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Original Article

Upregulated expression of CD30 protein in sclerosing angiomatoid nodular transformation (SANT): studies of additional 4 cases and analyses of 6 cases previously published cases

Jun Zhou[1,2*], Dandan Zhang[1,2*], Guiming Hu[1,2], Xiangyu Zheng[1,2], Qin Shen[3], Wencai Li[1,2], Gannan Wang[1,2], Yihui Ma[1,2], Jingjing Xu[1,2], Zhihua Zhao[1,2]

[1]Department of Pathology, The First Affiliated Hospital of Zhengzhou University, Zhengzhou, China; [2]Basic Medicine College of Zhengzhou University, China; [3]Department of Pathology, Jingling Hospital of Nanjing University, Nanjing, China. [*]Equal contributors.

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Abstract: Sclerosing angiomatoid nodular transformation (SANT) of spleen is a benign lesion with a distinct morphological and immunohisochemical characteristics. Only Weinred I et al (Virchow Arch 451: 73-9, 2007) reported 6 cases of SANT expressing CD30, of which positive for EBV by in situ hybridization (EBER). 4 cases of SANT were added to investigate the clinicopathological features and focused on the expression of CD30 and EBER combined with the previously published literature. Histologically, individual angiomatoid nodules were sharply delineated by fibrocollagenous stroma with numerous vascular lumens and surrounded by a different population of spindle and ovoid cells. Angiomatoid nodules of all of the 4 cases heterogeneously expressed CD34, CD8, CD68 and diffusely demonstrated CD31 and CD30, but none were positive for EBER. We added these cases with reviewed literature to emphasize and verify the fact that upregulated expression of CD30 in SANT is quite common, which should be taken into consideration when making differential diagnosis.

Keywords: Spleen, sclerosing angiomatoid nodular transformation, CD30, EBER, differential diagnosis

Introduction

Sclerosing angiomatoid nodular transformation (SANT), as a distinctive nonneoplastic vascular lesion exclusively involving the spleen, initially proposed by Martel et al [1]. A prominent characteristic of varying-sized nodules with superimposed dense fibrous stroma separation in morphology and the typical immunostaining by virtue of CD31, CD8 and CD34 illustrating three distinct types of vessels are quite impressive. Other than Weinred I et al, rare literature reported the frequent expression of CD30 in SANT which might be responsible for a matter of prudence when encountering the masqueraders bearing similar configuration and immunophenotypes [2]. Herein, we added 4 cases of SANT to further investigate and verify the expression of CD30 and discussed the relevant differential diagnosis.

Material and method

2 cases were derived from department of pathology of the first affiliated hospital of Zhengzhou University; another 2 cases were retrieved from department of pathology of the second affiliated hospital of Zhengzhou University and Jingling hospital of Nanjing University respectively. Complete clinical and follow-up data were obtained. The surgical specimen were fixed in 4% formalin, embedded routinely in paraffin and then stained with hematoxylin and eosin. Immuohistochemical studies were performed using commercial antibodies in the Ventana BenchMark XT instrument (Ventana System, Tucson AZ). The antibodies included CD31, CD34, CD8, CD68, CD30, CD15 (all above from Ventana, prediluted). Immunostaining for CD30 was carried out in the typical case of anaplastic large cell lym-

Table 1. 4 cases of clinical data, immnohistochemistry and follow-up

Case	Gender	Age (years)	Spleen size (cm × cm × cm)	Lesion size (cm × cm × cm)	Presentation	Positive Immunophenotype	Follow-up
1	Female	40	17 × 14 × 6.5	9.5 × 6.5 × 5.5	Left papillary thyroid carcinoma with metastasis to lymph nodes; multiple hepatic cysts; splenic mass	CD30/CD31/ CD34/Fascin/ FVIII/CD68/SMA	6 months, NER
2	Male	50	12 × 8 × 6	8 × 6.5 × 5	Hypertension; hepatitis A; multiple hepatic cysts; splenic mass	CD30/CD31/ CD34/Fascin/VIII/ CD8/CD68/SMA	9 months, NER
3	Male	65	13 × 7 × 5.5	6 × 4 × 3	Hypertension; multiple hepatic cysts; nephric and splenic mass	CD30/CD31/ CD34/Fascin/VIII/ CD8/CD68/SMA	48 months, NER
4	Female	59	15 × 10 × 5	5.5 × 5 × 4.5	Hypertension; diabetes; left nephric cysts; gallbladder polyps; mild fatty liver; splenic mass	CD30/CD31/ CD34/Fascin/VIII/ CD8/CD68/SMA	8 months, NER

NER, no evidence of recurrence.

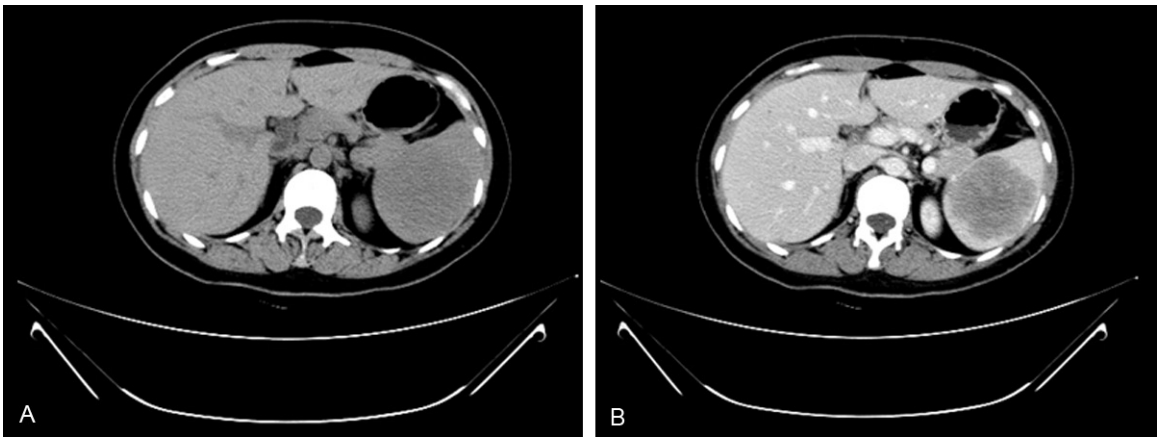

Figure 1. (A, B) CT plain scan showed an isodense mass involved the spleens (A) with a minimal enhancement revealed by contrast enhanced CT (B).

Figure 2. Typical general appearance of SANT. Note the obvious central fibrotic scar composed of bans of gray-white fibrous septa.

phoma as a positive control. In situ hybridization for EBV-encoded RNA (EBER) was performed on the automated Bond-max system (Leica Biosystems, Wetzlar, Germany) according to the manufacturer's instructions using the EBER probe (Catalog PB0589).

Results

Clinical findings

The main clinicopathologic data are summarized in **Table 1**. Cases included 2 males and 2 females, ranging in age from 40 to 65 with a median age 54 years. The splenic mass in all 4 cases were asymptomatic and revealed by imaging studies accidentally varying from 5.5 cm to 9.5 cm in the maximum diameter. Three fourth of the patients were presented with hypertension and multiple hepatic and nephric cysts, one of who suffered from left papillary thyroid carcinoma with metastasis to lymph nodes (case 1). All of the patients denied famil-

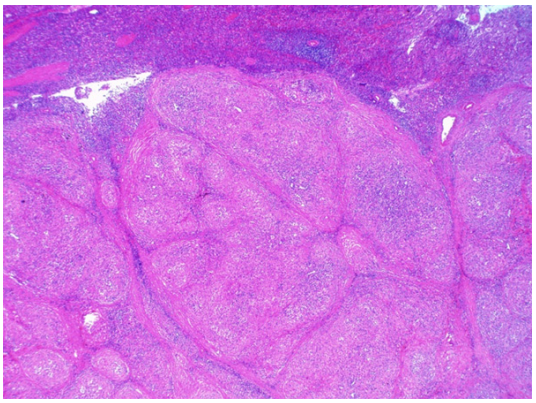

Figure 3. The angiomatoid nodules of SANT were variable in size, fine-contoured with partial coalescences with a relatively fine demarcation.

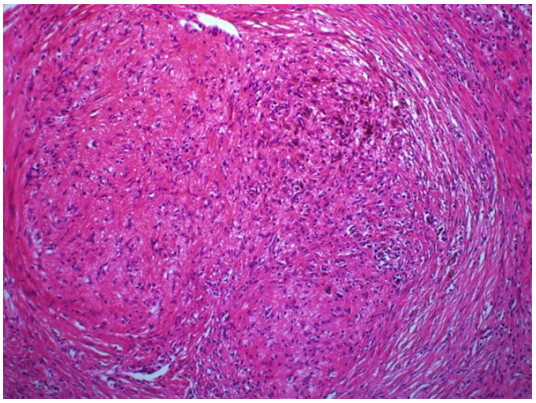

Figure 4. High magnification of details of SANT. The nodules were composed of slit-like vessels, small thin-walled veins and plump spindle cells with inflammatory cells infiltration and hemosiderin pigments.

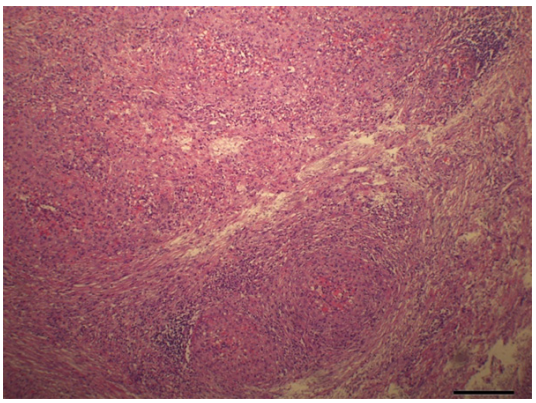

Figure 5. Case 1 took on an appearance of a florid proliferation with abundant epithelia.

ial heredity disease except for the pathetic plights of case 4 whose father died from the

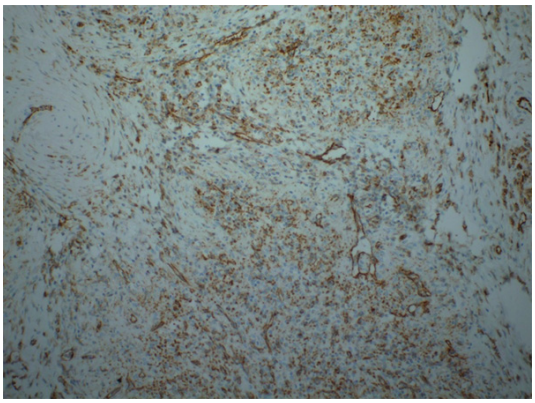

Figure 6. Angiomatoid nodules of SANT diffusely expressed CD31.

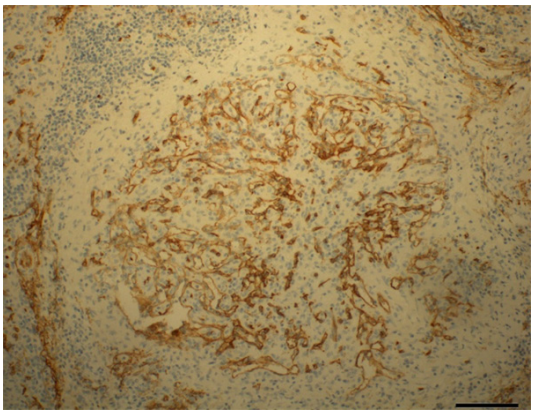

Figure 7. Nodules of SANT demonstrated a selectively positive staining of the narrow capillaries by CD34.

severe emphysema, departed mother subjected to lung carcinoma, and son succumbed to leukemia, but no further evidence to tell the genetic correlation to the disease of case 4. No significant laboratory markers were appreciated. Computed tomography (CT) scan showed isodense masses involved the spleens with a minimal enhancement revealed by contrast enhanced CT (CECT) (**Figure 1A**, **1B**). All of the patients underwent a splenectomy with a favorable prognosis without any evidences of relapse.

Pathological findings

Grossly, all of the lesions were solitary and fine demarcation, measuring 5.5 cm to 9.5 cm in size with a bulging cut surface and an obvious central fibrotic scar composed of bans of gray-white fibrous septa extending throughout to divide the lesion into lobules with multiple nod-

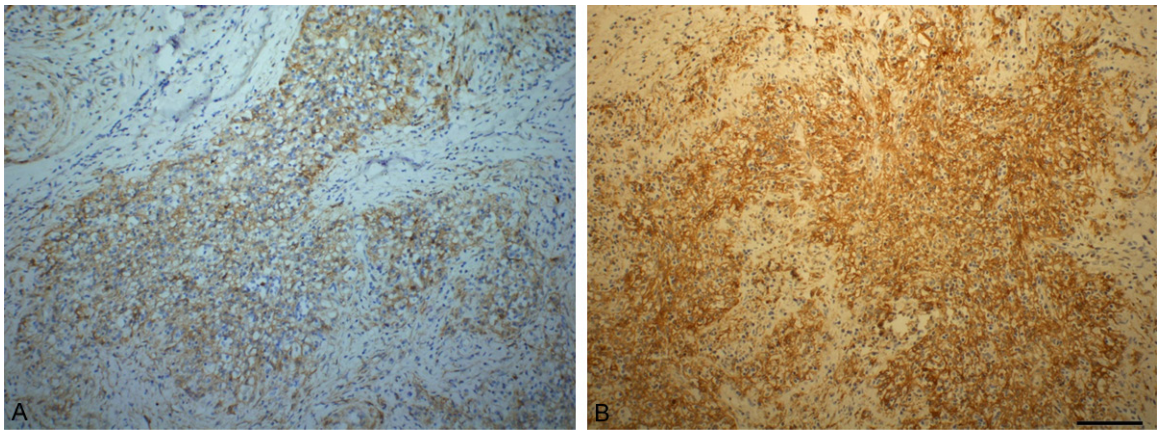

Figure 8. A. Angiomatoid nodules of SANT diffusely expressed CD30. B. More extensive expression of CD30 was appreciated in case 1 for its florid endothelium proliferation.

ules in different sizes (**Figure 2**). Histologically, the pathognomonic features of angiomatoid nodular appearance separated by the fibrotic tissues replicated the gross examination. The angiomatoid nodules were variable-sized, fine-contoured with partial coalescences (**Figure 3**). Within the nodules, there mixed slit-like or capillary-like vessels, small thin-walled veins and plump spindle cells in different proportion with more or less extraverted erythrocytes, hemosiderin deposition and inflammatory cells composed of small lymphocytes, plasma cells, histocytes and scant neutrophils (**Figure 4**). Compared to other 3 cases, case 1 took on an appearance of a florid proliferation with superimposed abundant and stratified endothelia of the small capillaries in the most angiomatoid nodules (**Figure 5**). Immunohistochemically, nodules demonstrated a diffuse positivity for CD31 labeling all the endothelia in any vascular type (**Figure 6**), but a selectively positive staining of the narrow capillaries by CD34 (**Figure 7**). CD8 variably expressed within the nodules with some lacking reactivity and CD68 heterogeneously stained some plump spindle cells or histocytes. Angiomatoid nodules in all the cases were diffusely positive for CD30 corresponding to the positive pattern of CD31, accentuating a sheet or latticed appearance composed of the numerous proliferated endothelia in each nodule (**Figure 8A**). In addition, more extensive expression was appreciated in case 1 for its florid endothelium proliferation (**Figure 8B**). Other than some inflammatory cells, CD15 was negative. EBV was not detected by EBER.

Discussion

Previously termed as “cord capillary hemangioma”, SANT is recognized as a distinctive noneoplastic vascular entity but different from littoral cell angiosarcoma, the conventional hemangioma, and hemangioendothelioma [1, 3]. But the exact nature of SANT is still controversial. Focal areas of some SANTs adopting large sheets of inflammatory fibrosis were observed, showing a close relationship with inflammatory pseudotumor (IPT) [4, 5]. The presence of a significant higher IgG4+ plasma cells and the IgG4/IgG ratio was reported by some literature [6-8]. However, no overwhelming evidences have represented SANT meeting the critical diagnostic requirements as one form of IgG4-related disease [9, 10]. In the literature published by Chiu et al, all 3 cases of splenic SANT showed nonrandom X-chromosome inactivation pattern suggesting the nature of clonality, which would be an evidence of SANT as a new true neoplasm [11]. However, further studies should be performed to evaluate the biological significance of that finding.

SANT usually affects 20-to 70-year group (mean age, 48 years), with a female predominance [1, 12]. Clinically, patients are usually asymptomatic and most lesions are found on imaging incidentally [4]. The gross appearance tend to be distinctive, which exhibits a solitary and demarcated lesion measuring 3 to 17 cm with a bosselated cut surface separated by the dense and gray-white fibrotic stroma with foci red hemorrhage, reminiscent of inflammatory

pseudotumor of the spleen [1]. Histologically, although there are usually deficient in a definite capsule merging with the peripherally normal splenic parenchyma, this abnormal fibrovascular lesion was interpreted as a relevant clear border in low magnification. A significantly multinodular growth pattern is haphazardly delineated by bands of fibrous or stromal tissue with scattered myofibroblasts, siderophages, and inflammatory cells infiltration [12-14]. Three distinct types of vessels intermixed in different proportion within the each angiomatoid nodule: well-formed cord capillaries bearing $CD34^{+}CD8^{-}CD31^{+}$, splenic sinusoids displaying $CD34^{-}CD8^{+}CD31^{+}$, and small veins possessing $CD34^{-}CD8^{-}CD31^{+}$. The spindle cells in the nodules and fibrotic septa can be immunoreactive with SMA and CD68 [6, 15]. The clinicopathologic features of our 4 cases corresponded to that we discussed above. Conspicuously, most of our cases suffered from multiple hepatic and nephric cysts and functional disorders such as hypertension. Similarly, several concurrent diseases were also described by some literature, but whether the correlation exists between these situation and SANT is unclear [1, 2]. Furthermore, the phenomenon of all the angiomatoid nodules expressing CD30 was found occasionally when we made a differential diagnosis by means of panel of immunohistochemical makers. As demonstrated above, our 4 cases of SANT, as well as the cases reported by Weinred I et al, consistently expressed CD30 in every angiomatoid module [2]. Interestingly, case 1 with a florid endothelium hyperplasia accentuated a much stronger positive staining of CD30 further suggesting the distinctive phenomenon that upregulated expression of CD30 in vascular endothelia of the SANTs indeed existed. Evidence from an in vitro experiment seemed to prove the possibility of CD30 expression in endothelial cells, which may suggests the formation of SANT is related with a cytokine-mediate process in more or less [2, 16]. When the nodules appear more cellular form with scattered binucleated endothelia set in the granuloma-like pattern with conspicuous fibrotic septa and, exceptionally, inflammation cells positive for CD15, the pitfall to be diagnosed as “nodular lymphocyte predominant Hodgkin lymphoma (NLPHL)” should be avoided. However, the diffusely immunoreactive pattern of CD30 in SANT is distinct from that in NLPHL with only the haphazard lacunar or binuclear cells expressing CD30 admixed with variable numbers of small lymphocytes, neutrophils, eosinophils, plasma cells, and histocytes. In addition, nuclear staining with EBER was detected in the spindle cells either within angiomatoid nodules or the fibrotic septa reported by some literature, which argued that SANT may be related to IPT [2, 4]. But identical to ours, not all cases were of compulsory EBER expression [5, 12]. Additionally, IPTs do not contain the significant angiomatoid nodules, the hallmark feature, in SANT, although they share the similar morphology focally in some cases [4, 12].

To rule out other vascular lesions is crucial including inflammatory granuloma, hemangioma, hemagiomoendothelioma, and angiosaroma. Resembling to their soft tissue counterparts, they are composed of vascular spaces in varying differentiation lined by bland or malignant endothelia in a single layer or stratified appearance, and express the typical vascular marker such as CD31, CD34, but not CD8, differing from the multiple types of blood vessels of SANT [1]. Littoral cell angioma (LCA), a benign vascular tumor exclusively developing in the spleen, comprises the anastomosing vascular spaces are lined by plump cells with bland oval to indented nuclei with interspersed pseudopapillary structure. Although LCA could also form multiple-nodule growth pattern to mimic SANT, a unique immunophenotype can tell them apart, with coexpression of histocytic and endothelial markers as CD31, factor VIII, CD68, but surprisingly negative for CD34 and CD8 [13, 14]. All of the lesions above we should take a consideration seemed to lack the diffusely positive CD30 expression, so CD30 may be a useful auxiliary makers to support the diagnosis of SANT [2]. However, further studies need to perform.

In conclusion, we added another 4 cases of SANT combined the 6 cases reported previously, with an emphasis on the real upregulated expression of CD30. To recognize this significant immunostaining characteristic contribute to avoid the pitfalls and dilemmas when making a differential diagnosis. Further studies are needed to elucidate the pathogenesis of diffuse expression of CD30 in SANT.

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Disclosure of conflict of interest

None.

Address correspondence to: Dr. Jun Zhou, Department of Pathology, The First Affiliated Hospital of Zhengzhou University, Zhengzhou 450052, Jianshe Road, China. Tel: 0086-186-255-08341; E-mail: zhoujun2006305@126.com

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