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ORIGINAL PAPER

An evidence-based aetiology study of synovial cyst of knee cruciate ligament: a real or pseudo-cyst

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Abstract

Purpose Synovial cyst of knee cruciate ligament (SCKCL) is a rare condition but can cause severe knee pain. The understanding of its aetiology is relatively poor. This current study aimed to elucidate the pathogenesis of SCKCL based on a series of histo- and cytopathological examination.

Methods Ten SCKCL patients who underwent arthroscopy were enrolled, amongst five patients claimed past knee injury. Haematoxylin & eosin staining was conducted to the cyst wall tissue sections and Papanicolaou staining to the cyst fluid smear. Prussian blue staining was employed to both the wall section and fluid smear. Immumohistochemical staining for mesothelial cells (MC), epithelial cells (CK), vascular endothelial cells (CD31), monocytes (CD68), and haematogenous stem cells (CD117) were taken to elucidate the possible involvement of various cell types in the development of SCKCL.

Results No erythrocyte was discovered in the fluid; however, Prussian blue stained haemosiderin particles were found in the cyst wall and fluid, suggesting past haemorrhage in all patients. Abundant lymphocytes and plasmocytes were observed in the cyst wall and fluid. In addition, the cyst lining was infiltrated with abundant CD68(+) monocytes while only few MC(+) mesothelial cells were sporadically observed in four samples. The cyst submucosa was also diffused with abundant CD68(+) monocytes and proliferated capillaries stained with CD31. CD117-positve haematogenous stem cells were sporadically observed in eight specimens.

Conclusion Our findings provided evidence that SCKCL is not a mature synovial cyst but rather an inflammatory pseudo-cyst. It may have resulted from past minor haemorrhage and intra-ligament chronic inflammation.

Keywords Immumohistochemical staining · Knee cruciate ligament · Synovial cyst

Introduction

Ganglion cyst (GC) is a benign, cystic lesion that commonly arises from tendon sheaths and joint capsules in the wrist or ankle joint. It is generally believed that knee cruciate ligament cyst (KCLC) is a special condition of GC [1–4] with a rare incidence rate of 1.06% [5] or 0.36% [3] only. However, KCLC is obviously different from GC in a few aspects.

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KCLC primarily appears on the surface of or inside the anterior cruciate ligament (ACL) or posterior cruciate ligament (PCL). Differing from GC, KCLC is not asymptomatic; it presents with obvious knee pain, claudication, and locking. Based on the pathological classification, a cyst can be recognized as a synovial cyst when its inner surface is covered with cells; otherwise, it is defined as ganglion cyst [6]. However, KCLC might be misdiagnosed as GC without presenting solid pathological evidence [1, 3, 5].

After all, the aetiology of KCLC remains unclear, which may account for the misunderstanding and controversy in the pathogenesis of KCLC. Several hypotheses have been proposed that it may arise from connective tissue degeneration after trauma [2, 4, 7], herniation of synovial tissue [4], ectopia of synovial tissue [8], and proliferation of pluripotent mesenchymal stem cells [9]. However, to our knowledge, there is still lack of sufficient evidence to support these theories.

In this study, we analyzed 10 KCLC patients and aimed to elucidate the pathological mechanism of KCLC. By histopathological examinations including haematoxylin and eosin (H&E) staining, Papanicolaou staining, Prussian blue staining, and immunohistochemistry staining, our study provided evidence to support a new understanding of KCLC.

Materials and methods

Cases selection

The study was carried out upon the approval of the Ethics Committee in our hospital. Ten symptomatic patients with KCLC who were diagnosed by magnetic resonance imaging (MRI) or arthroscopy (Fig. 1) in our department from June 2010 to January 2015 were included (Table 1). The mean age was 32 (ranging from 17 to 64 years old). There were seven male and three female patients, and six patients were diagnosed with ACL cyst while the rest were PCL cyst.

Surgical technique

Arthroscopic surgery was performed on all the patients. The operation was carried out by a senior surgeon and a junior resident. All the operations were performed under spinal anaesthesia. Anterolateral-anteromedial approach was employed in five ACL cyst patients and two PCL cyst patients whose cysts were located in front of the ligament. Posterior approach was used in two patients with the cyst in the back of PCL. The cyst was removed under arthroscopy and histological examination was performed on the cyst specimens. After the cyst resection, two ACL cyst patients received ACL reconstruction.

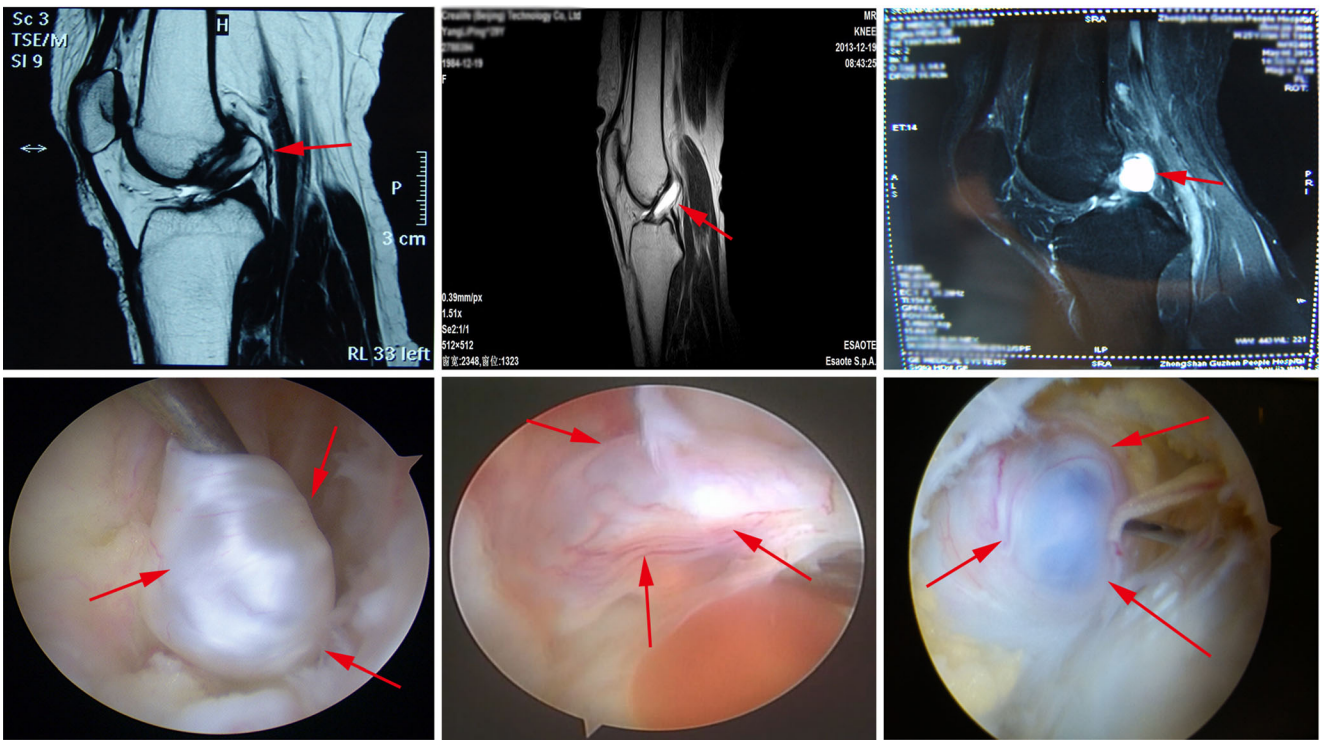

Fig. 1 Knee cruciate ligament cyst presented on magnetic resonance imaging (MRI) and under arthroscopy

Table 1 Demographic data of patients

Case	Age	Sex	Trauma history	Other lesions	Location	Cyst fluid color
1	41	Male	–	Lateral meniscus lesion	ACL	Red
2	20	Male	+	Synovitis	ACL	Yellowish
3	34	Male	+	ACL lesion	PCL	Yellowish
4	29	Female	–	–	ACL	Yellowish
5	22	Female	+	–	ACL	Red
6	24	Male	–	–	PCL	Yellowish
7	17	Male	+	ACL and Lateral meniscus lesion	PCL	Yellowish
8	64	Female	–	–	ACL	Red
9	31	Male	+	Lateral meniscus lesion	PCL	Yellowish
10	38	Male	–	–	ACL	Yellowish

ACL, anterior cruciate ligament; *PCL*, posterior cruciate ligament

Hematoxylin and eosin staining

H&E staining was conducted according to routine protocol. In brief, after deparaffinization and rehydration, 5-μm longitudinal tissue sections were stained with haematoxylin solution for five minutes. After dipping in 1% acid ethanol (1% hydrogen chloride in 70% ethanol) and then rinsing in distilled water, the sections were then stained with eosin solution for three minutes, followed by dehydration with graded alcohol and clearing in xylene. The mounted slides were then examined and photographed using a microscope.

Papanicolaou staining

The colour of the cyst fluid extracted by puncture under the arthroscopy was documented. The cyst fluid was briefly centrifuged at 1000 rpm for one minute and the 2/3 upper supernatant layer was discarded. The rest of the sample was made into smear. First, the smear was stained with Harris's haematoxylin after rehydration. Second, it was processed with Orange Gelb (OG)6 for two minutes and was dehydrated in isopropyl alcohol. After two times rinse in 95% isopropyl alcohol for two minutes each, it was immersed in Eosin Azure (EA)50 for three minutes. In the end, the sample was rinsed in 95% isopropyl alcohol for one minute and then air dried.

Prussian blue staining

For the specimens of the cyst wall, tissue blocks were sliced into sections and dewaxed before being rinsed in distilled water. The sections were immersed in a mixture solution containing equal ratio of 2% ferrous potassium hydride and 2% hydrogen chloride for ten minutes. The sections were then washed in distilled water before being counterstained with nuclear fast red for ten minutes, followed by sequential processes, i.e., water-washing, dehydration, clearing, and mounting.

For the cyst fluid, the specimens were centrifuged at 1000 rpm, followed by removal of supernatant and the smear was then made. The smear was fixed in methanol. After air dried, the smear was immersed in a mixture solution containing 3% ferrous potassium hydride and 2% hydrogen chloride and incubated at 37 °C for 30 minutes. After rinses with distilled water and phosphate buffer solution (PBS), the slides were counterstained with nuclear fast red for ten minutes. The positive result is blue mass or particle presented in the cyst fluid smear under microscopy indicating the sedimentation of haemosiderin.

Immunohistochemical staining

Immunohistochemical staining was performed using 4-μm thick, formalin-fixed, paraffin-embedded tissue sections. Five mouse anti-human monoclonal antibodies were chosen, including CD117 (haematogenous stem cells marker) (Leica, Germany), CD68 (monocyte-macrophages marker) (Leica, Germany), CD31 (vascular endothelial cells marker) (Leica, Germany), MC (mesothelial cells marker) (CM, America), and CK (epithelial cells marker) (Life, America).

Result

Trauma history of patients

Among the ten patients, only five had clear knee trauma history. The demographic data of patients are shown in Table 1. Despite low incidence of trauma history, most of the patients came to the clinic with clinical manifestations including knee pain, swelling, extension block, and limitation of knee flexion. However, no significant abnormality was found by preoperative X-ray test.

Histological characteristics of the cyst wall

By H&E staining, chronic inflammatory cells such as lymphocytes and plasmocytes, and a few epithelioid cells were

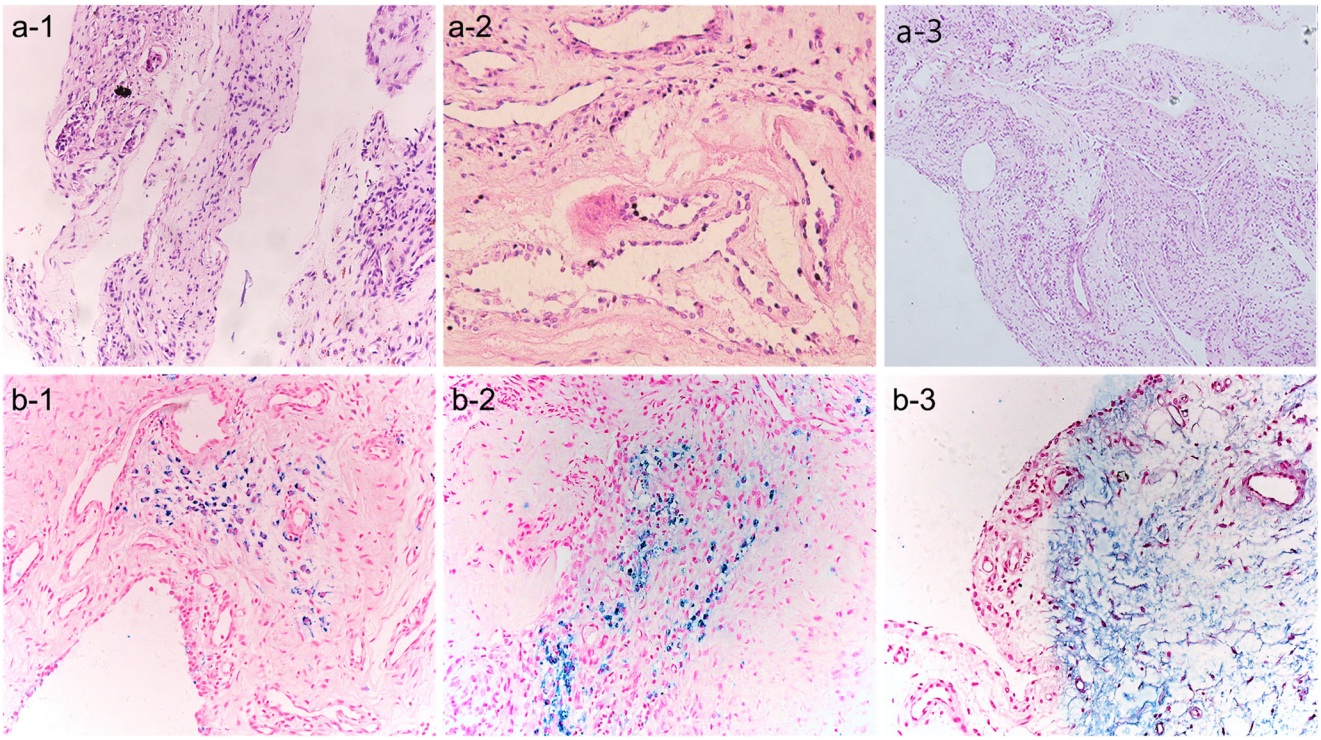

Fig. 2 Histological features of the cyst wall. (a-1–3) Haematoxylin and eosin (H&E) stain, interstitial capillaries, chronic inflammatory cells, and epithelioid cells were observed. (b-1–3) Prussian blue stain and positive stained blue particles were observed

observed in the cyst wall. Vascular endothelial cells and proliferous interstitial capillaries were observed in the submucosa (Fig. 2(a)). All of the 10 patients were confirmed with the diagnose of synovial cyst of knee cruciate ligament (SCKCL) according to the criteria [6].

By Prussian blue staining, abundant blue particles were observed sedimenting inside the cyst wall in all the samples examined. The blue particles aggregated and manifested with punctiform or blade shape (Fig. 2(b)), indicating the sedimentation of haemosiderin in the cyst wall.

Cytological characteristics of the cyst fluid

0.5~1 mL cyst fluid was extracted during the operation from each patient. Seven patients had yellowish fluid samples and the other three patients had red fluid samples. There is no correlation between patients' knee trauma history and the cyst fluid colour, i.e., not necessarily red cyst fluid appeared in patients with knee trauma history or yellowish fluid in patients without trauma history. Papanicolaou staining showed that the cyst fluid cells mainly consisted of inflammatory cells (including lymphocytes and plasmocytes), histocyte (monocyte), and quasi-circular mesothelial-like cells, which is in correspondence with the cell composition of the cyst wall (Fig. 3(a)). No red blood cell was found in the cyst fluid smear.

By Prussian blue staining, ample blue particles were also observed in the cyst fluid smear (Fig. 3(b, c)), indicating the sedimentation of haemosiderin in the cyst fluid.

Immunohistochemical characteristics

MC-positive cells were sporadically observed in only four samples examined while the rest showed negative result (Fig. 4(a)). CK marker was negatively stained in all the samples (Fig. 4(b)). Both CD68 (Fig. 4(c)) and CD31 (Fig. 4(d)) were positive in all samples, and the CD68-positive cells were distributed in both the epithelial and the submucosa layers of the cyst wall. The CD31-positive proliferous interstitial capillaries were found abundant in the submucosa. The CD117-positive cells were observed in eight samples, half of them were also positively stained by MC (Fig. 4(e) and Table 2). The results are summarized in Table 2.

Discussion

The pathogenesis of KCLC remains unclear and there are still many controversies on this topic. A study by Liu et al. suggested that KCLC is more likely a congenital abnormality rather than trauma-related [10]. Kang et al. also reported a case of ACL cyst patient without trauma history but rather a congenital abnormality [11]. Hameed et al. [9] and Feldmann et al. [12] showed that KCLC might be associated with a herniation derived from intra-ligament synovial membrane. However, many others regarded trauma as an important factor that was closely associated with the occurrence of KCLC [13, 14]. Nevertheless, the reported percentage of explicit trauma

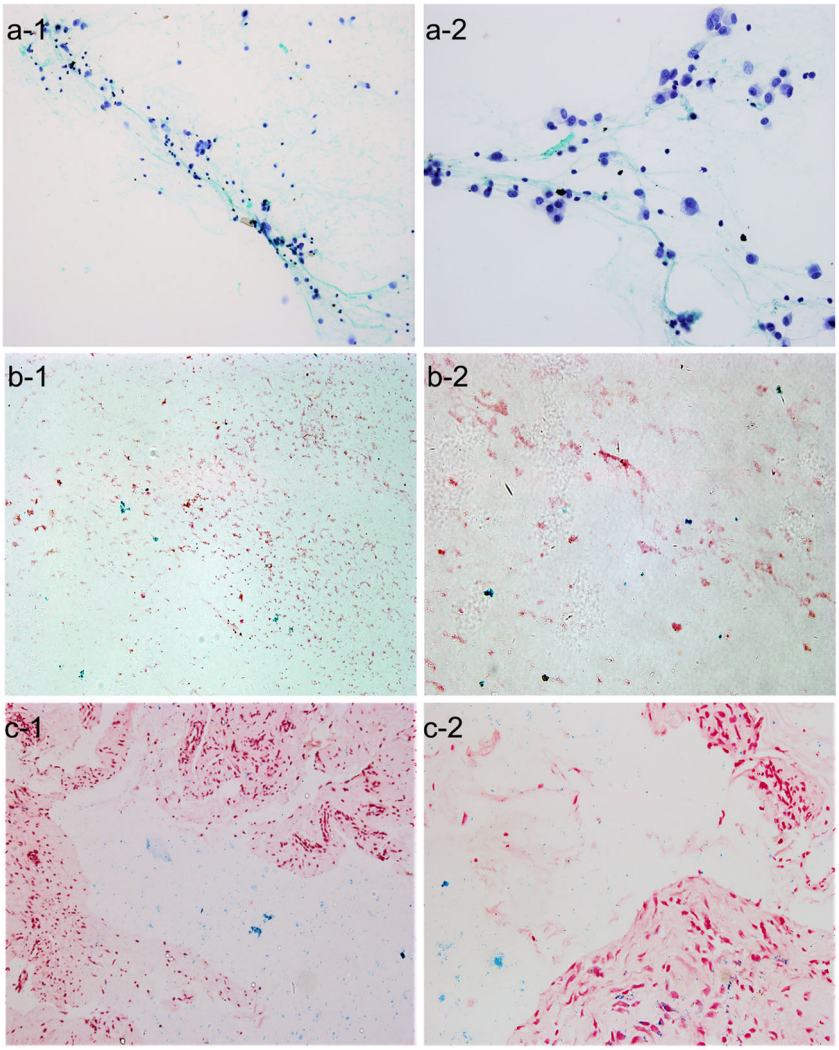

Fig. 3 Features of cyst fluid. (a-1–2) Papanicolaou stain, chronic inflammatory cells including lymphocyte and plasmocyte (green arrows), and histocytes like monocyte (red arrows) as well as some quasi-circular mesothelial-like cells (yellow arrow) were observed. (b-1–2 and c-1–2) Prussian blue stain and positive stained blue particles were observed

experience in KCLC patients was not as high as expected. More than half of the patients denied trauma experience in a study by Shetty et al. [4] and only two out of ten patients acknowledged trauma experience in another study by Garcia-Alvarez et al. [15]. None of the patients in a report by Sumen et al. had any trauma history [16].

Based on the low incidence rate of trauma history, the "trauma" hypothesis about KCLC is hardly accepted. Nevertheless, Mao et al. [3] discovered tissues of synovial membrane, collagen, and fascia inside the KCLC upon histological examination. They considered that repeated minor knee trauma might have contributed to the development of the cyst. Sloane et al. [17] also suggested the same. The study by Sloane et al. indicated that KCLC patients might have suffered from repetitive micro-trauma from knee joint and soft tissue motion during exercise, but such mild trauma history would be easily neglected.

Regarding the mechanism involved in the development of the cyst, Shetty et al. [4] suggested that haemorrhage overlying the cruciate ligament was a primary trigger to patients with antecedent history of knee trauma. They also discovered that the cyst fluid color may be correlated with the time when the bleeding occurred. Bloody fluid may reflect a recent haemorrhage, while the yellowish viscous fluid could be a result of resolution of a long-standing local haematoma. However, for objective assessment of a recent haematoma, the golden standard is to confirm the presence of red blood cells in the fluid smear. In our study, three patients showed red color cyst fluid and the other seven showed yellowish fluid. Interestingly, none of the above cyst fluid specimens were found with red blood cells by cytological examination. Taken together, SCKCL cannot be attributed to a fresh intra-ligament haematoma no matter the cyst fluid is red or not.

Prussian blue staining for both the cyst fluid smear and the cyst wall section was further performed to identify if an old haematoma was present. We noticed that considerable amount of haemosiderin particles was present in both the cyst fluid smears and the cyst wall sections of all patients. Hence, it indicated that minor focal haemorrhage ever occurred repetitively in the ligament and the transudation from the micro-

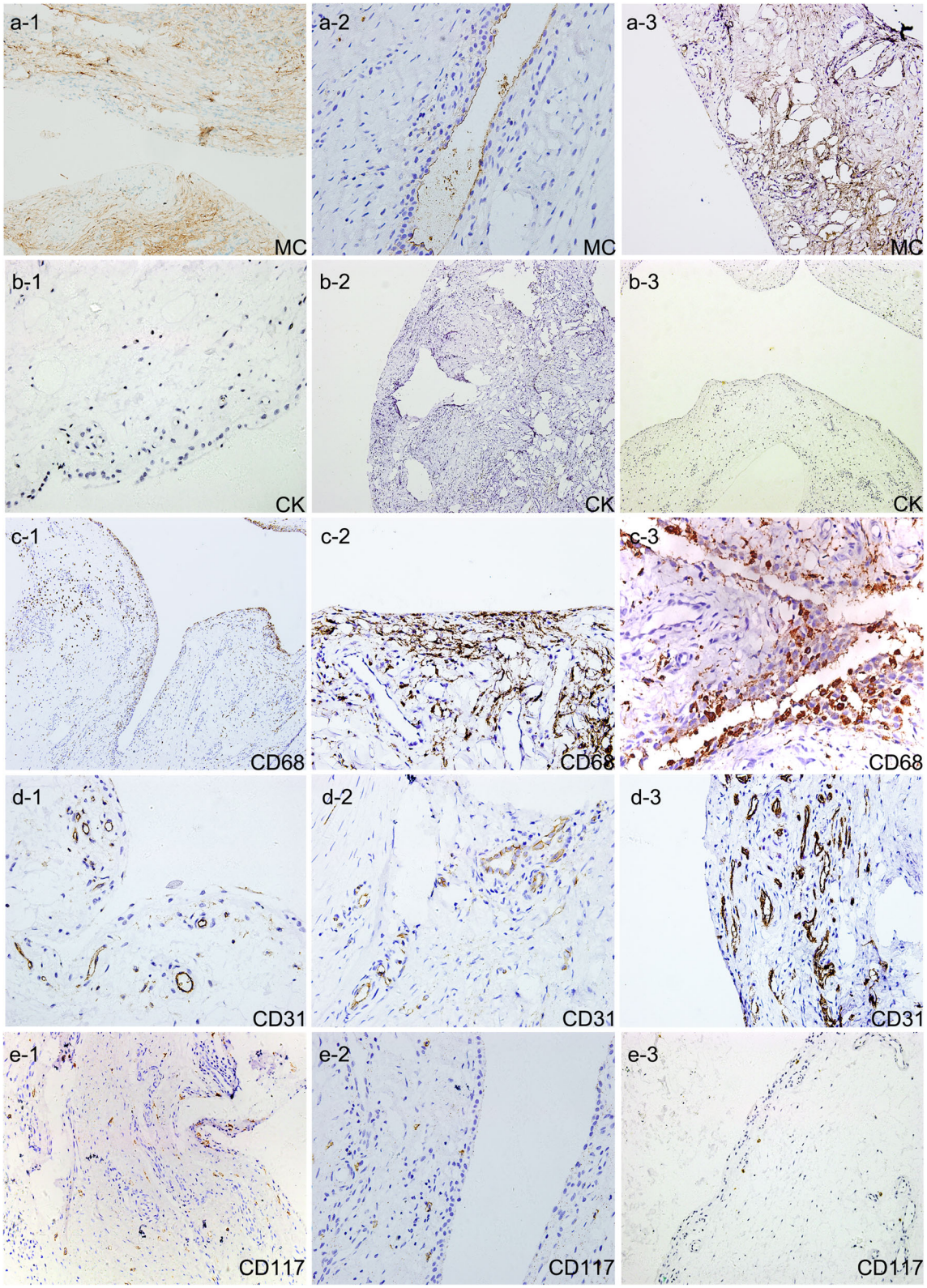

Fig. 4 Immunohistochemistry of the cyst wall. (a-1–3) Marker MC, most of the samples showed a negative result as shown in (a-1) and (a-2), while sporadic stained cells were observed in four samples, which is shown in (a-3). (b-1–3) Marker CK, negative results were present in all samples. (c-1–3) Marker CD68, monocytes that were stained was seen in the epithelial layer and the submucosa in all samples. (d-1–3) Marker CD31, positively stained cells are distributed around the capillaries in all samples. (e-1–3) Marker CD117, sporadic stained cells were observed in eight samples

Table 2 Summary of Prussia stain and immunohistochemistry for the cyst wall

Case	Prussian blue stain	MC	CK	CD68	CD31	CD117
1	+	+	–	+	+	+
2	+	+	–	+	+	+
3	+	–	–	+	+	–
4	+	–	–	+	+	+
5	+	–	–	+	+	–
6	+	–	–	+	+	+
7	+	+	–	+	+	+
8	+	+	–	+	+	+
9	+	–	–	+	+	+
10	+	–	–	+	+	+

vessels might be the archetype of the cyst fluid. The red blood cells in the haematoma disintegrated gradually and in the end, haemosiderin was left. Our findings supported the hypothesis that minor trauma may play a key role in the development of SCKCL. It is not surprising that minor trauma is easily unnoticed; thus, the reported incidence of trauma history is low.

Apart from haemosiderin particles that might result from disintegrating red blood cells, we noticed that the cyst fluid contained predominantly inflammatory cells, mainly lymphocytes and plasmocytes, as well as a few monocytes and quasi-circular mesothelial-like cells. To figure out the origin of the inflammatory cells and the quasi-circular cells in the cyst fluid, we also took immunohistochemical staining to the cyst wall. Interestingly, we found that MC-positive cells were only sporadically observed in four samples that were examined, and none of the patient's samples were stained positive with CK marker. This indicated that the lining of the cyst wall was neither dominantly consisted of mature mesothelial cells nor epithelial cells; only a few mature mesothelial cells were present in the cyst wall. This is consistent with our finding by Papanicolaou staining in the cyst fluid. Taken together, SCKCL cannot be a real synovial cyst, or at least not a developed synovial cyst.

As monocytes showed in the cyst fluid, the monocyte-macrophage marker CD68 was used to determine its distribution in the cyst wall. Remarkably, we discovered that CD68-positive cells were widely distributed in both the lining and the submucosa of the cyst wall in all the samples. Combined with the histological and cytological results, we believed that the proliferation was closely associated with the focal chronic inflammatory reaction and phagocytosis. Monocyte-macrophage plays an important role as the "scavenger" in vivo. With the continuous chronic inflammatory reaction in the cyst, monocytes increased locally and swallowed the cell fragment like erythrocytes or haemosiderin. CD31, a marker of vascular endothelial cell, positively stained the endothelium of the proliferous capillaries that were present abundantly in the cyst wall in all the samples, also indicating a remarkable chronic inflammation.

Our findings that chronic inflammatory cells (lymphocytes and plasmocytes), histocytes (monocytes) instead of conventional mesothelial cells, or epithelial cells are the main constitution of the cyst wall lining, which suggested that continuous inflammatory reaction, i.e., inflammatory cell infiltration, phagocytosis by monocytes, and capillaries proliferation following repetitive micro-trauma may have accounted for the development of KCLC. Therefore, the cyst was more likely a pseudo-inflammatory cyst than a synovial cyst. In consideration of very few quasi-circular mesothelial-like cells being discovered in the cyst fluid smear and sporadic MC-positive cells being discovered in the cyst wall, we speculated that these mesothelial cells might be developed from some specific cells in the cyst wall. However, the sporadic presence of these cells suggested that only a small part of cells had completed the differentiation. Most of the cells might differentiate but merely at a primary stage. In other words, the pseudo-inflammatory cyst might have a potential to differentiate into a synovial cyst. Nevertheless, the differentiation process was still far from complete.

To figure out what specific cells can differentiate into mesothelial cells in the cyst, a study by Kaotaoko et al. [18] attracted our attention. They reported that haematogenous stem cells could differentiate into mesothelial cells in vitro. In our study, CD117-positive cells were found scattered in the cyst wall of eight specimens examined. CD117 is a transmembrane receptor with tyrosine kinesis and has been acknowledged as the marker of haematogenous stem cells. Encouragingly, we also discovered that among those CD117-positive samples, the MC antigen-expressing cells were also present (Table 2). This supported our hypothesis that haematogenous stem cells originated from previous haemorrhage and inflammation might gradually differentiate to mesothelial-like cells to constitute the cyst wall.

To summarize, the pathogenesis of KCLC was investigated in the current study and we speculated that SCKCL was not a mature synovial cyst based on a series of histopathological evidence. SCKCL is more likely an inflammatory pseudocyst, with regional potential of synovial differentiation. Past repetitive minor trauma results in focal haemorrhage and recurrent inflammatory reaction. Chronic inflammation is generally characterized by inflammatory infiltration, exudation, and proliferation. Monocytes aggregate around the traumatic cruciate ligament and eliminate the red blood cell by phagocytosis. Gradually, the inflammatory cells, monocytes, the proliferous fibroblasts, and fibrous tissue constitute the cyst wall. Particularly, hematogenous stem cells may contribute to the presence of mesothelial-like cells in the development of SCKCL. Meanwhile, the inflammatory exudates containing the lysed erythrocytes form the cyst fluid. Eventually, recurrent inflammatory reaction leads to the SCKCL formation.

These current findings also support the efficacy and the low recurrence rate associated with arthroscopic excision [5, 19] for SCKCL treatment, which can theoretically remove the inflammatory cystic wall and inhibit subsequent exudation.

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Compliance with ethical standards

The study was carried out upon the approval of the Ethics Committee of Nanfang hospital. For this type of study, formal consent is not required.

Conflict of interest Jun Xiao receives a grant (81101389) from NNSFC (National Natural Science Foundation of China (CN)) to support the current research. For the rest of the co-authors, they declared that they have no conflict of interest.

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