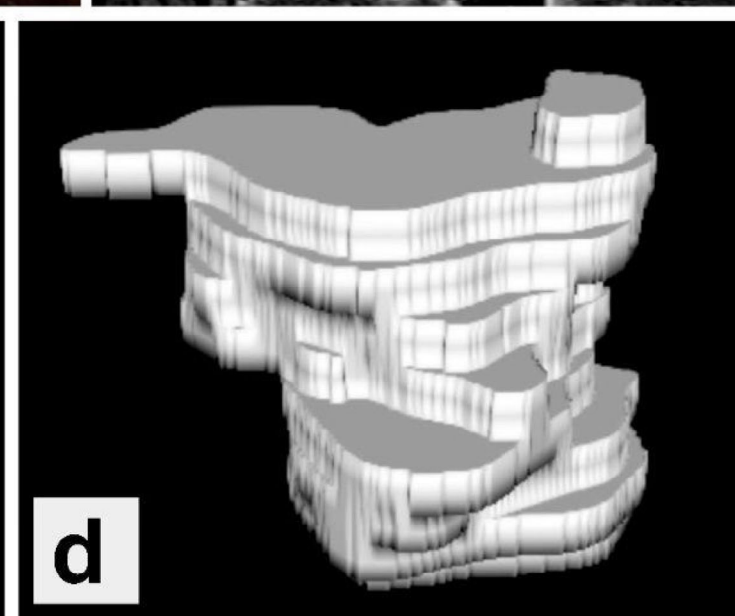
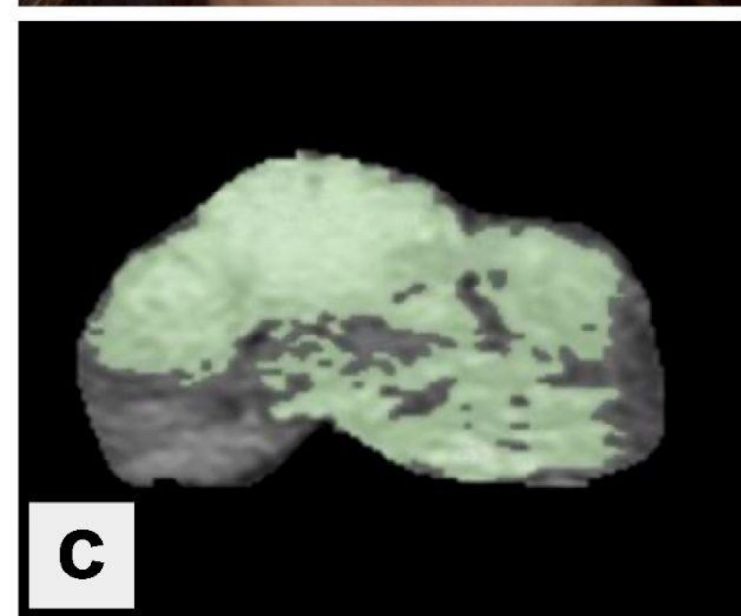
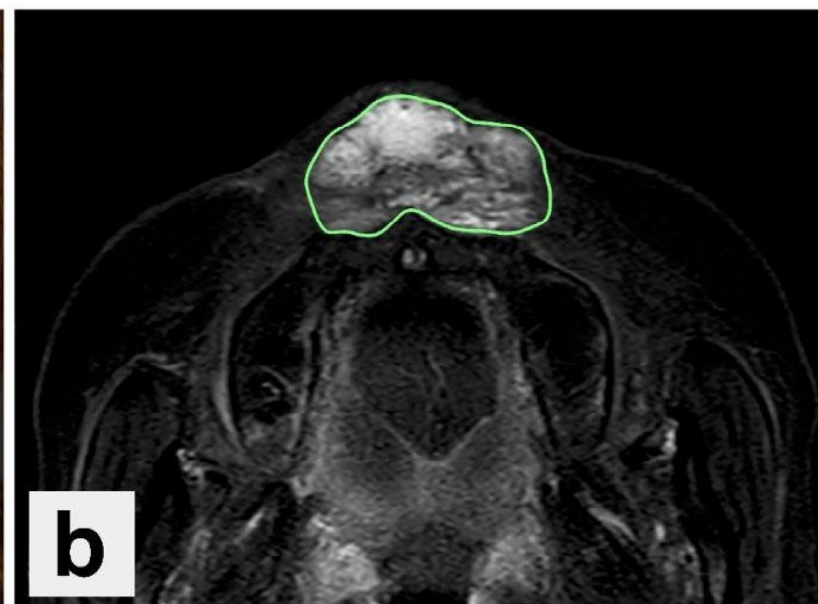
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Supplemental Table 1. List of sclerotherapy group patients.

Patient No.	sex	age	location	type	chief complaint	resection	sclerosing agents
1	male	17	lower left lateral and medial lip, tongue, chin, cheek	infiltrative	cosmetic deformities	partial	EO,PO
2	female	48	lower left lateral and medial lip	infiltrative	cosmetic deformities	partial	EO,PO
3	female	36	lower left lateral and medial lip	infiltrative	cosmetic deformities	partial	PO
4	female	19	lower left lateral and medial lip	focal	cosmetic deformities	partial	EO,PO,ET
5	female	45	lower right lateral and medial lip	infiltrative	cosmetic deformities	partial	EO,PO,ET
6	female	35	upper left lateral and medial lip	focal	cosmetic deformities	partial	EO,PO

ET:Absolute ethanol, PO:Polidocanol, EO:Monoethanolamine

Supplemental Table 2. List of surgical treatment alone group patients

Patient No.	sex	age	location	type	chief complaint	resection
7	male	68	lower lip~neck	infiltrative	cosmetic deformities	partial
8	female	73	upper left lateral lip	focal	cosmetic deformities	total
9	male	24	upper left lateral lip	focal	cosmetic deformities	total

Supplemental Table 3. Results of histopathological evaluation.

group	Patient No.	non-cystic area percentage	red-stained area percentage	purple-stained area percentage	yellow-stained area percentage	conducted therapy except sclerotherapy	No. of sclerotherapy before resection
sclerotherapy and surgery	1	92.47%	42.96%	16.85%	35.87%	partial resection	6
	2	88.88%	51.78%	30.21%	16.33%	partial resection	3
	3	95.65%	55.00%	27.50%	42.32%	partial resection, cryotherapy	13
	4	90.15%	53.05%	18.66%	43.02%	partial resection	3
	5	66.32%	50.25%	22.78%	13.90%	none	6
	6	80.75%	46.09%	38.50%	26.32%	none	2
average		85.70%	49.86%	25.75%	29.63%		
surgery	7	75.66%	31.51%	25.52%	18.69%	none	
	8	79.56%	37.70%	28.35%	11.05%	none	
	9	86.38%	38.68%	29.19%	11.97%	none	
average		80.53%	35.96%	27.69%	13.91%		
<i>p value</i>		<i>0.467</i>	<i>0.003</i>	<i>0.702</i>	<i>0.083</i>		

non-cystic area percentage: area without cystic lesion / total area including cystic lesion

red-stained area percentage: red stained area from EVG staining / non-cystic area

purple-stained area percentage: purple stained area from EVG staining / non-cystic area

yellow-stained area percentage: yellow stained area from EVG staining / non-cystic area

Supplemental Table 4. Results of MRI evaluation before and after
sclerotherapy.

Patient No.	pre sclerotherapy volume(cm3)	post sclerotherapy volume(cm3)	volume change	pre sclerotherapy cyst-to- total ratio(%)	post sclerotherapy cyst-to- total ratio(%)	cyst-to-total volume ratio change	Sclerotherapy sessions between MRI investigations
2	9.4	7.9	84.1%	73.5%	79.09%	107.57%	1
4	2.4	5.4	218.7%	72.8%	25.47%	34.97%	2
5	2.3	0.8	33.3%	97.21%	76.20%	78.39%	6
6	15.1	18.4	121.6%	67.41%	58.34%	86.55%	1
average	6.0	6.7	102.85%	73.17%	67.27%	82.47%	1.5
<i>p value</i>			0.595			0.207	