



ORIGINAL RESEARCH ARTICLE

Expression of chemokine receptors in angiosarcoma

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Abstract: Angiosarcoma is a rapidly progressing malignant skin tumor associated with a poor prognosis. We report an immunohistochemical investigation of chemokine receptors in angiosarcoma. The aim is to investigate chemokine receptor expression and, if it is expressed, to histologically discuss its association with age, sex, development site, and histological type. Analyzed were 12 angiosarcoma samples (mean patient age: 75.7 years old) collected by biopsy or surgery from patients at our department by comparing with 4 granuloma telangiectaticum samples (mean patient age: 37.5 years old) and 16 healthy skin samples as controls. CXCR4, CCR6, CCR7, and SDF-1 expressions were immunohistochemically investigated. CXCR4 expression was positive in 6 of the 12 samples. CCR6 and CCR7 were negative in all samples. SDF-1 was positive in 4 samples. Out of the 6 CXCR4-positive samples, 4 were SDF-1-positive. No significant difference associated with age, sex, development site, or histological type was noted in the expression of CXCR4, CCR6, CCR7, or SDF-1. Although CXCR4 is regarded as an index of tumor aggressiveness of malignant melanoma, it is suggested that chemokine receptors, such as CXCR4, do not serve as useful markers of angiosarcoma.

Keywords: CXCR4; chemokine receptor; angiosarcoma; immunohistochemistry

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Introduction

Angiosarcoma is a rare malignant skin tumor appearing as a pink-dark red erythema in the frontal region over the forehead initially and it frequently develops in the elderly. It is accompanied by edema and then forms an erosion, an exudation, and a crust; when it advances, nodules with ulceration occur. It is very malignant, recurring at a local site at a high rate, and causes hemothorax due to lung metastasis in many cases, while also being a disease associated with very poor prognosis^[1].

Chemokines are substances inducing the migration of specific white blood cells and lymphocytes, and it has been reported that chemokine and chemokine receptor expressions play important roles in the outgrowth, infiltration, and metastasis of malignant tumors^[2-8]. Among them, CXCR4 is a specific receptor of the chemokine SDF-1 (CXCL12), and it is expressed on the cell membrane of white blood cells and undifferentiated hematopoietic cells. Its stimulation with SDF-1 induces chemotaxis and promotes proliferation and survival. We previously investigated expressions of CXCR4, CCR6, CCR7, and SDF-1 in malignant melanoma, and reported that CXCR4 is a useful marker and serves as

an index of malignancy^[9]. We also reported that expressions of these chemokines may serve as markers of tumor advancement (predicting tumor aggressiveness) of malignant fibrous histiocytoma and dermatofibrosarcoma protuberans^[8]. Nakatsugawa stated that CXCR4 and SDF-1 expressions on squamous cell carcinoma of the tongue are associated with lymph node metastasis, thus serving as a prognostic factor^[10]. In reports on tumors other than mucocutaneous tumors, their expressions increased in cases of metastasis of breast and colorectal cancers, suggesting their involvement in tumor metastasis^[2].

Analysis of chemokines and chemokine receptors in angiosarcoma has not been performed. Once angiosarcoma develops, it rapidly progresses and results in a poor outcome; however, no useful marker has been identified, and the accumulation of knowledge is urgently needed. In this study, we immunohistochemically investigated chemokine receptors in angiosarcoma, particularly CXCR4, CCR6, CCR7, and SDF-1 (CXCL12).

Using tumor tissue of angiosarcoma, the expressions of CXCR4, CCR6, and CCR7 and a ligand of CXCR4, SDF-1 (CXCL12), were investigated. As clinical control, the expressions in a benign vascular proliferative tumor, granuloma telangiectaticum, were similarly analyzed immunohistologically. Normal control skin samples were also obtained. In addition, the presence or absence of an association with age, sex, development site, and histological type was also investigated.

Materials and methods

Along with 16 healthy skin samples as controls, 12 angiosarcoma samples (mean patient age: 75.7 years old) and 4 granuloma telangiectaticum samples (mean patient age: 37.5 years old) collected by biopsy or surgery at Wakayama Medical University Hospital's Department of Dermatology, were analyzed. The details of each case are shown in **Table 1**. The samples were immunohistologically analyzed after obtaining informed consent from the patients and their families. Immunohistochemical staining was performed following the method reported by Toyozawa *et al.*^[8,9]. Each sample was fixed with formalin and embedded in paraffin. After deparaffinization, 4- μ m-thick sections were prepared and subjected to immunohistochemical staining with control, anti-CXCR4 antibody (polyclonal, Gene TEX, Incorporated, San Antonio, TX, USA), anti-CCR6 antibody (polyclonal, Gene TEX), anti-CCR7 antibody (polyclonal, Gene TEX), and anti-SDF-1 antibody (polyclonal, Santa Cruz Biotechnology, Incorporated, Santa Cruz, CA, USA). The expressions were evaluated using the catalyzed signal amplification (CSA) method. For the evaluation, three specialized physicians from Department of Dermatology, who

Table 1. Patient and tumor characteristics

Patient characteristics		
	Angiosarcoma	Pyogenic granuloma
Total number of sites	12	4
Median age (years)	75.7	37.5
Range	61–89	15–54
Gender:		
Female	7/12	2/4
Male	5/12	2/4
Location:		
Head	8/12	0/4
Face	3/12	3/4
Trunk/neck/extremities	1/12	1/4
Feet	0/12	0/4
Histopathologic type:		
Cellular rich type	8/12	0/4
Vascular rich type	4/12	4/4
Biopsy/Operation:		
Biopsy	6/12	0/4
Operation	6/12	4/4

were unable to access the clinical findings of the patients, randomly selected and observed five visual fields in each section at 400X magnification^[9].

In terms of histological type, the histology tissue section judged as poorly differentiated angiosarcoma was defined as cellular rich type, whereas well-moderately differentiated angiosarcoma was defined as vascular rich type^[11].

The correlations of stainability of each antibody with age, sex, development site, and histological type were statistically analyzed using Fisher's exact test.

Ethics statement

This study was approved by the ethics review board of Wakayama Medical University.

Results

Immunohistologically investigated were 12 angiosarcoma samples, 4 granuloma telangiectaticum samples, and 16 normal skin control samples.

The results of staining of the normal skin, granuloma telangiectaticum, and angiosarcoma (vascular rich and cellular rich types) are shown in **Figures 1–4**, respectively.

The results of staining of the normal skin are shown in **Figure 1**. **Figures 1A–1D** show staining of CXCR4, CCR6, CCR7, and SDF-1 observed at 200X magnification, respectively. No specific positive reaction with any antibody was observed. **Figure 2** shows the results of staining of granuloma telangiectaticum. The hematoxylin and eosin-stained (HE-stained) sample observed at low

magnification (20X), at a high magnification (400X), and the control are shown in **Figures 2A–2C**, respectively. The results of CXCR4, CCR6, CCR7, and SDF-1 are shown in **Figures 2D–2G**, respectively; none of these were stained.

Figure 3A shows the clinical view of an angiosarcoma that developed on the head of a 78-year-old female. **Figure 3B** shows HE staining of a specimen collected from the lesion shown in **Figure 3A**, and it was diagnosed as the vascular rich type. **Figure 3C** shows the HE-stained sample observed at a high magnification (400X). **Figure 3D** shows the control. **Figure 3E–3H** shows the results of CXCR4, CCR6, CCR7, and SDF-1, respectively. CXCR4 and SDF-1 expressions were positive, but CCR6 and CCR7 expressions were negative.

Figure 4A shows the clinical view of an angiosarcoma that developed on the head of a 75-year-old female. **Figure 4B** shows HE staining of a specimen collected from the lesion shown in **Figure 4A**, and it was considered to be the cellular rich type. **Figure 4C** shows the HE-stained sample observed at a high magnification (400X). **Figure 4D** shows the control. **Figures 4E–4H** show the results of CXCR4, CCR6, CCR7, and SDF-1, respectively. CXCR4 expression was positive, but CCR6, CCR7, and SDF-1 expressions were negative.

As shown in **Tables 2** and **3**, CXCR4 was intensely stained in the cytoplasm or nucleus in 6 of the 12 angiosarcoma samples, being expression-positive. Cellular rich type were determined in 4 of these 6 samples, and 2 were the vascular rich type. CCR6 and CCR7 were negative. SDF-1 expression was positive in 4 of the 12 samples. All these SDF-1 expression-positive cases were also positive for CXCR4 expression. In contrast, all of CXCR4, CCR6, CCR7, and SDF-1 expressions were negative in granuloma telangiectaticum. CXCR4 and SDF-1 expressions are summarized in **Tables 2** and **3**, respectively. A significant correlation of the presence and absence of expression was noted between CXCR4 and SDF-1. Both CCR6 and CCR7 expressions were negative in all cases. Regarding the association of CXCR4, no significant difference due to age, sex, development site, or histological type was noted. Similarly, no significant difference was noted in CCR6, CCR7, or SDF-1.

Discussion

Chemokines represent cytokine proteins with chemotactic activity, and their study started in the late 1980s with the discovery of interleukin (IL)-8, a factor inducing neutrophil migration and activation. Subsequently, studies on the molecular pathology of inflammation and immunity have markedly advanced, and more than 50 chemokines have been identified.

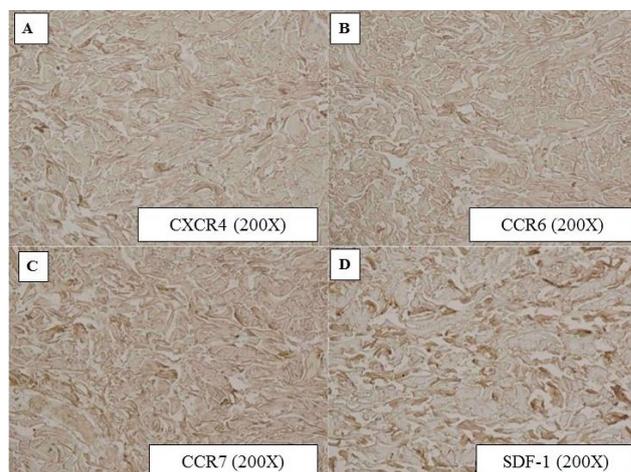
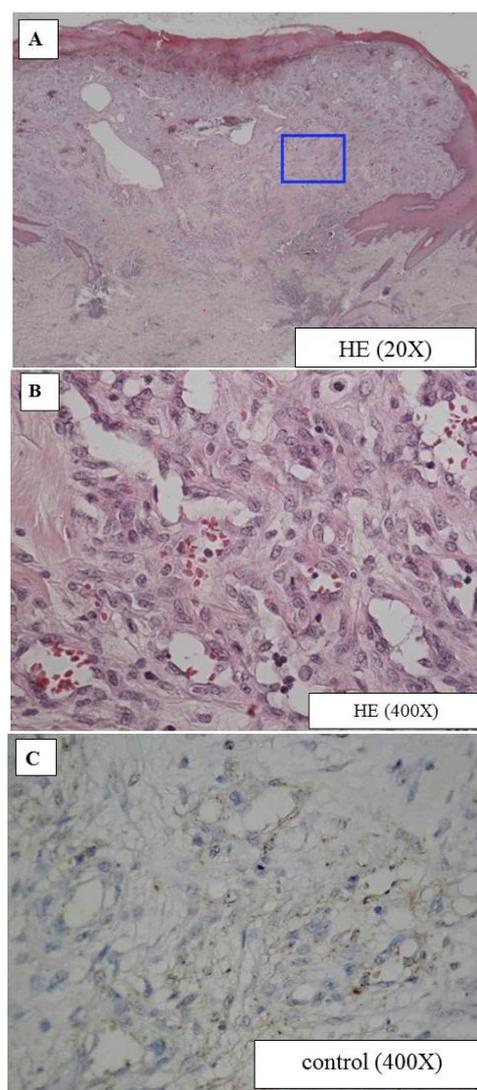


Figure 1. CXCR4, CCR6, CCR7, and SDF-1 immunoeexpression in the normal skin (200X). No expression of (A) CXCR4, (B) CCR6, (C) CCR7, and (D) SDF-1 were observed in the normal skin.



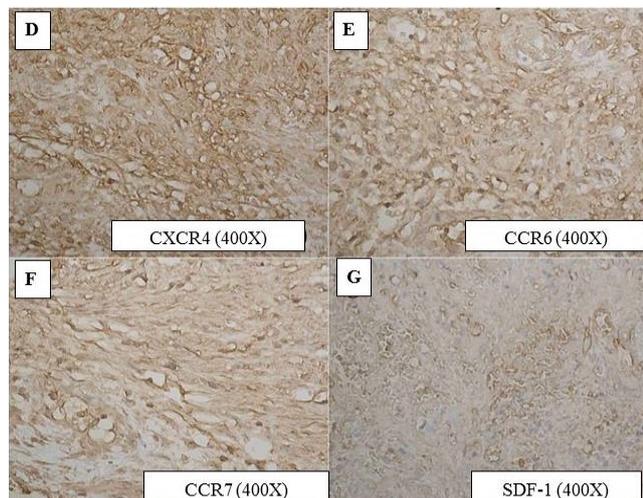


Figure 2. The HE-stained sample of granuloma telangiectaticum under (A) 20X magnification, (B) 400X, and (C) control (400X). No expression of (D) CXCR4, (E) CCR6, (F) CCR7, and (G) SDF-1 were observed in granuloma telangiectaticum.

CXCR4 is a specific receptor of the chemokine SDF-1, and it is expressed on the cell membrane of white blood cells and undifferentiated hematopoietic cells. Its stimulation with SDF-1 induces cancer cell chemotaxis and promotes further proliferation and survival of cancer cells^[2]. CCR6 is a receptor of CCL20 and it plays an important role in the movement of immunocompetent cells^[2]. Reduction of CCR6 expression in head-and-neck squamous cell carcinoma has been reported^[7]. Reportedly, CCR7 is a receptor of CCL19 and CCL21, and it is involved in lymphocyte migration^[2]; additionally, the enhancement of CCR7 expression in breast cancer, which is likely to metastasize to lymph nodes, has been reported^[2]. Regarding malignant skin tumors, the involvement of the expression of chemokine receptors such as CXCR4 and CCR7 in tumor growth and metastasis of squamous cell carcinoma, malignant melanoma, and fibrous system malignant tumors has been reported^[4,5,7,8], but the details of the distribution of expression and function have not been elucidated.

Toyozawa *et al.* investigated CXCR4, CCR6, CCR7, and SDF-1 expressions in 19 cases of malignant melanoma, and reported that the frequency of CXCR4/SDF-1 expression was significantly higher in the first of the following parameters: a tumor thickness of 2 mm or less, histological classification of the node or non-node type, and the presence or absence of distant metastasis within two years^[9]. On the other hand, CCR6 and CCR7 were associated with several parameters but these were not as favorable as markers compared with CXCR4/SDF-1.

Kaminaka *et al.* histologically investigated the effect of phenol therapy, which is a noninvasive treatment of solar keratosis, Bowen's disease, and squamous cell carcinoma, and they also investigated chemokine receptors immunohistochemically at the same time^[12]. Compared with normal skin and completely remitted cases, the tumor was thick and CXCR4 was strongly expressed on the first examination, similar to that in squamous cell carcinoma, and they reported that the tumor thickness and CXCR4 expression were associated with the treatment effect.

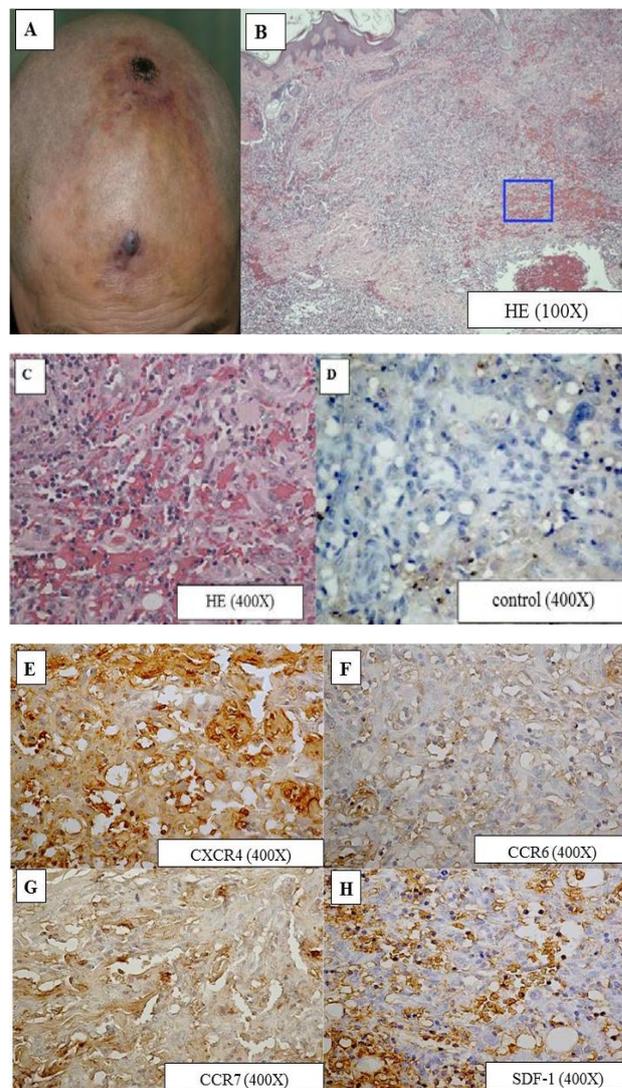


Figure 3. (A) Clinical feature of angiosarcoma in the head of a 78-year-old female. The HE-stained sample of angiosarcoma under (B) 100X magnification, (C) 400X, and (D) control (400X). It was diagnosed as the vascular rich type. The expressions were positive for (E) CXCR4 and (H) SDF-1, but (F) CCR6 and (G) CCR7 expressions were negative.

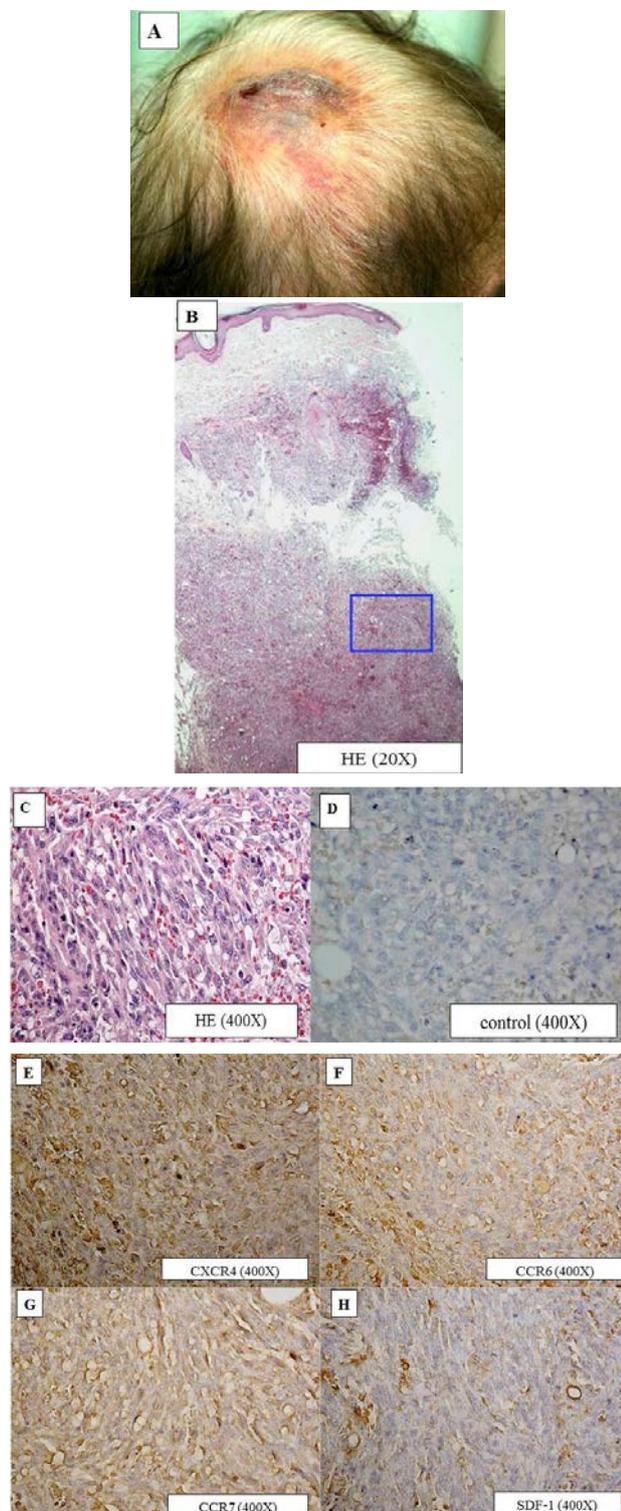


Figure 4. (A) Clinical feature of angiosarcoma in the head of a 75-year-old female. The HE-stained sample of angiosarcoma under (B) 20X magnification, (C) 400X, and (D) control (400X). It was diagnosed as the cellular rich type. The expression of (E) CXCR4 was positive, but (F) CCR6, (G) CCR7, (H) and SDF-1 expressions were negative.

Nakatsugawa investigated CXCR4 and SDF-1 expressions in 53 patients with squamous cell carcinoma of the tongue, in which the frequency of cervical lymph node metastasis was significantly higher in those with SDF-1 gene expression, and the five-year cumulative survival rate was significantly lower in patients with both SDF-1 and CXCR4 gene expressions, than those with no expression of either gene^[10]. Regarding the other factors, no association of SDF-1 and CXCR4 expressions with any clinicopathological factor was noted during gene analysis or immunohistochemical staining. Nakatsugawa stated that his findings suggest that the association between SDF-1 gene expression and lymph node metastasis of squamous cell carcinoma of the tongue and SDF-1/CXCR4 expression serves as a prognostic factor.

In our study on angiosarcoma, CXCR4 was expressed in half of the cases. CXCR4 was intensely stained in the cytoplasm or nucleus, being positive, in 6 of the 12 samples. Cellular rich type were determined in 4 of these 6 samples, and 2 were the vascular rich type. The association of CXCR4 with age, sex, development site, and histological type was investigated, but no significant difference due to any of these parameters was noted. In addition, CCR6 and CCR7 expressions were negative. SDF-1 expression was positive in 4 of the 12 samples, and all these SDF-1 expression-positive cases were also positive for CXCR4 expression. On the other hand, in granuloma telangiectaticum, which is a representative of benign vascular proliferative disease, CXCR4, CCR6, CCR7, and SDF-1 were negative in all cases.

CXCR4 and SDF-1 expressions were significantly correlated with the clinical views in malignant skin tumors such as malignant melanoma, solar keratosis, Bowen's disease, squamous cell carcinoma, and malignant fibrous histiocytoma; however, no correlation with the clinical view of angiosarcoma was noted. It was hence, suggested that CXCR4, CCR6, CCR7, and SDF-1 do not serve as a useful tumor marker.

Indeed, an investigation of a new chemokine and tumor marker for angiosarcoma is needed.

Conclusion

In our study on angiosarcoma, CXCR4 was expressed in half of the cases. The association of CXCR4, CCR6, CCR7, and SDF-1 with age, sex, development site, and histological type was investigated; however, no significant difference due to any of these parameters was noted. We have to collect more cases of angiosarcoma and analyze them immunohistochemically. Additionally, we have to investigate the usability of these chemokines as a marker. More development in the study of a new chemokine and tumor marker shall be needed for this disease.

Table 2. Clinicopathologic features and CXCR4 expression

		n (%)	Number of CXCR4 expression		p
			Positive n (%)	Negative n (%)	
Age (years)	< 70	3/12 (25%)	1 (33%)	2 (67%)	1.000
	≥ 70	9/12 (75%)	5 (56%)	4 (44%)	
Gender	Male	5/12 (42%)	3 (60%)	2 (40%)	1.000
	Female	7/12 (58%)	3 (43%)	4 (57%)	
Location	Head	8/12 (67%)	4 (50%)	4 (50%)	0.513
	Face	3/12 (25%)	2 (67%)	1 (33%)	
	Trunk/neck/extremities	1/12 (8%)	0 (0%)	1 (100%)	
	Feet	0/12 (0%)	0 (0%)	0 (0%)	
Clinical type	Cellular rich type	8/12 (67%)	4 (50%)	4 (50%)	1.000
	Vascular rich type	4/12 (33%)	2 (50%)	2 (50%)	

*p ≤ 0.05 between groups

Table 3. Clinicopathologic features and SDF-1 expression

		n (%)	Number of SDF-1 expression		p
			Positive n (%)	Negative n (%)	
Age (years)	< 70	3/12 (25%)	1 (33%)	2 (67%)	1.000
	≥ 70	9/12 (75%)	3 (33%)	6 (67%)	
Gender	Male	5/12 (42%)	2 (40%)	3 (60%)	1.000
	Female	7/12 (58%)	2 (29%)	5 (71%)	
Location	Head	8/12 (67%)	2 (33%)	6 (67%)	0.325
	Face	3/12 (25%)	2 (67%)	1 (33%)	
	Trunk/neck/extremities	1/12 (8%)	0 (0%)	1 (100%)	
	Feet	0/12 (0%)	0 (0%)	0 (0%)	
Clinical type	Cellular rich type	8/12 (67%)	2 (25%)	6 (75%)	0.548
	Vascular rich type	4/12 (33%)	2 (50%)	2 (50%)	

*p ≤ 0.05 between groups

Author contributions

The study was conceived and designed by Kishi T, Yamamoto Y, Kaminaka C, Toyozawa S and Furukawa F. The manuscript was prepared by Kishi T. The statistical analysis was performed by Matsunaka H.

Conflict of interest

The authors declare no potential conflict of interest with respect to the research, authorship, and/or publication of

this article.

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