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ARTICLE

Overlapping morphological, immunohistochemical and genetic features of superficial CD34-positive fibroblastic tumor and *PRDM10*-rearranged soft tissue tumor

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Superficial CD34-positive fibroblastic tumor (SCD34FT) is a recently recognized soft tissue tumor that is considered to be of borderline malignancy. The pathogenesis of this tumor remains incompletely understood, but it has been suggested that SCD34FT overlaps with tumors showing fusions involving the *PRDM10* gene. Previous analyses of *PRDM10*-rearranged tumors have demonstrated that they have a distinct gene expression profile, resulting in high expression of CADM3 (also known as SynCam3), which can be detected immunohistochemically. Here, we investigated a series ($n = 43$) of SCD34FT or *PRDM10*-rearranged tumors and potential mimics ($n = 226$) with regard to morphological, genetic, and immunohistochemical features. The results show that SCD34FT and *PRDM10*-rearranged tumor are morphologically indistinguishable; 41 of 43 tumors of both entities are CADM3-positive. Hence, we suggest that they constitute a single entity, preferably referred to as SCD34FT. Expression of CADM3 was only rarely seen in other soft tissue tumors, except in tumors with Schwann cell differentiation. Thus, IHC for CADM3, in combination with the characteristic morphological features, is a valuable adjunct in the diagnosis of SCD34FT.

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INTRODUCTION

Superficial CD34-positive fibroblastic tumor (SCD34FT) is a suprafascially located soft tissue tumor characterized by spindled cells with abundant, granular to glassy eosinophilic cytoplasm, hyperchromatic, bizarre-appearing nuclei, paradoxically low mitotic activity, diffuse CD34 and frequent aberrant keratin immunoreactivity and indolent clinical behavior [1]. The entity was first described in 2014 by Carter and colleagues and fewer than 60 cases have been reported in small series or case reports [2–6]. SCD34FT was incorporated in the latest edition of the World Health Organisation classification of soft tissue and bone tumors [1]. So far, aggressive clinical behavior of SCD34FT has not been reported. However, the presence of highly pleomorphic cells may be misinterpreted as evidence of a high grade undifferentiated pleomorphic sarcoma (UPS), especially on small biopsies [7, 8]. Although CD34 and keratin co-expression is quite characteristic of SCD34FT, neither marker is specific for this entity, and identification of more specific markers would be helpful in this sometimes challenging diagnosis.

Soft tissue tumors harboring fusion transcripts involving *PRDM10* have shown morphologic, immunohistochemical and clinical overlap with SCD34FT suggesting that *PRDM10* fusions are recurrent in SCD34FT [5]. A possible relationship between SCD34FT and *PRDM10*-rearranged tumors is further supported by overlapping morphological and molecular findings, as noted in a recent study of 20 SCD34FT and *PRDM10*-rearranged tumors [6]. *PRDM10*-rearranged tumors of soft tissue show a distinct transcriptional profile [9]. *CADM3*, coding for cell adhesion molecule 3, also known as synaptic cell adhesion molecule 3 (SynCAM3), nectin-like molecule-1 (NECL-1), TSLC1-like protein 1 (Tsll1), or immunoglobulin superfamily member 4B (Igsf4b), was one of the genes showing the highest differential expression in *PRDM10*-rearranged tumors when compared to other soft tissue tumors. *PRDM10*-rearranged tumors harboring *MED12::PRDM10* or *CITED2::PRDM10* fusion transcripts showed immunoreactivity for CADM3 and single cases of UPS did not [9].

We studied a large series of well-characterized soft tissue tumors previously classified as SCD34FT and *PRDM10*-rearranged soft tissue tumor, with the goals of comparing their morphologic,

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Table 1. Cohort of superficial CD34-positive fibroblastic tumors and *PRDM10*-rearranged tumors.

Case No	Sex age[a]	Location[a]	Size [mm] (duration of lesion)	Follow up [month][b]	IHC CADM3[c]	FISH *PRDM10*[d]	RNASeq[d]	RT-PCR[d]
1	F 50	Knee	70	NA	Positive ++ diffuse	NI	ND	ND
2	F 37	Chest wall	80	NA	Positive ++ diffuse	Negative	ND	ND
3	NA	NA	NA	NA	Positive ++ diffuse	Positive	ND	ND
4	F 33	Abdominal wall	25	NA	Negative	Negative	ND	ND
5	F 62	Thigh	42	NA	Positive ++ diffuse	ND	ND	ND
6	M 38	Thigh	22	NA	Positive +++ diffuse	Positive	ND	*MED12-PRDM10*
7	M 38	Hip	54	NA	Positive ++ diffuse	Positive	ND	ND
8	NA	NA	NA	NA	Positive ++ diffuse	ND	ND	ND
9	M 56	Arm	100 (20 yrs)	NA	Positive ++ diffuse	ND	ND	ND
10	F 35	Thigh	10	NA	Positive ++ diffuse	ND	ND	ND
11	F 55	Forearm	30 (many yrs)	NA	Positive +++ diffuse	Positive	ND	ND
12	F 23	Thigh	22	NA	Positive ++ diffuse	Positive	ND	ND
13	NA	NA	NA	NA	Positive ++ diffuse	Negative	ND	ND
14	NA	NA	NA	NA	Positive ++ diffuse	NI	ND	ND
15	M 48	Thigh	30	NA	Positive +++ diffuse	Positive	ND	ND
16	NA	NA	NA	NA	Positive +++ diffuse	ND	ND	ND
17	M 51	Abdominal wall	28 (8 yrs)	NA	Positive +++ diffuse	ND	ND	ND
18	F 64	Calf	25	NA	Positive +++ diffuse	ND	ND	ND
19	NA	NA	NA	NA	Negative	Negative	ND	ND
20	M 30	Buttock	35	NA	Positive ++ diffuse	Negative	ND	ND
21	NA	NA	NA	NA	Positive ++ diffuse	Positive	ND	ND
22[e]	F 53	Groin	34 (20 years)	NED [55]	Positive +++ diffuse	Positive	Negative	Negative
23[e]	M 26	Thigh	65	NED [7]	Positive +++ diffuse	Negative	Negative	Negative
24[e]	M 46	Hip	20	NA	Positive +++ diffuse	NI	Negative	Negative
25[e]	M 20	Thigh	30	Met to LN [84], NED [96]	Positive +++ diffuse	Negative	Negative	*MED12-PRDM10*
26	NA	NA	NA	NA	Positive ++ diffuse	NI	ND	ND
27	NA	NA	NA	NA	Positive ++ diffuse	Positive	Negative	Negative
28	M 53	Arm	15	NA	Positive ++ diffuse	NI	ND	ND
29	M 69	Groin	35	NED [29]	Positive +++ diffuse	Positive	*MED12-PRDM10*	*MED12-PRDM10*
30[e]	F 41	Shoulder	30	NED [120]	Positive ++ diffuse	Positive	*CITED2-PRDM10*	ND
31[e]	M 61	Thigh	50	NED [84]	Positive ++ diffuse	ND	*CITED2-PRDM10*	ND
32[e]	F 42	Knee	30	NED [18]	Positive ++ diffuse	Positive	*MED12-PRDM10*	ND
33[e]	M 42	Trunk	20	NED [204]	Positive ++ diffuse	Positive	Negative	ND
34[e]	M 20	Thigh	35	NED [12]	Positive +++ diffuse	Negative	*MED12-PRDM10*	ND
35[e]	M 25	Lateral foot	35	NA	Positive +++ diffuse	NI	NI	ND
36[e]	F 32	Thigh	45	NED [40]	Positive ++ diffuse	Positive	*MED12-PRDM10*	ND
37[e]	M 48	Perineum	60	NED [40]	Positive ++ diffuse	Negative	*CITED2-PRDM10*	ND
38	M 61	Thigh	70	NED	Positive ++ diffuse	ND	ND	ND

immunohistochemical and molecular genetic features. We additionally investigated the potential diagnostic utility of immunohistochemistry for CADM3 in the differential diagnosis of these rare soft tissue tumors.

MATERIALS AND METHODS

Tumor population

36 tumors classified as SCD34FT were retrieved from the archives of the participating pathological departments of the Mayo Clinic, Rochester, MN, USA, the Sahlgrenska University Hospital Gothenburg, Sweden, Lund University Hospital, Sweden, the Royal National Orthopedic Hospital, London Stanmore, UK, the University Hospital Nottingham, UK, the Royal Orthopedic Hospital Birmingham, UK as well as from the referral archives of the authors (ALF). Seven tumors from a previous cohort shown to harbor *PRDM10* fusions were used for morphological comparison [5]. Soft tissue lesions (n = 226) that may potentially mimic CD34-positive fibroblastic tumor were retrieved from the archives of the Sahlgrenska University Hospital Gothenburg, Sweden and the Royal National Orthopedic Hospital, London Stanmore, UK. A few tumors were included in previous studies (Table 1) [2, 5].

Immunohistochemistry

For IHC, antigen retrieval was performed on 3 µm sections from formalin-fixed paraffin-embedded tissue in pH 9 Tris/EDTA buffer retrieval solution (Agilent, Santa Clara, CA) at 68 °C for 16 h. For the detection of CADM3, the primary mouse monoclonal CADM3 antibody raised against human CADM3 Pro21-Tyr329 (R&D Systems, Minneapolis, MN, USA; clone #730004,) was used (1:100 dilution). The EnVision Dual Link system (Agilent Santa Clara, CA) was used for visualization. Surplus surgical tissue from cortical dysplasia including neurons and glial tissue was used as positive control.

The intensity of staining was graded as negative, weak, moderate, or strong; the extent of immunoreactivity was described as focal (scattered cells), partial (<50% of tumor cells) or diffuse (>50% of tumor cells).

Chromosome banding and fluorescence in situ hybridisation (FISH) analyses

Chromosome banding analysis of short-term cultured tumor cells from two samples and interphase FISH on FFPE sections from 33 cases with an in-house break-apart probe (BAP) for the *PRDM10* locus were performed as described [5, 10]. Around 100 nuclei were analyzed per case; split signals in >20% of the nuclei was used as cut-off for FISH-positivity.

SNP array analysis

The global copy number status of 10 samples was analyzed with Oncoscan CNV arrays, as described [11].

RNA-sequencing

RNA extraction, library preparation, and sequencing on fresh-frozen samples and FFPE tissue from a total of 17 samples were performed as described [5].

RT-PCR

The same RNA that had been extracted from FFPE tissue for RNA-seq was used for RT-PCR with primers specific for *CITED2*, *MED12*, and *PRDM10*, respectively, in 7 samples (Supplementary Table 1). The forward primers mapped to exon 2 of *CITED2* and exon 42 of *MED12*, respectively, and the reverse primer to exon 14 of *PRDM10*. Amplified products were Sanger sequenced.

RESULTS

Clinical details

Clinical details were available for 34 of 43 patients and are summarized in Table 1. Median age at presentation was 42 years (range, 20–69 yrs). The M:F ratio was 1.13 (18/16). Most ($n = 20$) tumors were located on the lower extremity, especially in the thigh ($n = 12$) and adjacent hip, buttock and knee regions ($n = 5$). Three tumors were found in the groin or perineum. Six tumors

Table 1. continued

Case No	Sex age[a]	Location[a]	Size [mm] (duration of lesion)	Follow up [month][b]	IHC CADM3[c]	FISH *PRDM10*[d]	RNASeq[d]	RT-PCR[d]
39[e]	F 30	Shoulder	40	NED [48]	Positive +++ diffuse	NI	Negative	ND
40[e]	F 42	Thigh	30	NED [48]	Positive +++ diffuse	NI	ND	ND
41[e]	F 40	Chest wall	30	NA	Positive +++ diffuse	Negative	Negative	ND
42	M 52	Forearm	60	NED [60]	Positive ++ diffuse	NI	NI	ND
43	F 42	Thigh	45	NED [114]	Positive ++ diffuse	ND	ND	ND

[a]NA, not available;
[b]Follow-up is given in months in square brackets; Met to LN, metastasis to lymph node; NED, no evidence of disease.
[c]+++, strong immunoreactivity; ++, moderate immunoreactivity; +, weak immunoreactivity.
[d]NI, not informative; ND, not done; NED, no evidence of disease.
[e]Previously published [2, 5].

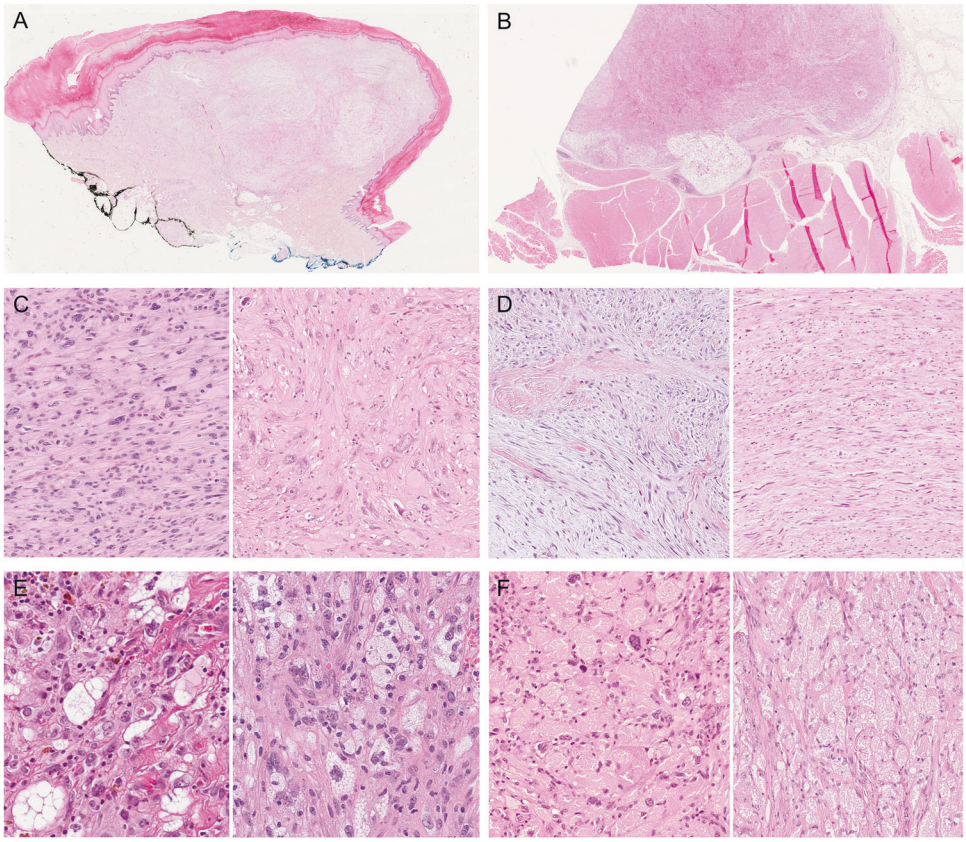

Fig. 1 Morphological spectrum of SCD34FT/*PRDM10*-rearranged soft tissue tumors. A All tumors were located above the fascia, at all levels of the cutis and subcutis: as polypoid lesions in the upper dermis (case 3); **B** or partly protruding into skeletal muscle (case 30). **C** Most tumors showed large cells with glassy cytoplasm, pronounced nuclear pleomorphism and multinucleation (cases 6, 34). **D** Three tumors showed more elongated cells or spindle cell morphology (cases 3, 26). **E** Cytoplasmic vacuolation was seen in scattered cells in 20 of 43 tumors (cases 31, 22). **F** Fourteen tumors contained small collections of large tumor cells with granular eosinophilic cytoplasm (cases 27, 12).

were on the upper extremity or shoulder region and 4 tumors were on the trunk. On average, tumors measured 40 mm in maximum diameter (median, 35 mm; range, 20–100 mm). Four patients reported that tumors had been present for several years, up to 20 y in 2 patients (Table 1). All patients were treated with surgical excision; one patient received radiotherapy. Clinical follow-up was available for 15 cases (median, 48 months; range, 5–204 months). All patients were alive without evidence of disease after excision. One patient had a lymph node metastasis to one iliac lymph node 7 years after excision of his primary tumor. That patient remained free of disease after a complete pelvic lymphadenectomy.

Morphological features

All tumors were suprafascial. The exact level could be identified in 35 out of 43 tumors. Eleven were located in the dermis/upper subcutis, one of which had a polypoid appearance (Fig. 1A), 14 in the subcutis, and 10 in the deep subcutis and were seen within the fascia or attached to fascial structures. Three of these deeper tumors were protruding into skeletal muscle, although the tumor cells were still surrounded by fascia (Fig. 1B). Except for 2 tumors with pronounced edematous stroma, all tumors were highly cellular. The predominant cells were large, polygonal or elongated, with eosinophilic glassy to fibrillary cytoplasm and highly pleomorphic nuclei (Fig. 1C). The cell borders were often distinct and cells were forming ill-defined fascicles. All tumors contained multinucleated tumoral giant cells. A spindle cell morphology was predominant in 2 tumors (Fig. 1D). Scattered tumor cells with xanthomatous or vesicular cytoplasm were noted in 20 of 43 tumors (Fig. 1E). Fourteen tumors contained small collections to confluent areas of very large tumor cells with granular cytoplasm (Fig. 1F). Tumoral necrosis was not noted. Mitotic figures were between 0 and 4 per 5 mm^2. Most tumors showed thin-walled dilated vessels. Focal areas with stromal edema were seen in 9 tumors, with 2 tumors showing stromal edema throughout (Fig. 2C). Hemosiderin-laden macrophages were noted in 9 tumors. These were focal in 5, but were a prominent feature in 4 tumors. An inflammatory infiltrate, consisting predominantly of lymphocytes and plasma cells, was present in all 43 tumors although with variable density. Two tumors contained collections of foamy macrophages. One tumor showed osseous metaplasia.

There was extensive morphological overlap between cases previously shown to harbor *PRDM10* fusions (cases 30–34, 36, 37) and cases described as SCD34FT (cases 1–29, 35, 38–43) [5]. There were no morphological features to separate these 2 groups, suggesting that they can be regarded as part of a morphological spectrum. The morphological features are listed in Supplementary Table 2.

CADM3 expression in SCD34FT/*PRDM10*-rearranged soft tissue tumor

Immunoreactivity for CADM3 was noted in 41 of 43 cases of SCD34FT/*PRDM10*-rearranged soft tissue tumor. Most ($n = 25$) tumors showed a diffuse staining pattern of moderate intensity (Fig. 2A, B). Sixteen tumors showed strong immunoreactivity (Fig. 3A). In contrast to vesiculated tumor cells, granular cells showed strong immunoreactivity to CADM3, which was more pronounced than in the surrounding spindle cell areas (Fig. 3E, F). Seventeen of 21 SCD34FT/*PRDM10*-rearranged soft tissue tumors tested expressed cytokeratins (AE1/AE3); immunoreactivity in

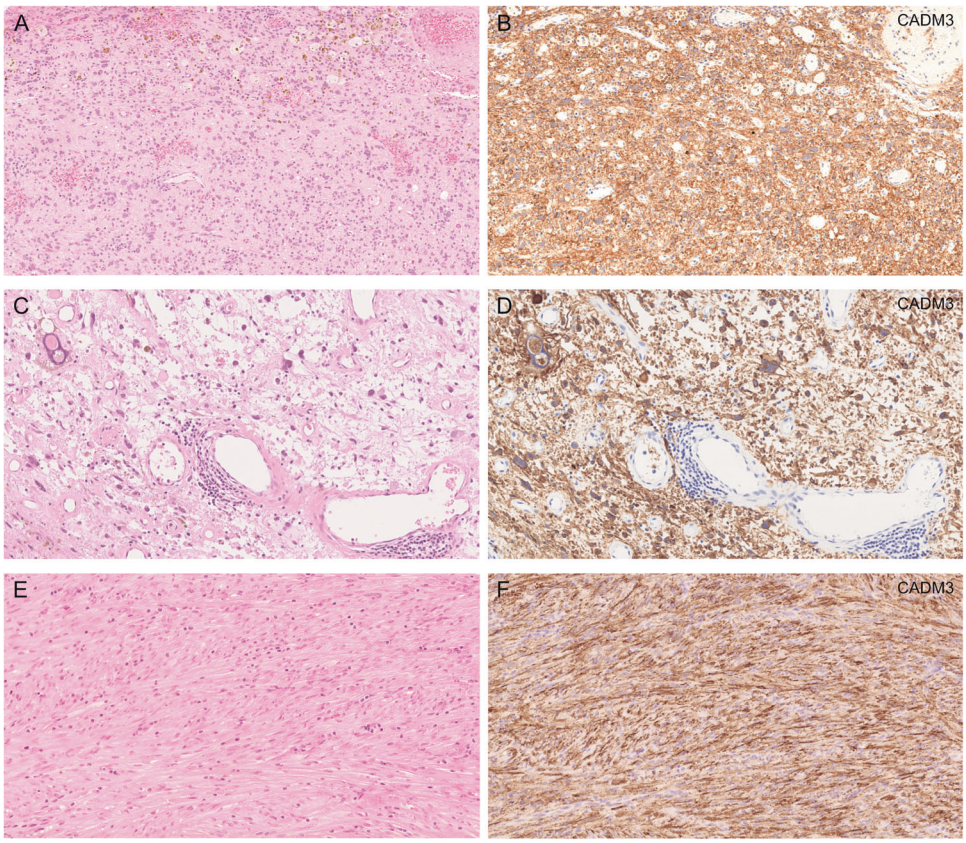

Fig. 2 Immunoreactivity for CADM3. A, B Moderate to strong immunoreactivity for CADM3 was seen in 41 of 43 SCD34FT/*PRDM10*-rearranged soft tissue tumors depicting classical morphology (case 9). **C, D** Immunoreactivity was also noted in cases with pronounced edema (case 42); **E, F** and in cases with predominant spindle cell morphology (case 18).

positive cases varied greatly, from diffuse and strong immunoreactivity to focal and faint staining of single cells (Fig. 4A, B).

CADM3 expression in other soft tissue tumors

In total, 226 cases of other soft tissue tumors were analyzed for CADM3 expression (Table 2). No staining was noted in benign fibrous histiocytoma (including deep fibrous histocytoma, "aneurysmal" fibrous histiocytoma, and *ALK*-rearranged fibrous histiocytoma), angiomatoid fibrous histiocytoma, dermatofibrosarcoma protuberans (DFSP), epithelioid hemangioendothelioma, epithelioid sarcoma, gastrointestinal stromal tumor, low-grade fibromyxoid sarcoma, nodular fasciitis, synovial sarcoma, solitary fibrous tumor, malignant peripheral nerve sheath tumor or inflammatory myofibroblastic tumor (Supplementary Fig. 1). Three of 26 myxofibrosarcomas (MFS) showed some immunoreactivity: one showed focal weak staining, and 2 showed moderate staining, either partly or diffusely. Focal and weak positivity was also noted in 2/9 dedifferentiated liposarcomas, 3/9 myxoinflammatory fibroblastic sarcomas (MIFS), 3/10 pleomorphic liposarcomas (PLS), and 1/31 UPS. Moderate positivity was seen in 1 PLS and 2 UPS (Fig. 4). Intensity and extent of CADM3 immmunoreactivity were less than that observed in SCD34FT/*PRDM10*-rearranged soft tissue tumor cases. None of the other soft tissue entities with limited immunoreactivity showed morphological features of SCD34FT.

In many lesions, CADM3 was expressed in peri- and intralesional nerves, which can serve as an internal positive control (Fig. 3A). In the external tissue control, brain, CADM3 stained axons and the dendrite network (not shown). Immunoreactivity was not observed in any other non-neoplastic soft tissue components (Fig. 3A). Because of the immunoreactivity in the central nervous system and in peripheral nerves, a number of nerve-derived tumors were investigated. Schwannomas showed moderate immunoreactivity as did nerve sheath myxomas (Fig. 5A, B). Granular cell tumor showed weak cytoplasmic positivity (Fig. 5C, D). In neurofibromas, CADM3 stained unmyelinated nerve fibers (Fig. 5E). No immunoreactivity was observed in non-Schwann cell-derived tumors, including cellular neurothekeomas and perineuriomas (not shown). In longitudinally-cut peripheral nerves, CADM3 immunoreactivity was seen in both axons and Schwann cells and showed a "crosstie"-like pattern consistent with Schmidt-Lanterman incisures (Fig. 5F).

Genetic findings

An overview of the molecular results is provided in Table 1 and Fig. 6. In brief, chromosome banding analysis of two samples showed structural rearrangements of chromosomes 6 and 11, compatible with a *CITED2::PRDM10* fusion. Interphase FISH for the *PRDM10* locus in 33 cases failed in 9, was negative (0–12% of the nuclei had split signals) in 10 samples, and was positive (22–68% of the nuclei had split signals) in 14 samples.

Oncoscan array analysis failed in one sample and did not disclose any copy number changes in four samples. Five samples showed copy number profiles with a single aberration (deletion near the *PRDM10* locus in two samples, loss of 14q material in two samples, and a deletion in 2q in one sample). RNA-seq on 17 cases failed in 2, and did not identify any *PRDM10* fusion, or any other relevant fusion transcript, in 8 samples. A *CITED2::PRDM10* or a *MED12::PRDM10* fusion transcript was found in 3 and 4 cases, respectively. Finally, RT-PCR for *CITED2::PRDM10* (exon 2-exon 14) and *MED12::PRDM10* (exon 42-exon 14) was negative in 4 cases (one only analyzed for the *MED12::PRDM10* fusion) and positive for *MED12::PRDM10* in three.

In summary, a total of 18 cases were positive for *PRDM10*-rearrangements by FISH, RNA-seq, and/or RT-PCR. In 11 of these,

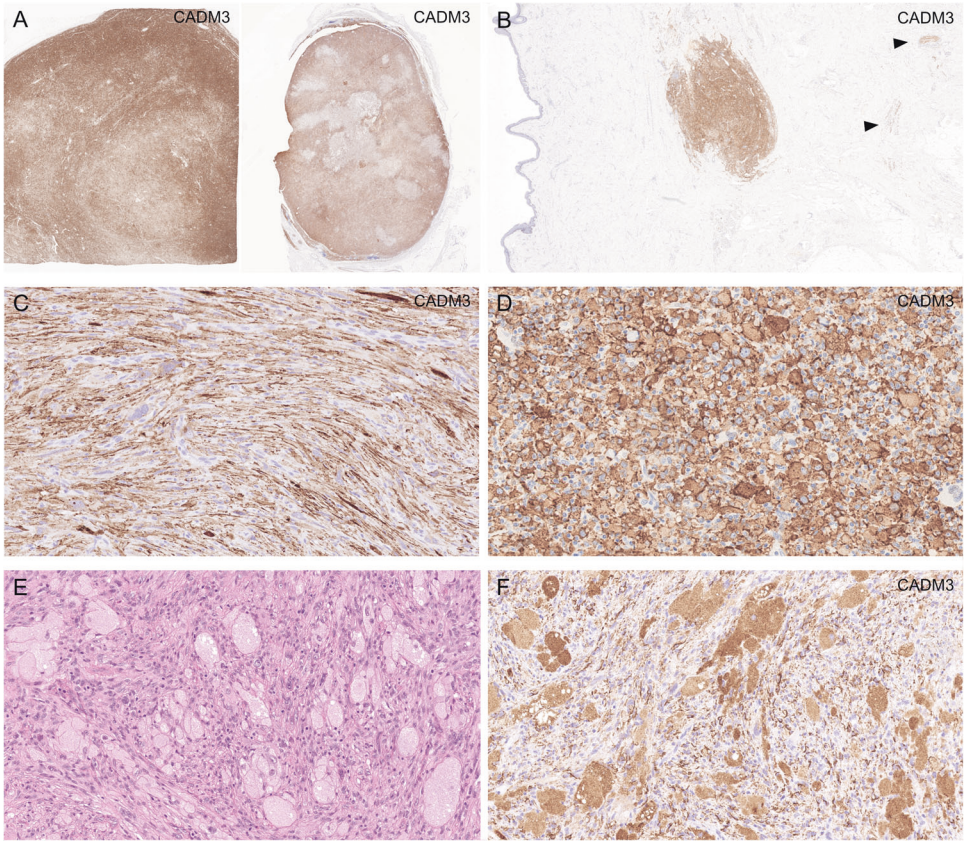

Fig. 3 Patterns of immunoreactivity for CADM3 in SCD34FT/*PRDM10*-rearranged soft tissue tumors. A 41 of 43 tumors showed strong (left) or moderate (right) immunoreactivity, with some geographical variety (cases 29, 33). **B** Except for peripheral nerves (arrowheads), there was no staining of non-tumoral soft tissue components (case 39, re-excision specimen). **C** Immunoreactivity was granular, with membranous accentuation (case 15). **D** Cytoplasmic positivity was slightly weaker and granular (case 39). **E, F** Granular cell change in tumor cells showed more pronounced immunoreactivity contrasting with surrounding spindle cell areas (case 23).

both FISH and RNA-based analyses had been performed, with discrepant results in 6 of them; three had positive FISH results but were negative at the RNA level and 3 were positive for a *PRDM10* fusion by RT-PCR or RNA-seq but negative by FISH.

Of these 18 cases with *PRDM10* rearrangements, 7 were originally classified as UPS ($n = 5$) or liposarcoma ($n = 2$) and 11 were classified as SCD34FT. All tumors with *PRDM10* rearrangement showed immunoreactivity for CADM3/SynCam3 (Table 1, Fig. 6).

DISCUSSION

SCD34FT is a recently recognized mesenchymal tumor of borderline malignancy (locally recurring, rarely metastasizing), first described by Carter and colleagues in 2014 [2]. Recurrent rearrangements of *PRDM10* were first described in 2015 in 3 tumors classified as UPS albeit with low grade features and indolent clinical behavior [10]. A larger series of soft tissue tumors with *PRDM10* rearrangement was described in 2019 by the same group and the morphological overlap with SCD34FT was noted [5]. Most of the tumors with *PRDM10* rearrangement were originally classified as UPS or PLS; however, long-term follow-up showed their indolent clinical behavior, and transcriptional profiling of these tumors showed their profile being distinct from UPS [5]. Analysis of tumors and conditional expression of the *CITED2::PRDM10* fusion in a fibroblast cell line identified *CADM3* as one of several highly and differentially expressed genes in tumors with *PRDM10* rearrangement. Expression was confirmed on the protein level in tumors with both *CITED2::PRDM10* and *MED12::PRDM10* fusions, but was not seen in single cases of other soft tissue tumors, including UPS [9].

Here, we investigated a larger cohort of SCD34FT and compared these with the original series of *PRDM10*-rearranged tumors, showing that SCD34FT and *PRDM10*-rearranged tumors have indistinguishable morphological features. Corroborating the morphological findings, we identified *PRDM10* rearrangements in a high proportion of SCD34FT tested, and show that SCD34FT and *PRDM10*-rearranged tumors display diffuse CADM3 expression in almost all instances. Notably, all tumors with identified *PRDM10* rearrangements expressed CADM3. Given the overlapping morphological, immunohistochemical and genetic features we believe that *PRDM10*-rearranged tumors and SCD34FT are the same entity and should be best referred to as SCD34FT. Furthermore, in this series we describe some additional morphological features of SCD34FT. Granular cell change, predominant spindle cell morphology, extensive stromal edema, and prominent hemosiderin-laden macrophages are newly recognised features of SCD34FT. Although most tumors were located in the subcutis, they were located quite deep, protruding into skeletal muscle and appearing intramuscular on clinical/radiological assessment [5]. This observation is in agreement with Perret and colleagues who describe 2 intramuscular SCD34FT, one harboring a *CITED2::PRDM10* fusion, in a recent series [6].

CADM3 expression was also observed in 41 out of 43 SCD34FT, including cases with unusual features. IHC for CADM3 may therefore aid not only in separating SCD34FT/PRDM10-rearranged tumor from other lesions, but also in further defining the morphological spectrum of this relatively new tumor entity.

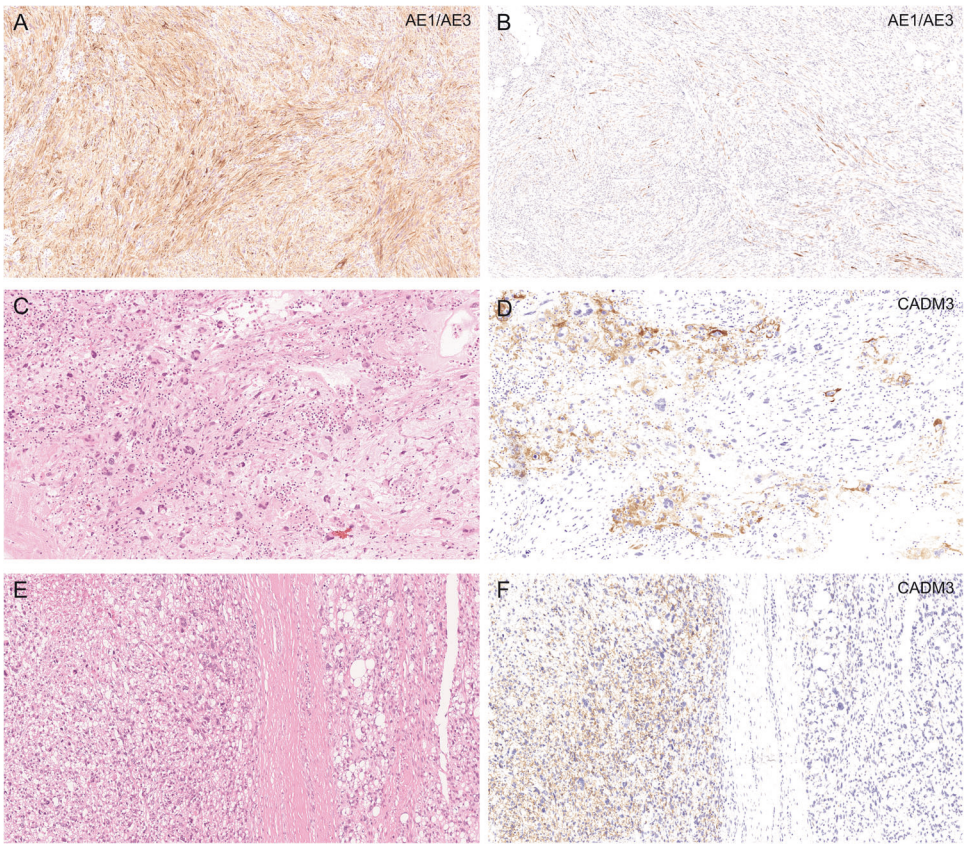

Fig. 4 AE1/AE3 immunoreactivity in SCD34FT/*PRDM10*-rearranged soft tissue tumors and CADM3 in other soft tissue tumors. A, B AE1/AE3 immunoreactivity in SCD34FT/*PRDM10*-rearranged soft tissue tumors varied from diffuse (case 40) to very focal (case 15) to negative. **C, D** Focal immunoreactivity for CADM3 was noted in 3 out of 26 myxofibromsarcomas tested. **E, F** Four out of 10 pleomorphic liposarcomas showed focal positivity for CADM3.

When the 2 IHC-negative SCD34FT were reviewed retrospectively, they had unusual morphological features like extensive diffuse infiltration into subcutaneous fat, absence of large, well-delineated cells with glassy cytoplasm, and unusually high cellularity when compared to the CADM3-positive tumors. These 2 tumors were negative at FISH analysis for *PRDM10*-rearrangement, suggesting that they might represent other tumor types.

Correct classification of SCD34FT is important as these tumors can recur locally, but very rarely metastasize, in line with the classification of SCD34FT as a borderline tumor [2]. Owing to their striking pleomorphism, the distinction of SCD34FT/PRDM10 tumors from fully malignant soft tissue tumors can be quite challenging, especially as expression of CD34 is not specific and mitotic counts may be underestimated in limited biopsies. Thus, the absence of CADM3 expression in potential morphologic mimics may be of considerable value in their distinction from routinely positive SCD34FT/PRDM10 tumors. The differential diagnosis of SCD34FT includes various pleomorphic sarcomas of both superficial and deep soft tissue, e.g. UPS, PLS, MFS, "atypical" fibrous histiocytomas, and tumors expressing CD34 and/or keratins (e.g. such as epithelioid sarcoma, DFSP). Of these, limited and weak expression of CADM3 was noted only in a small minority of UPS, MFS and PLS, suggesting a potentially important role for this marker in the distinction of SCD34FT from potential mimics.

In the present study, immunoreactivity was absent or very focal in the majority of tumors that enter the differential diagnosis of SCD34FT/PRDM10 rearranged tumors: epithelioid sarcoma shares the glassy cytoplasm of its tumors cells, the immunopositivity for AE1/AE3 cytokeratins, and CD34-positivity (in approximately 50% of cases) with SCD34FT, although expression of INI-1 (SMARCB1) was shown to be consistently retained by SCD34FT [2]. No immunoreactivity for CADM3 was noted in DFSP or benign fibrous histiocytoma or variants thereof. MIFS enters the differential diagnosis of SCD34FT, although MIFS are usually CD34-negative [2]. Immunoreactivity for CADM3 was weak and focal in 3 of 9 MIFS showing a different staining pattern than in our population of SCD34FT. Some degree of CADM3 immunoreactivity was also noted in 3 out of 31 cases of UPS. The extent and intensity were lower than in SCD34FT apart from in one case, which did not show any classical hallmarks of SCD34FT. A similar pattern was noted in PLS, where only 1 in 10 cases showed partial positivity and 3 cases showed scattered positive cells. Like SCD34FT, MFS is mainly located suprafascially and can be CD34-positive [2]. Our study population of 26 MFS contained 3 CD34-positive MFS, none of which showed immunoreactivity for CADM3. One low-grade superficial CD34 negative MFS showed moderate immunoreactivity. This case showed classical MFS morphology with infiltrative growth pattern and abundant myxoid matrix production. Higher levels of CADM3 expression were also noted in some MFS at the RNA level; however, these levels were still lower than in SCD34FT [9]. The IHC results therefore appear to complement the RNA data. Although the sensitivity of IHC for CADM3 seems to be high, occasional immunoreactivity in a small fraction of MFS, PLS and UPS has to be kept in mind; as always, IHC results should be assessed in conjunction with morphological features and other markers. A number of other superficially located lesions that may mimic SCD34FT in some circumstances showed no immunoreactivity for CADM3: epithelioid hemangioendothelioma, nodular fasciitis, solitary fibrous tumor and angiomatoid fibrous histiocytoma. Additionally, there was no immunoreactivity in lesions that are typically deep-seated lesions, like low-grade fibromyxoid sarcoma and synovial sarcoma.

Table 2. Immunohistochemical staining for CADM3/SynCAM3.

Tumor type	Total cases	neg	Pos +	Pos ++	Pos +++	Staining pattern
Superficial CD34-positive fibroblastic tumor	43	2	0	25	16	Diffuse
Angiomatoid fibrous histiocytoma	5	5	–	–	–	–
Dermatofibrosarcoma protuberans	13	13	–	–	–	–
Dedifferentiated liposarcoma	9	7	2	–	–	Very focal
Epithelioid hemangioendothelioma	5	5	–	–	–	–
Epithelioid sarcoma	8	8	–	–	–	–
Fibrous histiocytoma, superficial, deep, epithelioid	16	16	–	–	–	–
Gastrointestinal stroma cell tumor	11	11	–	–	–	–
Granular cell tumor	7	0	6	1	–	Weak (6) and moderate (1) cytoplasmic staining
Low grade fibromyxoid sarcoma	8	8	–	–	–	–
Myxoinflammatory fibroblastic sarcoma	9	6	3	–	–	Very focal
Myxofibrosarcoma	26	23	1	2[a]	–	Scattered positive cells (1), partly positive (2)
Nerve sheath myxoma	2	0	–	2	–	–
Neurofibroma	10	0	10	–	–	Staining of intralesional nerves and scattered Schwann cells
Malignant peripheral nerve sheath tumor	1	1	–	–	–	–
Neurothekeoma	3	3	–	–	–	–
Nodular fasciitis	9	9	–	–	–	–
Perineurioma	2	2	–	–	–	–
Pleomorphic liposarcoma	10	6	3	1[b]	–	Scattered positive cells (3), partly positive (1)
Schwannoma	13	0	5	8	–	Diffuse membranous staining
Solitary fibrous tumor	18	18	–	–	–	–
Synovial sarcoma	9	9	–	–	–	–
Undifferentiated pleomophic sarcoma	31	28	1	2[a]	–	Diffuse weak staining (1), scattered positive cells (1), partly positive (1)
Inflammatory myofibroblastic tumor	1	1	–	–	–	–

[a]One case negative for *PRDM10* break apart on FISH, one case not informative.
[b]Negative for *PRDM10* break apart on FISH.

Although the striking pleomorphism, CD34 expression and occasional presence of ectatic blood vessels may occasionally raise the question of pleomorphic hyalinizing angiectatic tumor of soft parts (PHAT), careful morphologic study and appropriate ancillary studies should allow the confident distinction of CD34FT from PHAT in almost all instances. Unlike SCD34FT, essentially all bona fide cases of PHAT show at their periphery areas of hemosiderotic fibrolipomatous tumor, consisting of relatively bland, hemosiderin-laden spindled cells infiltrating adipose tissue, with variable stromal myxoid change, and small aggregates of damaged blood vessels [12]. Additionally, PHAT lacks the distinctive "glassy" cytoplasm often seen in SCD34FT, and is "keratin-negative". Additionally, the *TGFBR3* and/or *MGEA5* rearrangements, frequently found in cases of PHAT with an associated HFLT component are not seen in SCD34FT [13].

Regrettably, 16 cases of SCD34FT could not be assessed by molecular methods or yielded inconclusive results, mainly due to degraded RNA or lack of material. In 18 tumors, 11 of which were initially classified as SCD34FT, evidence of *PRDM10* rearrangement was identified, with discrepant results between RNA-based analysis and FISH in 6. We have previously shown that *PRDM10*-fusions result in low expression levels with few fusion-supporting reads, which were difficult to detect when using fusion-calling algorithms with default settings [9]. However, when investigated with simple RT-PCR with primers for the most prevalent *PRDM10* fusions, a *MED12::PRDM10* fusion was identified in 1 SCD34FT case negative by FISH or RNA-seq. This suggests that a proportion of the tumors may harbor unidentified *PRDM10* fusions and that neither FISH nor RNA-Seq is sensitive enough to detect all fusions in FFPE material. Indeed, Perret and colleagues detected 2 variant *PRDM10*-fusions—*PRDM10::ARHGAP32* and *PRDM10::RAB30*—which, however, had the *PRDM10* gene as the 5′-partner instead of as 3′-partner, making their significance unclear [6].

PRDM10 is a sequence-specific zinc finger transcriptional regulator. One of its direct targets is *CADM3* [14]. CADM3 is also referred to as Necl-1 and belongs to the family of Nectin-like proteins (Necls) which has five members. These transmembranous proteins have three Ig-like extracellular domains, and are widely and highly expressed in the central and peripheral nervous systems [15, 16]. In the peripheral nervous system, CADM3 is mainly located on the axons interacting with Necl-4 on the Schwann cells and thereby mediating adhesion between the axon and its myelin sheath. In rodents, mRNA expression of *CADM3* in Schwann cells is very low, but, at the protein level, CADM3 is seen in Schwann cells along the axon, especially in the Schmidt-Lanterman incisures, pockets of Schwann cell cytoplasm within the tightly packed myelin sheath [17]. The immune-staining pattern observed in perilesional myelinated nerves and unmyelinated axons was in accordance with published findings. Interestingly, we also observed CADM3 immunoreactivity in

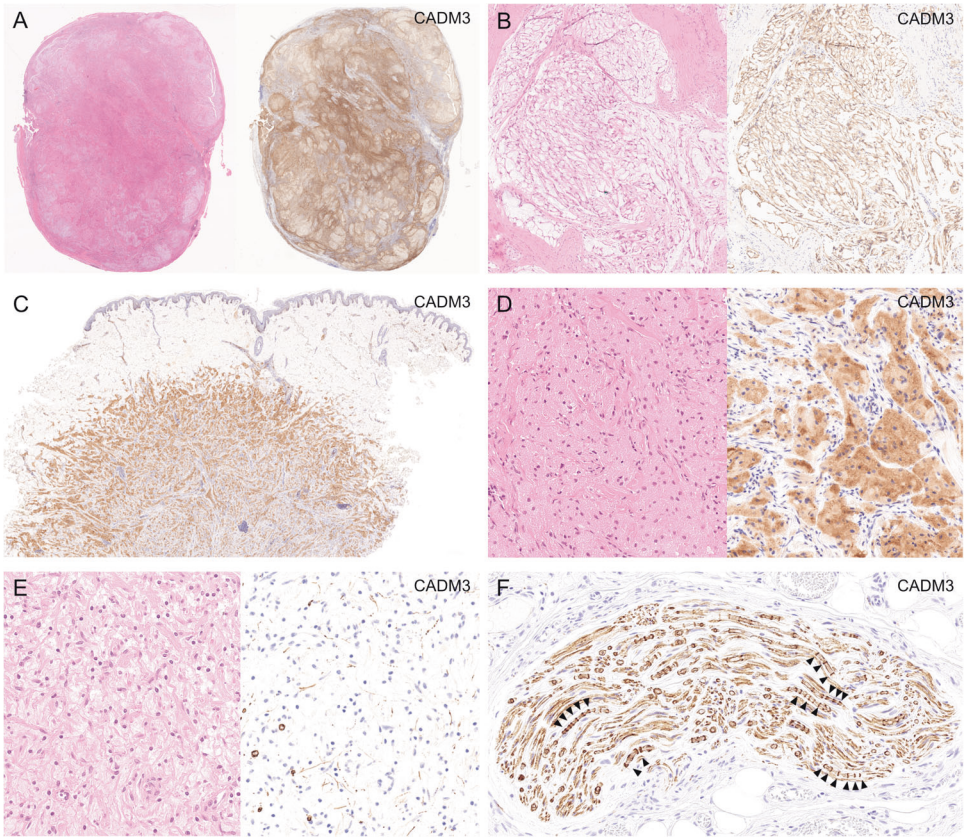

Fig. 5 CADM3 immunoreactivity in tumors containing Schwann cells and nerves. A There was membranous immunoreactivity in schwannomas, **B** and nerve sheath myxomas. **C, D** Granular cell tumors showed granular cytoplasmic positivity. **E** In neurofibromas, CADM3 stained intralesional myelinated and unmyelinated axons but no lesional cells. **F** In longitudinal sections of myelinated peripheral nerves, crosstie-like immunreactivity for CADM3 highlighted Schmidt-Lanterman incisures (arrowheads).

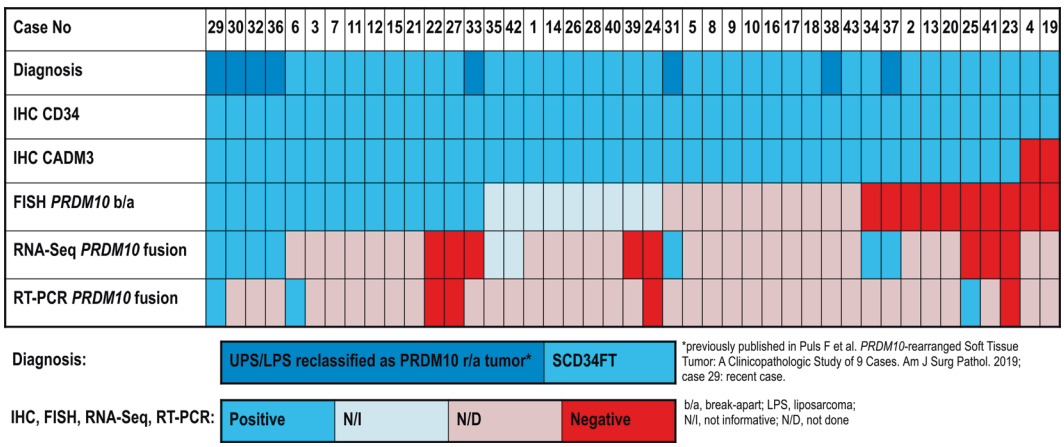

Fig. 6 Overview of cases with diagnoses, immunhistochemical and molecular findings. Of 18 cases with *PRDM10* rearrangements, 7 were originally classified as undifferentiated pleomorphic sarcoma (5) or liposarcoma (2) and 11 were classified as SCD34FT. All tumors with *PRDM10* rearrangement showed immunoreactivity for CADM3.

tumors with Schwann cell differentiation such as schwannomas, nerve sheath myxomas and granular cells tumors. Positivity for CADM3 in SCD34FT and *PRDM10*-rearranged tumor can be explained by expression of *PRDM10*-fusion transcripts as demonstrated by inducible expression in transfected fibroblast-derived cell lines [9]. It is not a sign of Schwann-cell derivation, for which there is no morphological evidence.

In summary, the results of the present study strongly suggest that SCD34FT and *PRDM10*-rearranged tumor represent a single entity, sharing similar morphologic, immunohistochemical and molecular genetic features. Although an argument could be made to modify the name "superficial CD34-positive fibroblastic tumor" to reflect the more specific immunohistochemical and molecular genetic features of these distinctive tumors, not all are demonstrably *PRDM10*-rearranged, and CADM3 immunohistochemistry is not widely available. Thus, at this time, we recommend maintaining the current name of this entity, recognizing that future advances may necessitate changes in nomenclature.

DATA AVAILABILITY

The datasets used and/or analyzed during the current study are available from the corresponding author on reasonable request.

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AUTHOR CONTRIBUTIONS

J.M.C., N.P., T.M., V.P.S., P.R., H.F., M.H., F.A., R.T., and A.M.F. contributed cases, performed data acquisition, data analysis and interpretation. F.P., J.N., and L.M. performed experiments, data acquisition, data analysis and interpretation. F.P., A.L.F., and F.M. performed study design, data acquisition, data analysis and interpretation and wrote the manuscript. All authors read and approved the final manuscript.

COMPETING INTERESTS

The authors declare no competing interests.

ETHICS APPROVAL/CONSENT TO PARTICIPATE

All analyses were performed in accordance with the ethical guidelines of the contributing institutions.

ADDITIONAL INFORMATION

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