

The Significance of Immunohistochemical Staining, Including that for Glucose Transporter Protein Isoform 1, as Related to the Clinical and Angiographic Features of Adult Soft-Tissue Hemangioma and Arteriovenous Malformation in the Head and Neck¹

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Purpose: Glucose transporter protein isoform 1 (GLUT1) has been introduced to diagnose the hemangiomas of infancy. We investigated the usefulness of several immunohistochemical markers, including GLUT1, as related with the clinical and radiologic findings for making the diagnosis of adult subcutaneous vascular lesions in the head and neck.

Materials and Methods: The 24 patients who underwent operations for soft tissue vascular lesion during the previous 7 years were included in this study. We analyzed the angiographic data, the clinical data and the immunohistochemical study results, including the GLUT1, S-100 protein and Movat pentichrome staining.

Results: Twenty-two patients were confirmed to have arteriovenous malformation (AVM) and two hemangiomas, respectively. The number of lesions with positive Movat pentichrome, S-100 and GLUT1 staining in the patients with AVM and those patients with hemangioma were 22/22, 20/22 and 0/22, and 0/2, 0/2 and 0/2, respectively. For the 22 patients with AVMs, eight had a soft tissue vascular lesion at birth, 13 had cutaneous change and 15 had a change of the size of the lesion. For the 2 patients with one hemangioma each, neither patient had a soft tissue vascular lesion at birth, and both patients had cutaneous change and a change of the size of the lesion. The angiograms revealed a focal hypervascular mass (19/24) or diffuse staining (5/24) without showing significant features for making the definitive differential diagnosis.

Conclusion: Our study revealed that none of the patients with AVM or hemangioma had GLUT1 positivity, and an arteriovenous malformation was more common than the adult-type hemangioma.

Index words : Head and neck, hemangioma
Angiography
Glucose transporter type 1
Arteriovenous malformations

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Mulliken and Glowacki in 1982 first introduced a new classification of vascular malformations based on their clinical and histologic characteristics (1-3), and since then two categories have been used to classify these vascular anomalies, i.e., hemangioma and arteriovenous malformation (AVM). This classification was adopted in 1996 and then it was expanded by the International Society for the Study of Vascular Anomalies (4-6). They suggested that the terms "hemangioma of infancy" or "infantile hemangioma" be used to denote the classical type of hemangioma that appears in early infancy and has a rapid growth phase and it shows a positive expression of glucose transporter protein isoform 1 (GLUT1) (7). GLUT1 is currently being introduced to definitively diagnose he-

mangiomas of infancy in questionable cases (8).

Vascular lesions are also common in adults, and vascular tumors are the most common childhood tumors.

Table 1. The Clinical and Pathologic Presentation of Adult Subcutaneous Vascular Lesions in the Head and Neck

	AVM (n=22)	Hemangioma (n=2)
Size change/growth of the lesion	15 (68%)	2
Presence of an overlying skin lesion	13 (59%)	2
Presence of elastic fibers	22 (100%)	0
Presence of nerve fibers	20 (91%)	0
GLUT1 positivity	0	0

Note: AVM = arteriovenous malformation, presence of elastic fibers = positive Movat pentichrome staining, presence of nerve fibers = positive S-100 staining

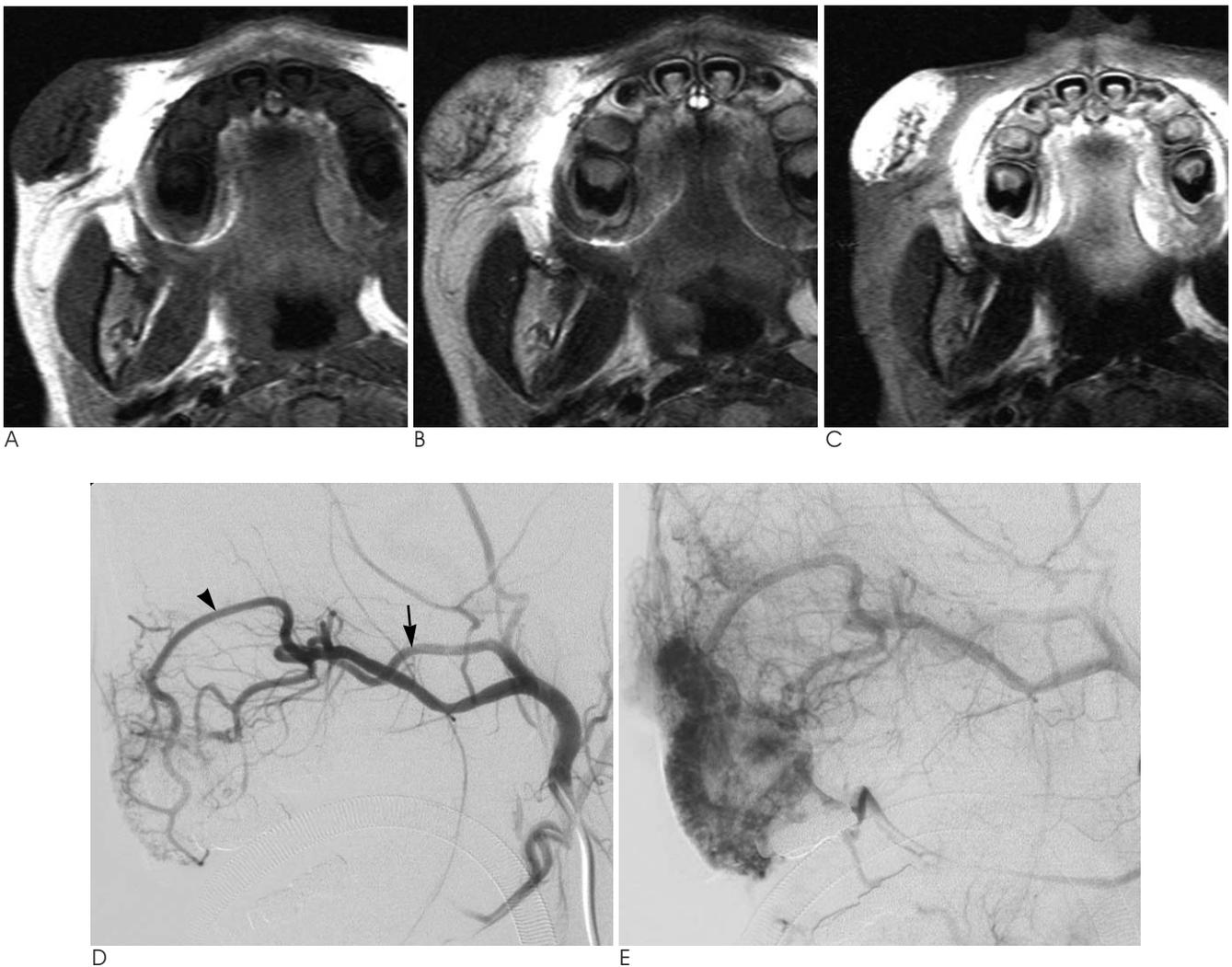


Fig. 1. A nine-year-old boy presented with a vascular mass that was confirmed as an arteriovenous malformation in his right cheek. The MR image shows a low-signal-intensity lesion on the T1-weighted image (A), high signal intensity on the T2-weighted image (B), and strong enhancement after Gadolinium administration (C). The external carotid arteriograms show multiple feeders of the external carotid arterial branches (D) and a rather well-circumscribed hypervascular mass with early draining veins (E). The lesion was supplied by both the transverse facial artery (arrow in D) and the infraorbital artery (arrowhead in D) and the lesion drained into both the facial vein and the superficial cutaneous vein.

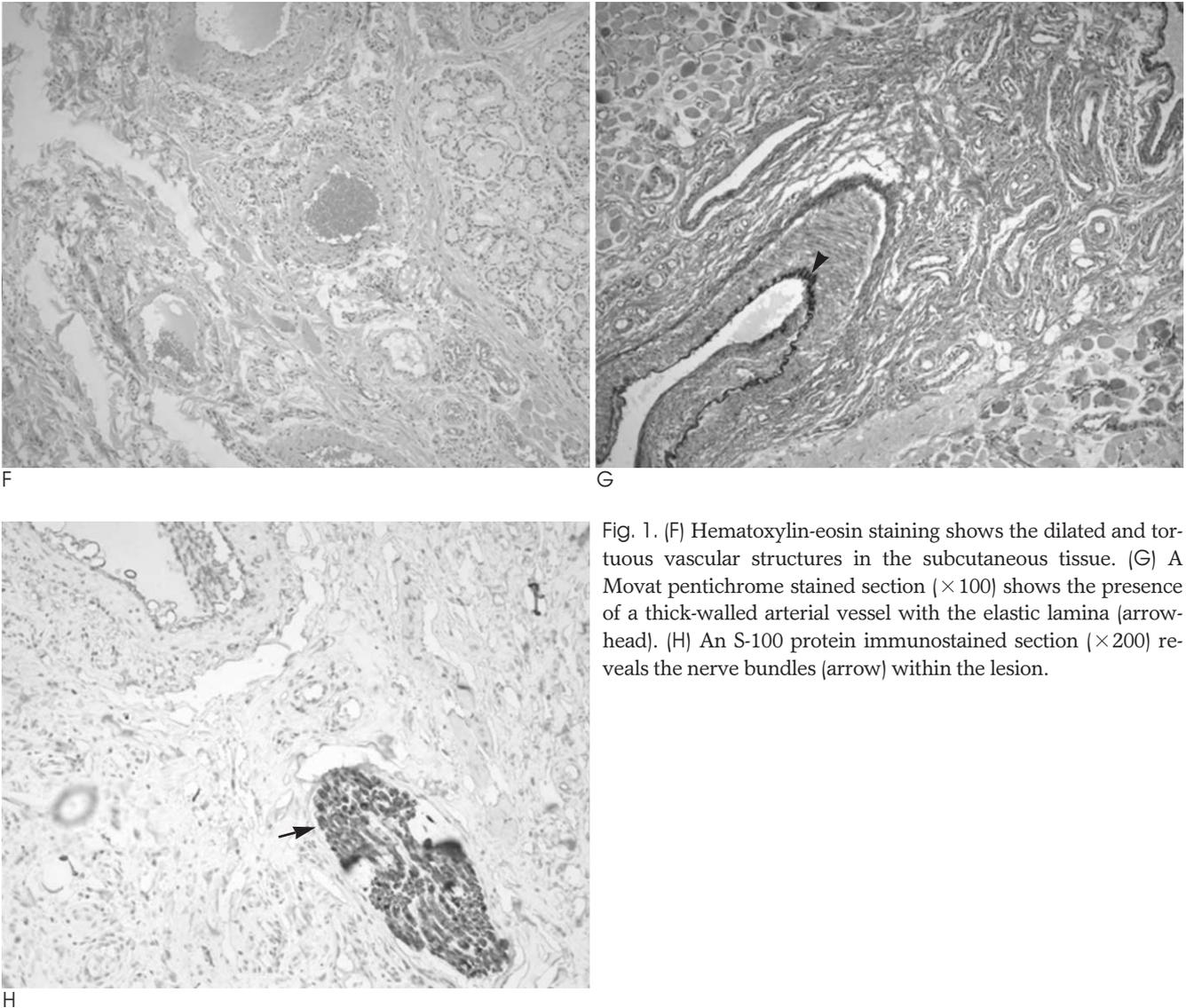


FIG. 1. (F) Hematoxylin-eosin staining shows the dilated and tortuous vascular structures in the subcutaneous tissue. (G) A Movat pentichrome stained section ($\times 100$) shows the presence of a thick-walled arterial vessel with the elastic lamina (arrowhead). (H) An S-100 protein immunostained section ($\times 200$) reveals the nerve bundles (arrow) within the lesion.

They can cause significant morbidity and even mortality in both children and adults (9). Although the patient's history and the findings on a physical examination can distinguish vascular tumors from AVMs with a diagnostic accuracy greater than 90% (1), the diagnosis and management of vascular lesions continue to present diagnostic and therapeutic challenges, and their management is made more difficult by the use of inconsistent nomenclature. Many authors use the term hemangioma to describe AVMs, although the term hemangioma is also frequently applied generically and indiscriminately to refer to vascular lesions that have entirely different histology and behavior from hemangiomas (6-8, 10). Cavernous hemangioma is a classic example of this discrepancy because in reality there is no such entity as this term refers to either a deep hemangioma or to a venous malformation (5, 11).

We hypothesize that adult-type subcutaneous vascular lesions may differ from the vascular birthmarks of childhood. We attempted to differentiate adult-type vascular anomalies from infantile hemangiomas by using immunohistochemical staining for GLUT1 and S-100 protein, as well as Movat pentichrome histochemical stain (elastic stain). In addition to the pathologic diagnosis, we analyzed the clinical features and the angiographic findings of adult-type soft tissue vascular lesions of the head and neck.

Materials and Methods

Patient Population

Our study materials were retrieved from the prospectively collected neuroangiographic data registry. This study included 24 consecutive patients (August, 1999 to

January, 2006) who had benign vascular lesions of the head and neck; there were nineteen male and five female patients with an age range between 8–56 years and a mean age of 30 years. Patients were included in the study when they presented with a longstanding large subcutaneous mass without distinctive skin color change and then they underwent pre-operative angiography and/or preoperative embolization or sclerotherapy followed by surgical resection and immunohistochemical staining. The definition of adult-type hemangioma in our study was a large soft-tissue hemangioma in the head and neck that did not show proliferative and involutionary phases at a patient age of more than two years. Patients were excluded when the vascular lesions were identified as being the infantile type or the pathologic studies were incomplete.

The lesions were located in the scalp, cheek, upper lip, lower lip, forehead, submandibular area, auricular area and neck. This study was approved by our institutional review board.

Specimens & Immunohistochemistry

We acquired twenty-four tissue samples with the corresponding hematoxylin-eosin (H & E) glass slides for review and the formalin-fixed, paraffin-embedded tissue blocks for conducting further histochemical and immunohistochemical studies. Immunohistochemical staining was performed by a conventional streptavidin-biotin method using a LSAB kit (Dako, Denmark) for GLUT1 (Neomarkers, CA, U.S.A., 1:100) and S-100 protein (Zymed, CA, U.S.A., 1:500). For all the antibodies, the tissue sections were pre-treated for antigen retrieval by steam heating in 10 mM citrate buffer (pH 6.0) at 90 °C for 60 minutes. The samples with cytoplasmic GLUT1 positivity and S-100 protein-reactive nerve fibers were examined.

Movat pentachrome histochemical staining was performed using a conventional method to identify the presence of dense elastic lamina in the vascular walls (12–14).

Clinical Features

We investigated the following clinical findings: (1) the

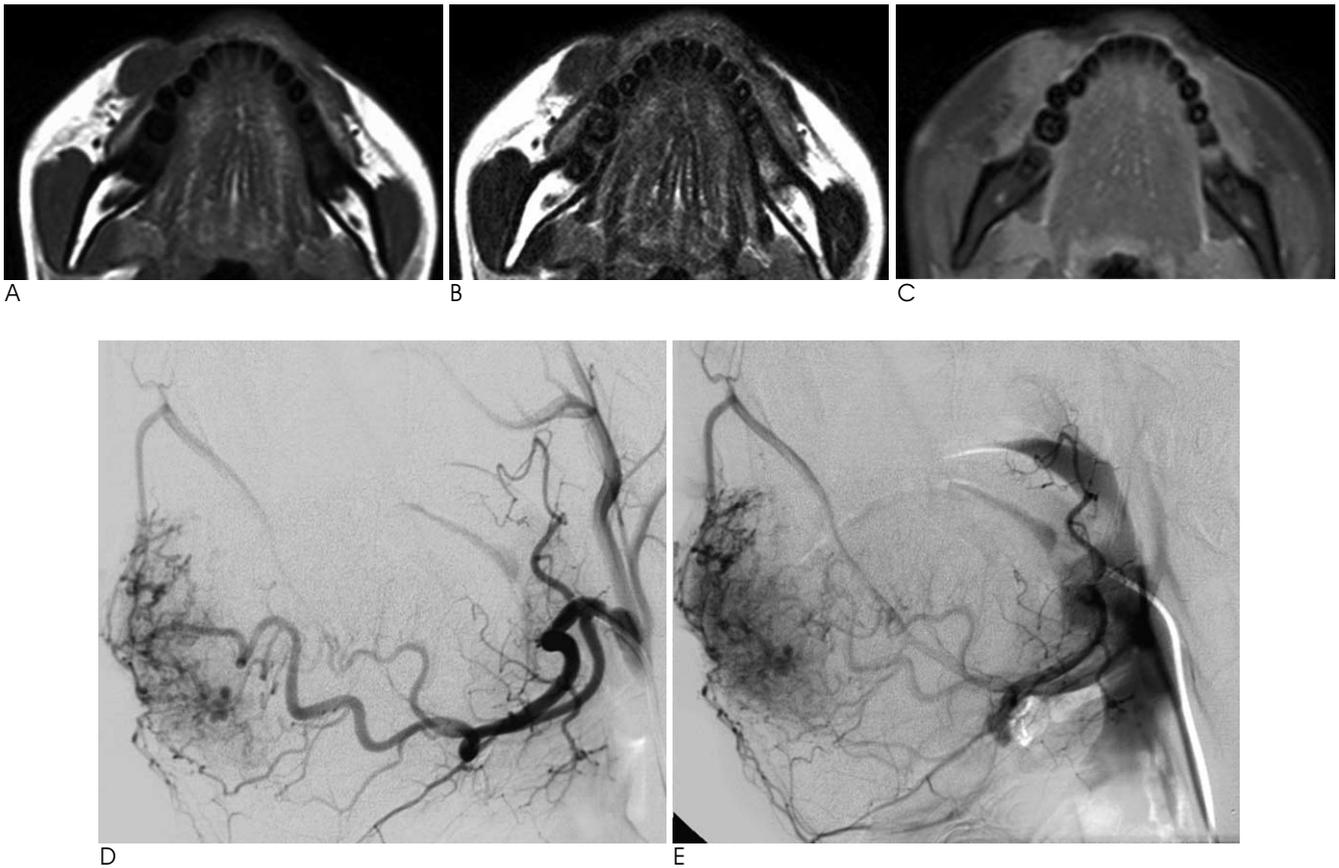
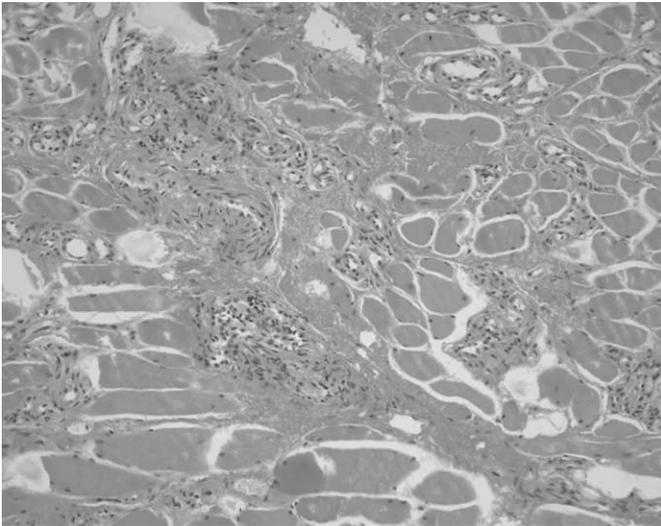
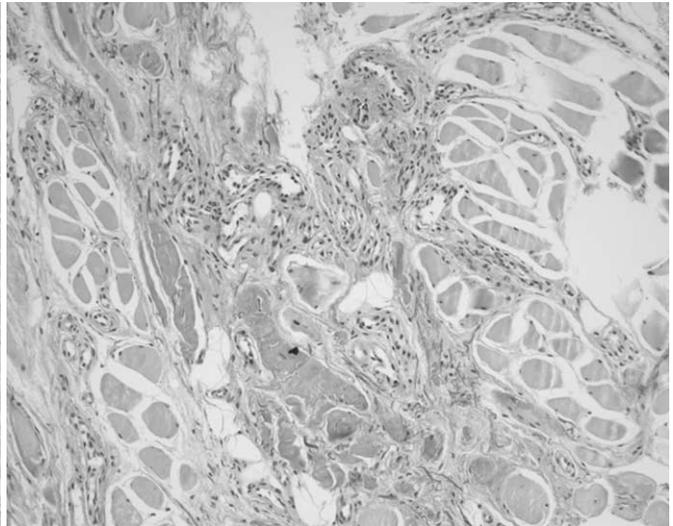


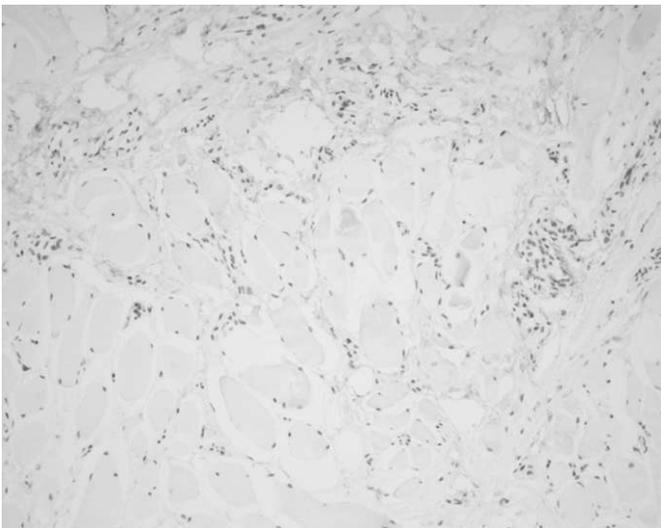
Fig. 2. A thirty-year-old female with an intramuscular hemangioma in the right cheek. The hemangioma shows isosignal intensity similar to that of muscle on the T1-(A) and T2-(B) weighted images. (C) An enhanced T1-weighted image shows minimal enhancement of the lesion. (D & E) Lateral views of the facial arteriogram reveal hypervascular staining and the early draining veins.



F



G



H

Fig. 2. (F) There were multiple microvascular formations between the skeletal muscle fibers (H & E staining, $\times 100$). Negative elastic staining (G, $\times 100$) as well as the lack of nerve tissue on the S-100 protein immunostaining (H, $\times 100$) confirmed the diagnosis of hemangioma.

presence of an overlying skin lesion although the skin color change had no clinical significance for the diagnosis, (2) the presence of the lesion at birth and (3) the size change of the lesion.

Angiographic Findings

The conventional angiography images were retrospectively reviewed by two radiologists who worked in consensus. The angiography was assessed for the following features: (1) lesion distribution, i.e., localized (mass forming) or diffuse (15), (2) the presence of parenchymal staining, (3) the size and number of the arterial feeders and draining veins, and (4) early filling of the draining vein.

Results

Histological and Immunohistochemical Features

The histological and immunohistochemical features confirmed there were twenty-two AVMs and two hemangiomas without any overlap in the pathologic findings (Table 1). The AVMs histologically showed scattered or loosely conglomerated medium-sized to large-sized, thick-walled vessels in the dermis or subcutaneous tissue. All 22 cases were proven to contain arterialized vascular components on elastic staining. Non-neoplastic structures, including peripheral nerve fibers, were identified in between the vascular lumina either on H & E staining (22 cases) or on S-100 protein immunostaining (20 cases). The two hemangiomas consisted of pyogenic granuloma (lobular capillary heman-

gioma) and intramuscular hemangioma. None of the specimens showed reactivity for GLUT1.

Clinical Features

The clinical presentations of the vascular lesions in the head and neck are summarized in Table 1. A significant size change of the AVM lesions occurred for 15 patients (68%) at the median age of 18 years (range: 6–33); a significant change of the lesion occurred at 16 and 29 years of age, respectively, for the two patients with hemangioma. Overlying skin lesions with faint discoloration

were noted in 13 patients (59%) with AVMs and in the two patients with hemangiomas.

Angiographic Findings

Most of the patients (77%, *n* = 17) with AVMs had a localized hypervascular mass lesion, except for five AVMs for which the lesion was diffusely distributed in large facial areas. The AVMs were of various sizes and they had a number of arterial feeders and early draining veins (Fig. 1). Two hemangiomas that were a pyogenic granuloma and an intramuscular hemangioma (Fig. 2)

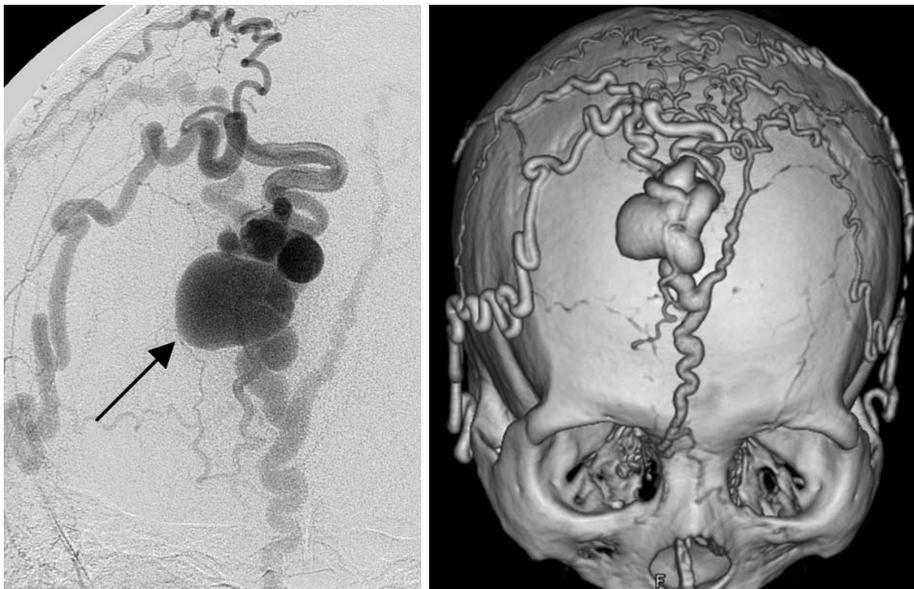
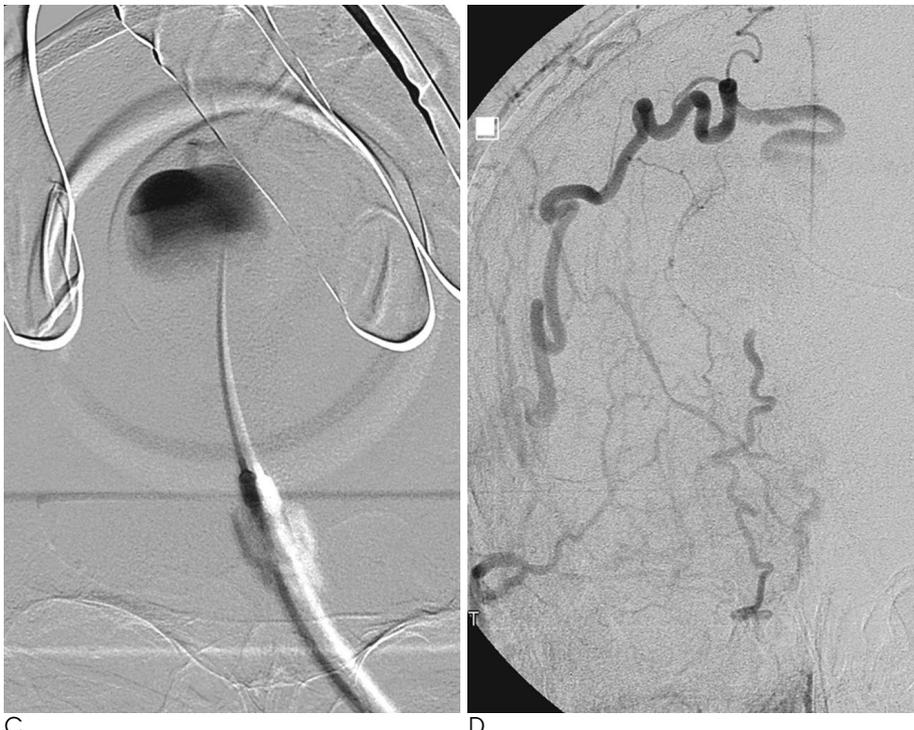
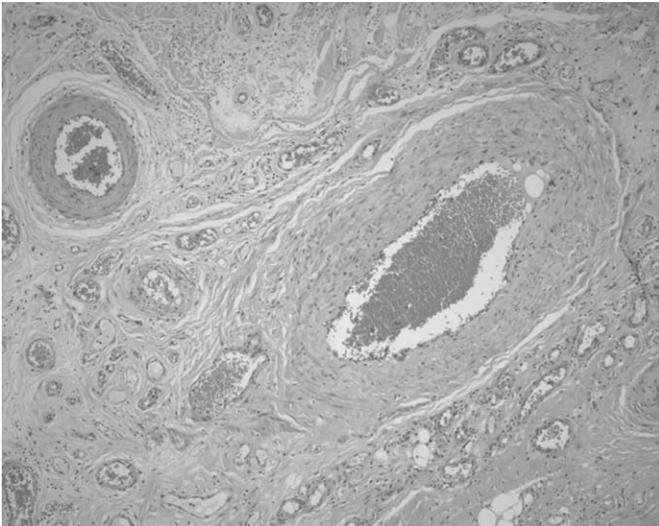
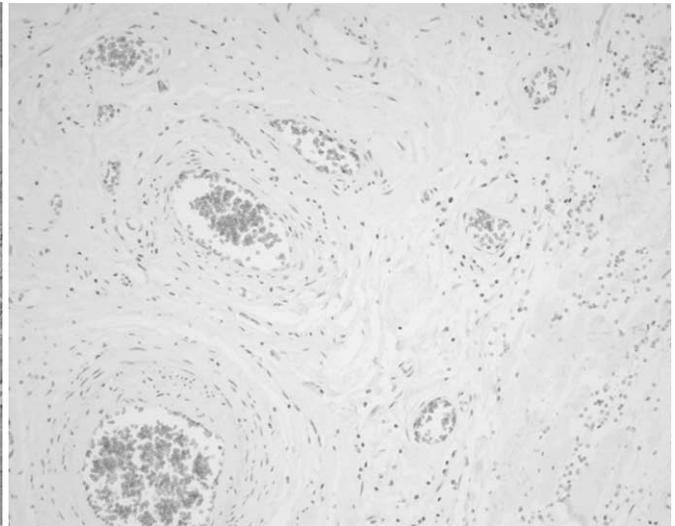


Fig. 3. A 56-year-old male with a progressively growing pulsatile mass on his forehead. (A) The anteroposterior view of the external carotid arteriogram shows a dilated superficial temporal artery supplying a high-flow arteriovenous fistula and a dilated vein (arrow) on the forehead. (B) CT angiography reveals the feeder and draining veins. (C) Note the stasis of the contrast agent on the direct puncture venogram obtained while compressing the draining veins with using a ring compression device before injecting a NBCA and lipiodol mixture. (D) The final angiogram obtained after completed glue embolization reveals no residual fistula.

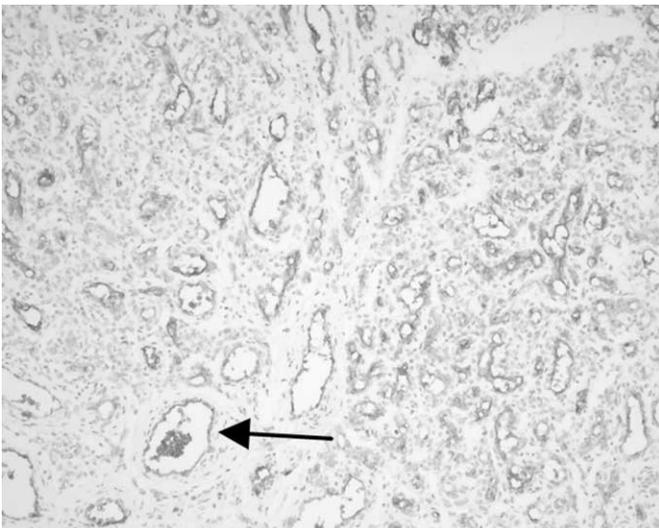




E



F



G

Fig. 3. Multiple malformed arteries (E) on H & E staining ($\times 100$) reveal a negative endothelial immunoreaction for GLUT1 (F, $\times 200$), as compared to the positive immunoreaction for GLUT1 (arrow), in an infantile hemangioma from a control patient who was not included in this study (G, GLUT1 $\times 200$).

were located in the left nasal cavity and in the right cheek of two patients, respectively. The angiographic images showed localized vascular lesions with parenchymal staining. The lesions were supplied by multiple arterial feeders and each had early draining veins (Fig. 3).

Discussion

Previous studies have focused on the differential diagnosis of AVMs and infantile hemangiomas without giving any special attention to discussing the adult-type vascular lesions in the head and neck (1, 2). Our study revealed that most adult subcutaneous vascular lesions are AVMs. None of the adult AVMs and hemangiomas demonstrated GLUT-1 positivity.

Adegboyega et al. studied 91 hemangiomas and 76

AVMs and they reported that nerve bundles are consistently present in AVMs, but these are absent in hemangiomas (13). In our study, immunostaining for S-100 protein, which is a neural marker, was used to boost the credibility of diagnosing AVMs (7, 13, 16). As arteriovenous malformations are a complex network of arterial and venous structures, an elastic stain was also used to confirm the presence of arterialized vessels, thereby establishing the diagnosis of arteriovenous malformation (12, 13). In our study, a combination of nerve fiber staining and dense elastic fiber staining was helpful for establishing the differential diagnosis between AVM and hemangioma.

GLUT1 (Glucose transporter isoform 1) is an immunohistochemical marker that is normally restricted to the endothelial cells that have a blood-tissue barrier function, such as those in the brain and placenta. Specific

microvascular GLUT1 (erythrocyte-type glucose transporter protein) immunoreactivity is present in normal brain cells, placental chorionic villi and placental trophoblasts, but this is undetectable in the vasculature of normal skin and the subcutis layer (3, 7, 8). This suggests that placental migration is a possible etiology for infantile hemangioma. North et al. reported intense immunoreactivity for GLUT1 in all juvenile hemangiomas (7). However, no lesional GLUT1 expression has been detected in any malformations, pyogenic granulomas, tufted angiomas or hemangioendotheliomas, as was also noted in our study (7, 11). Although the positivity for GLUT1 has not yet been generally determined for all types of vascular lesions, our study suggests that adult-type subcutaneous vascular lesions may differ from the birthmarks of childhood as the latter can be GLUT1 positive.

We were able to determine that that hemangioma had a relatively rarer incidence than AVM as our study revealed this tendency. Unlike the infantile hemangioma, adult-type hemangioma showed a negative result for GLUT1 staining even though the number of patients who were enrolled in our study was relatively small, and especially the number of patients with hemangioma. We can possibly add an explanation for the difference between adult-type and infantile hemangioma in terms of the pathogenesis according to the negative GLUT1 staining in the adult-type hemangioma. Further studies are needed to prove this assumption.

This study revealed that AVMs are usually detected at birth and they progressively grow until puberty (9). In contrast, the hemangiomas detected at puberty are growing masses that did not present at birth. The angiographic findings of hypervascularity and early draining veins in our study were not specific for differentiating either of these types of lesion (15).

The accurate diagnosis of vascular lesions of the head and neck remains a challenge for a multidisciplinary clinic. In our study, most of the adult subcutaneous vascular lesions were AVMs, while the incidence of hemangioma was very low for the adult-type subcutaneous vascular lesions. In addition, adult subcutaneous vascular lesions do not present GLUT1 activity, thereby suggesting that they do not originate from the migration of placental tissue. Because of the non-regressive nature

of adult-type vascular lesions, choosing their appropriate management should be determined based on their clinical features.

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성인 두경부 연부조직에서 혈관종과 동정맥기형의 진단에 있어 임상양상 및 혈관조영촬영술 소견과 면역조직화학염색의 의의¹

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목적: Glucose transporter protein isoform 1(GLUT1)은 면역조직화학 염색 표지자로 영유아 혈관종의 확정 진단법으로 최근 알려졌다. 저자들은 임상적, 방사선학적 소견과 더불어 성인두경부의 연부조직 혈관병변에 대한 진단 시 이용되는 GLUT1을 포함한 염색 표지자의 의의를 밝히고자 하였다.

대상과 방법: 최근 7년간 안면혈관병변을 가진 24명에서 수술 전 혈관조영소견과 임상양상을 분석하였으며 병리진단으로 GLUT1, S-100 protein, Movat pentichrome 면역조직화학 염색을 시행하였다.

결과: 병리 상 동정맥기형 22 및 혈관종 2예로 진단되었다. 혈관기형은 모두 Movat pentichrome염색양성, 20예에서 S-100 양성이었다. 반면 혈관종은 Movat pentichrome 및 S-100 염색 모두 음성이었다. GLUT1 은 모든 환자군에서 음성이었다. 태생부터 병변이 있었던 경우는 혈관기형에서 8명이었고(36%), 혈관종 환자에서는 없었다. 피부병변은 혈관기형 13(59%), 혈관종 2예에서 있었다. 병변크기 변화는 혈관기형15(68%), 혈관종 2예에서 보였다. 혈관조영술 상 국소적인 과혈관성 종괴(19/24, 77%) 혹은 광범위한 과혈관성 병변(5/24)으로 보여 진단구분이 어려웠다.

결론: 성인 두경부의 연부조직 혈관병변은 영유아에 비해 상대적으로 혈관기형이 더 많았으며 GLUT1 는 혈관기형 및 혈관종에서 모두 음성을 보였다.