Sclerosing angiomatoid nodular transformation in the spleen

A case series study and literature review

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Abstract

To assess the clinicopathologic features, differential diagnosis, and pathogenesis of sclerosing angiomatoid nodular transformation (SANT) of the spleen.

The clinical characteristics of 7 cases with SANT were retrospectively analyzed. Histochemical and immunohistochemical (EnVision method) examinations were performed. Moreover, quantitative assessment of IgG4 and IgG-positive cells was performed.

The 7 SANT patients included 1 female and 6 males, with ages ranging from 7 to 60 years (mean 33.4 years). They showed no specific clinical manifestations. Macroscopically, the surface of the excised masses was gray-white, and vague nodularity was observed. Mass sizes ranged from 3.0 to 7.0 cm (mean 5.5 cm). Microscopically, all cases were characterized based on multiple angiomatoid nodules of various sizes embedded in a fibrosclerotic stroma. The nodules were round and sometimes convoluted. Moreover, the nodules were composed of slit-like, irregularly-shaped, or slightly dilated vascular spaces lined by plump endothelial cells, and interspersed with a population of spindly or ovoid cells. Immunohistochemical examination showed a heterogeneous staining pattern, with the lining cells of small capillaries expressing CD34 and those of sinusoid-like structures expressing CD8. CD31 was stained in the lining and interspersed cells, thus resulting in a complex meshwork. Additionally, the lining cells were focally positive for CD68. Vimentin and smooth muscle antibody were expressed in all 7 cases, whereas no desmin or CD21 was detected. IgG4-positive cells accounted for 2 to 5 per high-power field (mean 3.7). Furthermore, the IgG-positive cells accounted for 2 to 8 per high-power field (mean 4.2).

SANT is a rare primary benign tumor-like lesion of the spleen, and has characteristic histopathological features and immunohistochemical profiles. SANT should be distinguished from other angiomatoid tumors and tumor-like lesions. Moreover, SANT could be treated by splenectomy, with favorable prognosis. The relationship between SANT and IgG4-related sclerosing lesions remains to be clarified.

Abbreviations: CT = computed tomography, H&E = hematoxylin and eosin, PBS = phosphate buffer saline, SANT = sclerosing angiomatoid nodular transformation, SMA = smooth muscle antibody.

Keywords: immunohistochemistry, sclerosing angiomatoid nodular transformation of spleen, splenic disease

1. Introduction

Sclerosing angiomatoid nodular transformation (SANT) is a rare primary benign tumor-like lesion found in the spleen. In 2004, the SANT designation was first reported by Martel et al,[1] and the report described the pathological features and immunophenotypes of 25 cases. Recently, Wang et al[2] stated that 133 SANT cases have been described so far in approximately 50 reports published in English. SANT is frequently misdiagnosed as splenic inflammatory pseudotumor, hamartoma, or even epithelioid hemangioendothelioma mainly due to lack of knowledge regarding SANT. Moreover, the pathogenesis of SANT remains unclear. In this work, we summarized the clinicopathologic manifestations and differential diagnosis of SANT based on 7 cases in our center, and performed a literature review. Furthermore, we discussed the relationship between SANT and IgG4-related sclerosing lesions.

2. Materials and methods

2.1. Patients

IN all, 7 patients with SANT, including 6 males and 1 female aged from 7 to 60 years (median age of 33.4 years), were recruited from the First Affiliated Hospital of Xi'an Jiaotong University from January, 2009 to December, 2014. One case was diagnosed via occasional examination for dull pain in the lower abdomen, 2 via medical examination for trauma, and the remaining 4 via routine examination. All 7 patients underwent preoperative ultrasound examinations and showed splenic space-occupying lesions. Two cases were considered angiomas, whereas 4 and 1

were considered inflammatory pseudotumors and hamartoma, respectively. All patients underwent splenectomy. The study was approved by ethics committee of the First Affiliated Hospital of Xi'an Jiaotong University. All the 7 patients agreed to participate in this study and signed written informed consent forms.

2.2. Histological detection

The specimens obtained via surgical removal were fixed in 10% formalin, and then underwent conventional dehydration and paraffin embedding. Afterwards, 4-μm sections were prepared for hematoxylin and eosin (H&E) staining and observed by light microscopy. Immunohistochemical staining was performed by the EnVision 2-step method, with antibodies that targeted vimentin, smooth muscle antibody (SMA), CD34, CD31, CD8, CD68, desmin, CD21, IgG, and IgG4 (Fuzhou Maixin Biotech. Co., Ltd.). Phosphate buffer saline (PBS) was used as the blank control for primary antibodies, and positive tissue slices were used as positive control.

2.3. Analysis of immunohistochemical staining results

Yellow or brown particles were identified as positive signals for vimentin, SMA, desmin, CD34, CD31, CD21, and CD68, which were all located in the cytoplasm; moreover, CD8 signals were mainly found in the cytoplasmic membrane. A total of 10 high-power fields were selected randomly in each slice, and we counted 100 cells in all. Afterwards, the percentages of cells positive for each indicator were averaged. For assessment, $<5\%$, 5% to 25%, and $>25\%$ were defined as negative, slightly positive, and positive, respectively. IgG/IgG4-positive cells were accessed by counting at 3 high-power fields ($\times 400$ magnifications) in the same high-density area.

2.4. Clinical data and follow-up

Demographic data were collected using standardized epidemiological questionnaires. At 3-month intervals, follow-up information about patient death was updated by a trained clinical specialist through on-site interview, direct calling, or medical record review. The latest follow-up data in this analysis were obtained in January, 2017.

3. Results

3.1. Pathological examination

The clinical characteristics of the 7 patients with SANT are summarized in Table 1. Upon visual inspection, the removed spleens showed normal or slightly enlarged sizes in the 7 patients. Additionally, the lesions in the parenchyma showed diameters of 3 to 7 cm (5.5 cm in average) and were roughly round or lobulated, solid, nonencapsulated, and tough, with clear boundary and gray-maroon areas with many spots, but without hemorrhage or necrosis (Fig. 1).

In the microscopic examination, normal spleen structure was not found in the lesion area, and multiple angiomatoid nodules in highly proliferated and hardened interfibrillar materials were unevenly distributed, and singly or multiply fused and encapsulated by proliferated fibrous tissue. The angiomatoid nodules showed different sizes and encapsulation areas. Complete, round encapsulations were generally found in the small nodules and showed concentric circles formed by spindle cells and fibrinoid deposits in the surrounding area (Fig. 2). Incomplete encapsulations were found in some larger nodules that were infiltrated with the surrounding spindle cells, and showed no fibrinoid deposit. In some cases, a red pulp-like structure with an irregular shape was the only matter that formed, and it was divided and encapsulated by fibrous tissue. A slit, sinus-shaped, or irregular slightly expanded vascular lumen was found in the center of nodules, with swollen lining endothelial cells. Varying amounts of red blood cells could be observed in most of the small lumens. Some spindle or oval cells were spread around the lumens, without abnormal shapes (nuclear splitting was rarely found). Some inflammatory cells, such as lymphocytes, plasmacytes, and histocytes, were scattered. Pyknotic fiber or fiber myxoid tissues and numerous vessels with different wall thicknesses were observed among the nodules. Hemosiderosis, a giant cell reaction to foreign matter and calcification, could be found in some cases. Furthermore, lesions with margin areas linked to normal splenic tissues showed clear boundaries and developed in a pushing way. Spindle cell infiltration into the splenic tissue was locally found under high-power microscope, and formed rough and jagged edges (Fig. 3).

Table 1

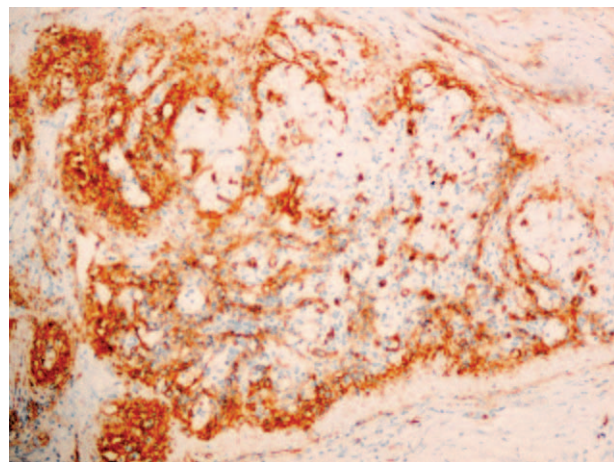Clinical characteristics of the 7 patients with SANT.

Case no.	Sex	Age (y)	Clinical detection	Gross size	Preoperative diagnosis	Postoperative diagnosis	Follow-up	IgG4 (mean ± SD)	IgG (mean ± SD)
1	Male	7	B-mode ultrasound examination for trauma	$9.5 \times 5 \times 8$ cm	Hamartoma	SANT	No recurrence	5.6 ± 1.2	6.3 ± 1.5
2	Male	35	Physical examination	$7 \times 7 \times 5$ cm	Inflammatory pseudotumor	SANT	No recurrence	3.6 ± 1.5	6.6 ± 2.9
3	Male	26	Physical examination	$6 \times 4 \times 5$ cm	Inflammatory pseudotumor	SANT	No recurrence	3.7 ± 1.2	5.3 ± 2.5
4	Male	32	Physical examination	$5 \times 5 \times 4$ cm	Hemangioma	SANT	No recurrence	2.9 ± 0.9	6.2 ± 2.8
5	Female	23	Physical examination	$6 \times 5.5 \times 5.5$ cm	Inflammatory pseudotumor	SANT	No recurrence	3.9 ± 1	5.6 ± 1.2
6	Male	51	Physical examination	$3 \times 3 \times 2.8$ cm	Inflammatory pseudotumor	SANT	No recurrence	3 ± 1.7	3 ± 1.7
7	Male	60	B-mode ultrasound examination for trauma	$4.5 \times 4.5 \times 4$ cm	Hemangioma	SANT	No recurrence	3.3 ± 1.5	4.3 ± 1.5

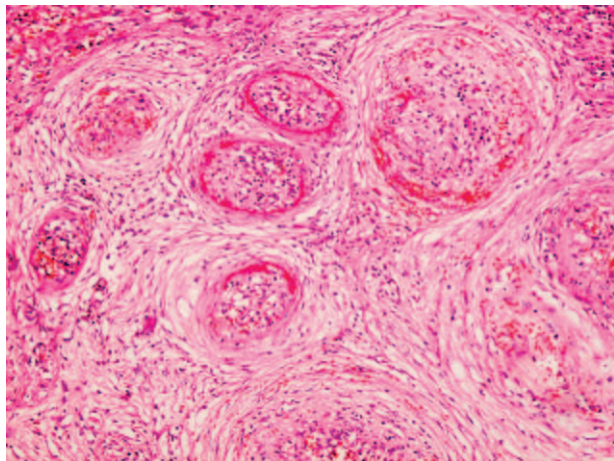SANT = sclerosing angiomatoid nodular transformation.

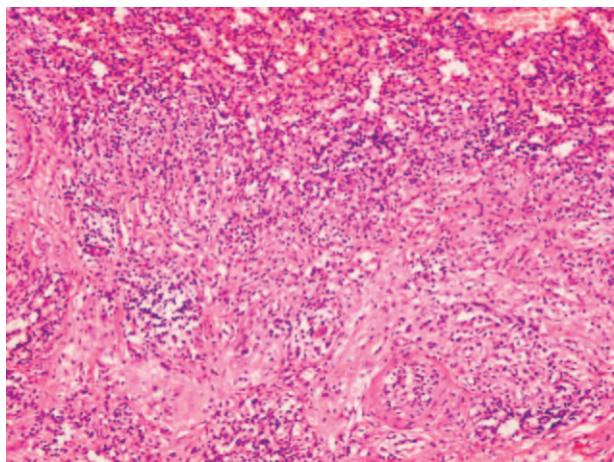**Figure 1.** Lobulated lesion, with a clear boundary, and gray-maroon-colored section.

Figure 2. Roughly round-shaped angiomatoid nodules, encapsulated by fusiform cells in concentric circles.

Figure 3. Proliferated fusiform cells infiltrating into the splenic tissue, forming rough jagged edges.

Figure 4. CD34 is expressed in the lining epithelial cells in the lumens around the angiomatoid nodules (EnVision 2-step method).
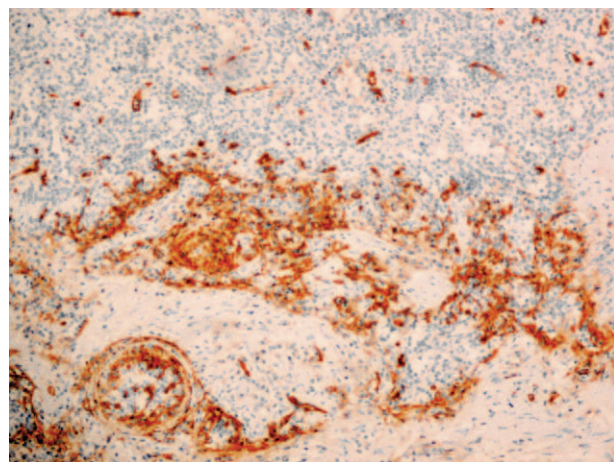
3.2. Immunophenotypes

In all patients with SANT, high expression levels of CD31 were found in the lining and infiltration cells in the lumen of angiomatoid nodules, in which a reticular structure was formed similar to the splenic tissue. Moreover, CD34 was characteristically expressed in the spindle cells surrounding the nodules and the lining epithelial cells in the lumen in most cases. This led to a vascular structure similar to that of sinusoidal endothelial cells in liver cancer (Fig. 4), which was also found at the junctional area of the lesion and splenic tissue (Fig. 5). In contrast, CD8 was often expressed in lining epithelial cells in the central lumen of the angiomatoid nodules (Fig. 6). Vimentin and SMA were mainly detected in the spindle cells surrounding the nodules. Additionally, positive SMA cells were found in proliferating spindle cells inside the nodules and at junctional areas. CD68 was expressed in histocytes inside and around nodules. CD21 and desmin were not expressed in the spindle cells inside and around the nodules. IgG4-positive cells accounted for 2 to 5 per high-power field (mean 3.7). Furthermore, IgG-positive cells accounted for 2 to 8
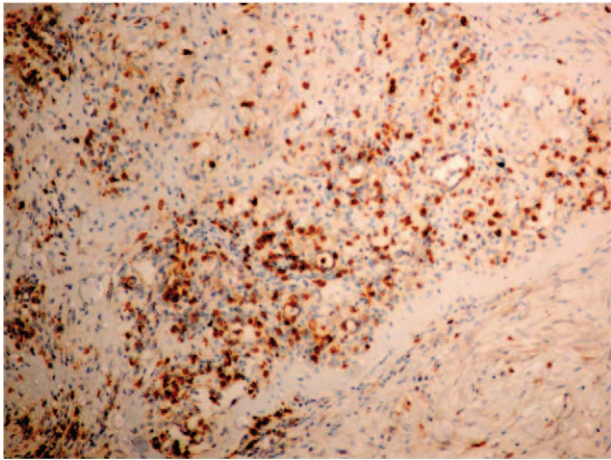

Figure 5. CD34 is expressed in the sinusoidal endothelial cells (capillarization) at the junction between the lesion and the splenic tissue (EnVision 2-step method).

Figure 6. CD8 is expressed in the lining epithelial cells in the central lumens of the angiomatoid nodules (EnVision 2-step method). (A) This case (case 3) shows scattered IgG4-positive cells (5 cells/HPF). (B) The corresponding area also shows scattered IgG-positive cells (8 cells/HPF). (C) The corresponding hematoxylin and eosin region also shows that the scattered plasma cells are mainly located in interfibrotic stroma.

per high-power field (mean 5.3). The IgG4/IgG-positive cells were few and were not sufficient (Fig. 7) (most articles recommend >30 IgG4+ cells/HPF, or more specifically >50 IgG4+ cells/HPF) for diagnosing IgG4-related sclerosing lesion; thus, the IgG4/IgG ratio was not calculated.[3–4]

3.3. Clinical outcomes

All 7 patients were followed up for 15 to 76 months, and were alive without recurrence, metastasis, or other concomitant neoplastic diseases.

4. Discussion

In 1978, this disease was first described and considered a splenic hamartoma by Silverman and LiVolsi.[5] In 1993, Krishnan et al designated it "splenic cord capillary angioma," which was later redefined as a special kind of splenic hamartoma by the same authors. As described in "*Rosai and Ackerman Surgical Pathology*" (Edition 9), the disease is a "multinodular hemangioma." In 2004, the SANT designation was provided by Martel et al[1] with an in-depth study of 25 cases, and their study has

been widely accepted. According to Martel et al's concept, SANT is related to the red pulp instead of white pulp, and is possibly a hamartoma-like lesion. Different from common hamartomas, SANT shows characteristic angiomatoid nodules.

The incidence of SANT is low. Less than 200 cases have been reported since 2004.[2,6] Moreover, no obvious clinical symptoms have been observed in patients with SANT, and in most cases, this disease is diagnosed unexpectedly in routine physical or imageological examinations. In a few cases, discomfort in the upper abdomen or backache occurs. Additionally, fever and elevated erythrocyte sedimentation rate have been observed in a small proportion of SANT patients. With the aid of imageological examination, SANT can be diagnosed nonspecifically. In addition, solid low-echo masses in the splenic parenchyma are often indicated by ultrasound, which is the most commonly applied method for this case. In the current cases assessed in our center, most patients with SANT were diagnosed with angiomas or inflammatory pseudotumors. Low-density signals in the spleen were detected by computed tomography (CT) scanning, and moderately or less obviously enhanced signals were obtained in contrast scanning; these results indicated a clear boundary between the mass and the splenic parenchyma. To date, pathological examination remains the gold standard for the diagnosis of SANT. Generally, a single mass is reported, with multiple masses found in a few cases.[7] The lesions are round or lobulated, nonencapsulated, and have clear boundaries with the surrounding splenic tissues, thus showing multiple gray or brownish red nodules (with different sizes) that are divided by scar-alike gray cords. Under a microscope, the lesions are characterized by multiple angiomatoid nodules. The hardened interfibrillar materials and the nodules are singly or multiply fused and encapsulated by proliferated fibrous tissue. Single angiomatoid nodules consist of slit-shaped, round, or irregularly shaped lumens with swollen lining endothelial cells. Some small nodular lesions are surrounded by collagenous fibers ranged in concentric circles, with exuded red blood cells and fibrinoid deposits. The interstitial substance in the nodules consist of a myxoid to extensively collagenized fibrous tissue and vessels with different sizes, Moreover, the interstitial substance is occupied by scattered swollen myofibroblasts, plasmacytes, lymphocytes, and monocytes. Previous reports have suggested 3 vascular lumen subtypes in angiomatoid nodules based on immunohistochemical properties: capillaries with CD34+/CD8-/CD31+; sinusoid spaces with CD34-/CD8+/CD31+; and small veins with CD34-/CD8-/CD31+. In the current 7 patients, however, we only observed capillaries and sinusoid spaces in immunohisto-
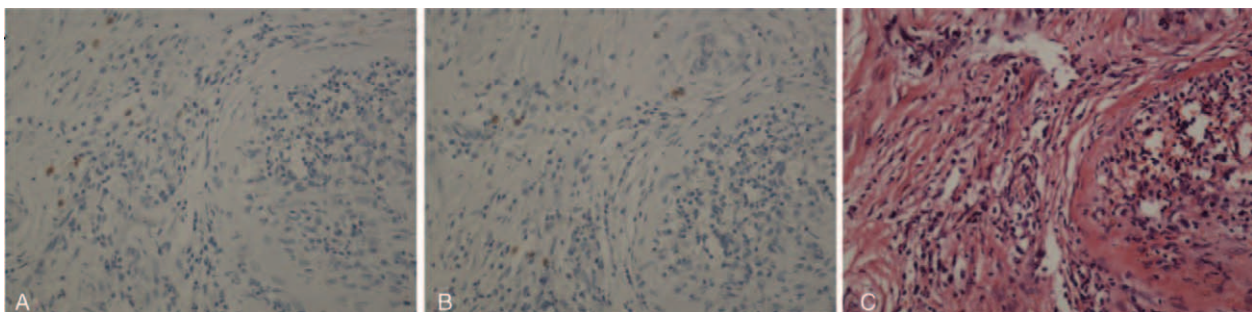
Figure 7. The IgG4/IgG-positive cells were few and were not sufficient. (A) This case (case 3) shows scattered IgG4-positive cells (5 cells/HPF). (B) The corresponding area also shows scattered IgG-positive cells (8 cells/HPF). (C) The corresponding hematoxylin and eosin region also shows that the scattered plasma cells are mainly located in interfibrotic stroma.

chemical staining, except for small veins. In these patients, CD34 was often expressed in the spindle cells surrounding the nodules and the lining epithelial cells in the slit-shaped lumens. These findings formed a vascular structure similar to those of sinusoidal endothelial cells in liver cancer, related to "vascular transformation in lymphatic sinuses," as reported by Ostrowski et al[8] and Cook et al.[9] This finding has also been observed at the junctional area of the lesion and the splenic tissue. In contrast, CD8 was frequently expressed in the lining epithelial cells within the central lumen of the angiomatoid nodules. Therefore, this observation could be considered a reactive change of sinusoidal endothelial cells.

Recently, the relationship between SANT and IgG4-related sclerosing lesions has received attention. IgG4-related sclerosing lesions have displayed sclerosing inflammation and infiltration of numerous IgG4+ plasma cells that affect multiple organs with increased serum levels of IgG4. Clinically, some patients with SANT show high blood levels of IgG4.[10–11] Microscopically, some SANT lesions contain dense IgG4+ plasma cells[12–13] within fibrous stroma and erythrocyte extrasation, thus sharing features with IgG4-related sclerosing lesions. Recently, Nagai et al[14] described a rapidly growing SANT after adrenalectomy, and this phenomena may be due to the decrease in glucocorticoid concentrations, thus mimicking steroid-responsive IgG4-related sclerosing lesions. These data raise the possibility that SANT is an IgG4-related sclerosing lesion. Chang et al[15] described 22 cases of SANT. Only 2 cases out of 22 showed an IgG4/IgG ratio >40%. In our case series, too few IgG4+ plasma cells were observed for diagnosing IgG4-related sclerosing lesion.[3–4] Chang et al's and our present study did not corroborate the IgG4-related sclerosing lesions in these SANT cases. Thus, the relationship between SANT and IgG4-related sclerosing lesions remains to be clarified.[16]

A significant difference was found in the number and morphological characteristics of angiomatoid nodules among the 7 SANT patients. Only a few typical changes were observed in the limited number of cases. Morphological changes implied that the lumens had blood sinus, similar to red pulp, and also slit-shaped lumens and granulomatous nodules with limited lumens. These observations indicated gradual progression of angiomatoid nodules that ended with collagenized fibers. We observed that highly collagenized fiber areas actually existed in all patients, as reflected by positive immunohistochemical staining of vimentin and SMA, and often without the expression of other mesenchymal markers. According to previous studies, the fusiform cells in the nodules are considered to be myofibroblasts, as verified in this study. Fusiform cells with high expression levels of SMA were found around and inside the angiomatoid nodules. These active, proliferating cells existed even at the junction between the lesion and the splenic tissue, and infiltrated the nodules and residual splenic tissue, even dividing and encapsulating the splenic tissue. Fusiform myofibroblasts might be nourished by numerous small vessels growing in the fibrotic area of the lesion. Therefore, myofibroblast proliferation may be responsible for this pathological condition, and this observation deserves confirmation via molecular pathology techniques. We hypothesized that certain factors, such as inflammation, trauma, and hemorrhage, may lead to myofibroblast proliferation, splenic tissue division and encapsulation, small vessel growth in the interstitial space and vascular structures in sinusoidal endothelial cells, the development of angiomatoid nodules, and consequently fibrosis and hyaline degeneration. Overall, SANT may be a reactive or neoplastic lesion related to the proliferation of myofibroblasts. Further studies are required to confirm the pathogenesis of SANT.

SANT should be distinguished from other primary splenic diseases, including the following: Inflammatory pseudotumors consisting of proliferating fibroblasts (arranged in bundles or disordered) and various inflammatory cells, including lymphocytes, plasmacytes, and histocytes; these are partly similar to SANT but do not possess angiomatoid nodules; splenic hamartoma, a neoplastic lesion mainly consisting of highly proliferated red pulp, with irregularly arranged cells, and has no multiple angiomatoid nodules and proliferating myofibroblasts[17]; littoral cell angioma, which mainly consists of sinus-shaped lumens with different sizes that are mutually linked in a labyrinthine manner or ballooned, with papillae inside; additionally, the surfaces of the lumens and papillae are coated with endothelial-like cells. Different from SANT, no surrounding fibrosis or hardening is observed. Follicular dendritic cell tumors, in which fusiform tumor cells are arranged alternately, with the background infiltrated by many lymphocytes. No multiple angiomatoid nodules are present, unlike in SANT. As it originates from follicular dendritic cells, CD21 and CD23 as the markers of such cells are expressed on these tumor cells.[18] In suspected cases of SANT, fine needle aspiration biopsy could be performed to identify the nature of the lesion and to avoid overtreatment.

5. Conclusions

SANT, a benign hyperplasic neoplastic disease, can be cured by clinical treatment with surgical excision, and generally without recurrence and metastasis. The 7 patients followed up until January, 2017 lived in good conditions, without recurrence or metastasis.

Author contributions

Conceptualization: Pei-long Cao, Chun-bao Wang.
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Resources: Pei-long Cao.
Validation: Pei-long Cao.
Writing – original draft: Pei-long Cao.
Writing – review & editing: Pei-long Cao, Kai Wang, Chun-bao Wang, Hong-yan Wang.

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