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Clinicopathological analysis of myeloid sarcoma with megakaryocytic differentiation

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Summary

Myeloid sarcoma (MS) is defined as a tumour mass consisting of myeloid blasts that occurs at an anatomical site other than bone marrow. MS with megakaryocytic differentiation (MSmgk) is extremely rare and its clinicopathological features have not been well described.

We reviewed 11 cases in 11 patients of extramedullary mass-forming malignant tumours composed of immature non-lymphoid haematopoietic cells expressing CD41 with or without concurrent bone marrow lesions.

The patients consisted of seven men and four women (1.75:1 male-to-female ratio). The mean and median ages at diagnosis were 50 and 62 years, respectively, ranging from 2 to 78 years. Extramedullary mass lesions were solitary in three cases (27%) and multiple in eight cases (73%). Tumour locations were lymph nodes (6 cases), subcutaneous tissue (3 cases), intramuscular (1 case), and bone (1 case). Seven of the 11 patients (64%) had a history of myelodysplastic syndrome (MDS) or myeloproliferative neoplasm (MPN). Three patients (27%) developed MS during remissions of acute myelogenous leukaemia, and one patient had a recurrence of MS at other sites. Follow-up data were available for four cases. Tumour cells were positive for CD41, CD33, CD34, MPO, and CD68 in 11 (100%), three (27%), seven (64%), four (36%), and seven (64%) cases, respectively. Cytogenetic analysis was successfully performed in two cases. Complex but inconsistent abnormalities were evident. When compared with cases of MS without megakaryocytic differentiation, the survival of MSmgk was significantly shorter (p=0.0033).

Compared to MS without megakaryocytic differentiation, MSmgk is more likely to follow MDS/MPN, to involve multiple sites, and to be associated with poorer outcomes. More detailed studies, including genomic or gene expression analyses, could confirm the characteristics of MSmgk.

Key words: Myeloid sarcoma; extramedullary AML; megakaryocytic differentiation.

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INTRODUCTION

Myeloid sarcoma (MS) is defined by the World Health Organization (WHO) as a tumour mass consisting of myeloid blasts occurring at an anatomical site other than bone marrow.[1] As long as a mass lesion effaces the normal tissue architecture, the lesion is classified as MS regardless of leukaemia background. MS can also precede or follow the development of acute myeloid leukaemia (AML), myeloproliferative neoplasm (MPN), or myelodysplastic syndrome (MDS).

Since first being described in 1811,[2] efforts to subclassify this rare tumour have been made, with the goal of better stratifying patients to predict their clinical courses. In the WHO classification published in 2001,[3] MS was subclassified depending on its histological morphology into mature, immature, and blastic, and the remaining minority cases were divided into monoblastic, trilineage haematopoiesis, erythroid, and megakaryocytes. Subsequent studies failed to prove the clinical relevance of this morphology-based subcategorisation.[4,5]

Another way to subclassify MS is based on its chronological relationship with leukaemia and other bone marrow pathologies. Pileri *et al.* divided 92 cases of MS into isolated MS (*de novo*), 27%; MS with simultaneous AML, MDS, or myeloproliferative disorder (MPD), 35%; MS with associated prior history of AML, MDS, or MPD, 38%; and MS in patients with a previous history of non-haematopoietic tumours, 6%. The authors concluded that there were no differences in clinical behaviours and therapeutic responses between these groups.[5] Kawamoto *et al.* studied 131 cases of MS, dividing them into types 1 to 4: *de novo* MS, 27%; MS with concurrent AML without previous AML or MDS, 28%; MS developed during the course of MDS or MPN, 24%; and MS as recurrence of AML after remission, 21%. The authors described that underlying MDS or MPN was a poor prognostic factor.[6]

While there has been several large case series published on MS, fewer than 20 case reports of MS with megakaryocytic differentiation (MSmgk) have been published to date. To elucidate this very rare subtype of MS, we present the clinicopathological features of 11 cases of MS with immunohistochemical megakaryocytic differentiation.

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MATERIAL AND METHODS

Patients

The 11 patients were diagnosed at the Department of Pathology, Kurume University between 2006 and 2019, for extramedullary mass-forming malignant tumours composed of immature non-lymphoid haematopoietic cells with megakaryocytic differentiation. We determined megakaryocytic differentiation by blastic morphology, no evidence of lymphoid differentiation, and expression of CD41, even focally upon immunohistochemistry. Clinical information obtained from the submitting clinicians or pathologists includes age, sex, tumour site, number of lesions, past medical history, and prognosis. The glass slides of all cases were re-reviewed by two expert haematopathologists (HM and KO). We divided the 11 cases into four groups according to previous reports.[6,7] Type 1 comprised MS without evidence of leukaemic presentation in peripheral blood and bone marrow. Type 2 comprised MS concurrent with AML, which was not previously diagnosed with AML or MDS/MPN/chronic myeloid leukaemia (CML). Type 3 comprised MS that developed during the course of MDS/MPN/CML. Type 4 comprised MS as recurrence after the remission of AML. This study was conducted in accordance with the Helsinki Declaration, and the Ethics Review Committee of Kurume University approved the study.

Immunohistochemistry (IHC) and flow cytometry (FCM)

Sections from formalin-fixed, paraffin-embedded blocks were stained with haematoxylin and eosin and subjected to IHC. The antibodies (clone) tested include: CD41 (EPR4330; Abcam, UK), factor VIII (F8/86; Dako, Denmark), CD34 (QBEnd10; Beckman Coulter, USA), CD68 (KP-1 or PG-M1; Agilent), CD3 (F7.2.38; Agilent), CD20 (L26; Agilent), CD13 (38C12; Leica, Germany), CD33 (PWS44; Leica), myeloperoxidase (rabbit polyclonal; Agilent), terminal deoxynucleotidyl transferase (TdT; EP266; Agilent), CD4 (SP35; Roche, USA), CD8 (C8/144B; Agilent), TIA1 (2G9A10F5; Beckman Coulter), spectrin (RBC2/3D5; Cell Marque, USA), PAX5 (1EW; Leica), CK AE1/AE3 (AE1/AE3; Agilent), CD30 (Ber-H2; Agilent), CD79a (JCB117; Agilent), CD45RO (UCHL1; Agilent), CD138 (MI15; Agilent), CD123 (7G3; Becton Dickinson, USA), CD163 (10D6; Leica), CD56 (1B6; Leica), CD71 (rabbit polyclonal; Atlas Antibodies), and glycophorin A (JC159; Dako).

Except for CD41 and factor VIII, the IHC results were interpreted as 'positive' when $\geq$30% of tumour cells were positive, and 'focal positive' when 1–29% were positive. For CD41 and factor VIII, we recorded the proportion of positive tumour cell, and any staining was considered positive.

In FCM analysis, fresh specimens at the biopsy were used. The expressions of CD2 (T11:SFCI3Pt2H9; Beckman Coulter), CD5 (UCHT2; BD Biosciences, USA), CD7 (3A1; Beckman Coulter), CD10 (J5; Beckman Coulter), CD11c (BU15; Beckman Coulter), CD13 (MY7; Beckman Coulter), CD16 (3G8; Beckman Coulter), CD25 (2A3; BD Biosciences), CD34 (581; Beckman Coulter), and CD56 (NHK-1:N901; Beckman Coulter) were assessed.

Cytogenetic analysis

The G-banding method was used for the cytogenetic analysis. Karyotypes were described according to the International System for Human Cytogenetics Nomenclature (1995) as previously described.[6] The fresh specimens at the initial diagnosis were also used for cytogenetic analysis by G-banding.

Statistical analysis

Overall survival (OS) was defined as the time from the day of MS diagnosis to the day of death or the last follow-up. Twenty-four MS cases proven to have no evidence of megakaryocytic differentiation were selected as the control cases from the authors' previous studies.[6,7] Kaplan–Meier estimates were used to predict OS by comparing the survival curves using the log-rank test. All calculated p values were two-sided, and values <0.05 were considered statistically significant. Statistical analysis was performed using JMP version 15 (SAS, USA).

RESULTS

Clinical presentations

Patient demographics are shown in Table 1. The male-to-female ratio was 1.75:1. The mean age of the patients at diagnosis was 50 years, and the median age was 62 years (range 2–78 years). Extramedullary mass lesions were solitary in three cases (27%) and multiple in eight cases (73%). Tumour locations were lymph nodes in six cases (55%), subcutaneous tissue in three cases (27%), intramuscular (chest wall) in one case, and bone (femur and humerus) in one case. Seven of 11 cases (64%) were Type 3, three cases (27%) were Type 4, and only one case (9%) was Type 1. There were no Type 2 cases.

Type 3

Seven patients (4 males and 3 females) were classified in this category. Two of the seven cases had a history of carcinoma of the breast and oesophagus, respectively, in addition to MDS/MPN. Age ranged from 35 to 78 years, with an average age of 63 years, and a median age of 69 years. The extramedullary lesions were multiple in five cases and single in two cases. The location of the lesion was the lymph node in four cases, intramuscular in one case, subcutaneous in one case, and bone in one case.

Two patients were diagnosed with acute megakaryoblastic leukaemia (AMgkL) in the bone marrow almost simultaneously. In one of these (Case 3-2), bone marrow and lymph node biopsies were performed on the same day. In the other case (Case 3-4), bone marrow biopsy was performed 2 weeks before her death and she was diagnosed as AMgkL at our department. The lymph node showing MSmgk was sampled during the autopsy.

The underlying diseases were MDS in three cases, MPN in four cases [primary myelofibrosis (MF) in 1 case, essential thrombocythaemia (ET) in 1 case, both MF and ET in 1 case, and CML in 1 case]. The time to develop MS varied from 2 months to 8 years.

Treatments administered to each patient before the development of MS included stem cell transplant (SCT) 60 months before the development of MS in one case, chemotherapy in three cases, immunosuppressive drugs in three cases, and periodic transfusions in one case. Non-haematopoietic diseases were also present in three cases: breast carcinoma treated with surgery and chemotherapy; esophageal carcinoma with multiple recurrences treated with surgery, chemotherapy, and radiation therapy; and rheumatoid arthritis treated with methotrexate.

Follow-up data were available in four cases, excluding the autopsy case (Case 3-4). Three patients died of disease within 6 months after the diagnosis of MS. One patient died almost immediately. The remaining patient (Case 3-5) received SCT twice but died 6 months after the diagnosis of MS.

Type 4

Three patients, all males, developed MS after AML remission. Age ranged from 2 to 62 years. Two were paediatric patients and the other was an elderly patient. The type of AML was JMML (monosomy 7, NRAS mutation-positive) and subsequent AML (M6) in Case 4-1 and AMgkL in Case 4-2. In Case 4-3, detailed information regarding AML was not available. All patients received some kind of transplant, multiple times in paediatric patients, before the development of MS. The time between remission and MS development was 2 and 18 months in the paediatric patients and 4.5 months in the elderly patient. In all cases, the MS was developed in multiple locations. Follow-up data were

Table 1 Clinical features of the present cases

Case no.	Age/ sex	Past medical history	Time to MS development	Treatment before MS	Transplant before MS	Site	Tumour multiplicity	Follow-up
Type 3								
3-1	66 F	ET × 7y, MF × 5y, breast ca × 3y	6 y	SCT	+	Soft tissue	Solitary	N/A
3-2	55 M	MDS-RAEB × unknown length	Unknown	Immunosuppression	–	LN, BM(AMgkL)	Multiple	N/A
3-3	78 M	MF × 4y	4 y	Transfusions	–	LN	Multiple	N/A
3-4	70 F	ET × 8y, RA × 5y	8 y	Chemo, MTX	–	LN, BM(AMgkL)	Multiple	Autopsy case
3-5	35 M	CML × 14m	14 m	Dasatinib, rad, chemo	–	Bone	Multiple	DOD 6 m
3-6	69 M	Oesphageal ca × 14y, MDS × 2m	2 m	Chemo/rad for ca, Aza	–	Subcutaneous	Solitary	DOD 2 d
3-7	71 F	MDS-RA × unknown length	Unknown	Unknown	Unknown	LN	Multiple	Alive 2 w
Type 4								
4-1	3 M	JMML, M6	2.5 y	Allo-BMT	+	LN	Multiple	N/A
4-2	62 M	AMgkL	8 m	Idarubicin, allo-PBSCT	+	Subcutaneous	Solitary	DOD 3 m
4-3	2 M	AML	2 y	Allo-BMT, PBSCT	+	LN	Multiple	N/A
Type 1								
1-1	41 F	MS (ovary)	1 y	Surgery, chemo	–	Subcutaneous	Multiple	N/A

AMgkL, acute megakaryoblastic leukaemia; AML, acute myeloid leukaemia; Aza, azathioprine; BMT, bone marrow transplant; ca, carcinoma; chemo, chemotherapy; CML, chronic myelogenous leukaemia; d, days; DOD, died of disease; Eso, oesophageal; ET, essential thrombocythaemia; F, female; JMML, juvenile myelomonocytic leukaemia; LN, lymph node; m, months; M, male; MDS, myelodysplastic syndromes; MDS-RA, MDS refractory anaemia; MF, myelofibrosis; MS, myeloid sarcoma; MTX, methotrexate; N/A, not available; PBSCT, peripheral blood stem cell transplantation; RA, rheumatoid arthritis; rad, radiation therapy; RAEB, refractory anaemia with excess blasts; SCT, stem cell transplantation; w, weeks; y, years.

available only in the adult case, in which the patient died of disease ≤6 months after the MS diagnosis.

Type 1

One of 11 cases was Type 1. It presented as a recurrent MS. The patient was a 41-year-old woman who developed MS in a subcutaneous mass in the right shin and multiple inguinal and intra-abdominal lymphadenopathy. The previous MS was in the ovary 8 months prior. The ovarian MS was surgically removed when the diagnosis of granulocytic sarcoma was made histologically and AML-type chemotherapy regimens were initiated. The detailed pathology of ovarian MS was not available. Whether there was megakaryocytic differentiation was unknown. No bone marrow pathology was identified during the course of the disease.

Pathological findings

The pathological findings of the 11 cases are shown in Fig. 1 and Table 2. In eight cases (72%), the lesion was composed of diffuse proliferation of medium to large atypical mononuclear cells, morphologically resembling malignant lymphoma. Large multinucleated atypical cells, which are often described as a typical pathological finding of MSmgk, were observed in three cases (27%). All were Type 3. Smaller tumour cells with abundant eosinophilic cytoplasm, which have been described as eosinophilic myelocytes[8–10] were readily evident in one case (Case 3-4; Fig. 1C,D). These myelocytes were not prominent features in the remaining 10 patients.

Tumour cells were polymorphous with variation in cell size in six cases but relatively monotonous in five cases. The maximum cell size varied from case to case. In Type 4 MS cases, tumour cells appeared to be relatively small (average 9.8 μm in greatest dimension) and monotonous (Fig. 1E,F).

The backgrounds were relatively monotonous. Eight cases (73%) showed scattered small lymphocytes. Three cases, all Type 3 MS, showed small numbers of plasma cells (Case 3-3), eosinophils (Case 3-5), and neutrophils (Case 3-2) mixed with inactive lymphocytes.

IHC demonstrated that tumour cells were positive for CD41, CD33, CD34, MPO, and CD68, in 11 (100%), three (27%), seven (64%), four (36%), and seven (64%) cases, respectively. The proportion of tumour cells expressing CD41 positivity varied, ranging from 1% to 80%, without apparent association with clinical and pathological characteristics. Factor VIII was positive focally in all stained cases.

FCM was performed in six cases (Table 3). In four cases, the majority of cells expressed CD34. The CD41 antibody was not evaluated.

Cytogenetic analysis was successfully performed in only two cases, which both showed complex but inconsistent abnormalities (Table 4).

Comparison of OS with and without megakaryocytic differentiation

Follow-up data were available for four cases. According to the log-rank test for the analysis of OS, the cases showed significantly worse OS compared with 24 MS cases without evidence of megakaryocytic differentiation (p=0.0033; Fig. 2).

DISCUSSION

We present the clinicopathological features of MS cases with morphological and immunohistochemical megakaryocytic differentiation. Although the number of cases is relatively small, the present study provides clinicopathological insights, including their poor prognosis in this extremely rare condition.

In the present study, two-thirds of the cases (7/11 cases, 64%) were categorised as Type 3 MS. In previous case series, Type 3 MS was much less frequent in all MS cases: 24% of 131 MS cases,[6] 43% of 61 cases,[10] and 38% (Type 4 combined) of 92 cases.[5] In contrast, MS without underlying bone

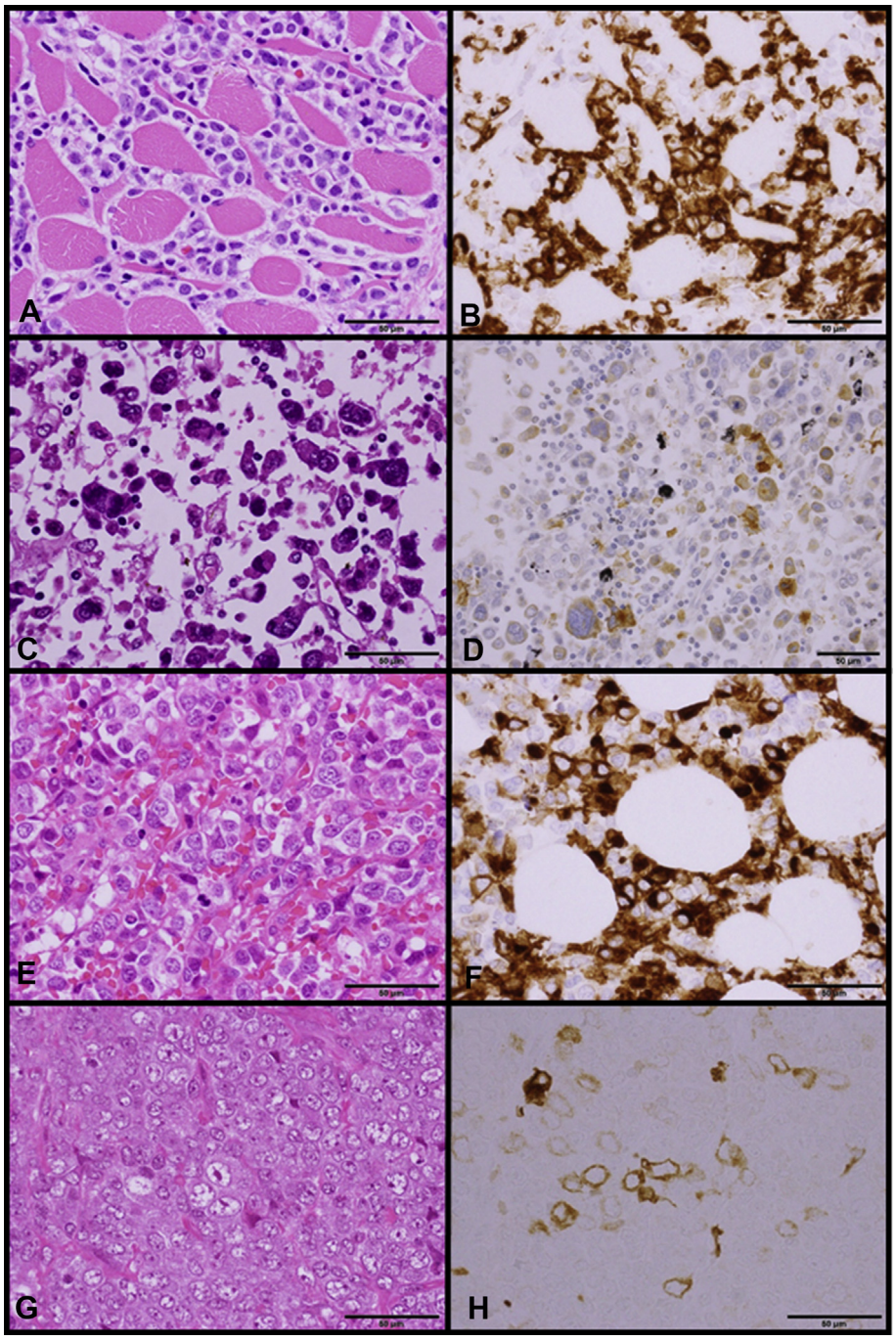

Fig. 1 Microscopic images. (A,C,E,G) Haematoxylin and eosin stain. (B,D,F,H) CD41 immunohistochemical stain. Scale bar = 50 μm. (A,B) Type 3 (Case 3-1). Tumour cells are medium-sized atypical lymphoid cells infiltrating skeletal muscle tissue. Nuclear pleomorphism is minimal with occasional nuclear membrane irregularity and single nucleoli. The background shows only a few small lymphocytes and plasma cells. Twenty percent of the cells are positive for CD41. (C,D) Type 3 (Case 3-4). Bone marrow and lymph node show similar histology consisting of many scattered megakaryoblastic cells in a background of small to medium eosinophilic myocytes. Fifty percent of atypical cells, including megakaryoblastic cells, are positive for CD34 and CD41. (E,F) Type 4 (Case 4-1). Tumour cells have relatively ample pale eosinophilic to clear cytoplasm. Nuclei are round and vary in size with fine chromatin and occasionally one to several nucleoli. Mitosis and apoptotic bodies are scattered. No multinucleated cells are seen. The background shows scattered small lymphocytes with no plasma cells nor eosinophils. Ninety percent of tumour cells are positive for CD34, and 50% are positive for CD41. (G,H) Type 1 (Case 1-1). Tumour cells have scant to moderate amount of frothy cytoplasm and markedly atypical nuclei with prominent nucleoli, vesicular chromatin pattern, and very irregular nuclear contour. Five percent are positive for CD41.

marrow pathology (Type 1 MS) was reported to comprise approximately 25% of MS cases,[1,6] which is much more frequent than the 9% in the present study. These data suggest that MSmgk is more likely to be associated with MDS or MPN and less likely to develop *de novo*, compared to MS without megakaryocytic differentiation. This finding is consistent with previous studies.[1,5]

The male predilection is a constant finding in MS cases in general,[5,6,10] with a male:female ratio of up to 3.14.[11] Male predominance was also evident in the present study, with a male:female ratio of 1.75. The median age of the patients seems to be comparable to previous MS case studies. MSmgk might also have a male predilection, although the reason for this is unknown.

Table 2 Pathological findings

Case no.	Morphology	Mgk−blast	Tumour cell polymorphism	Cell size (μm)	Background	CD41 (%)	Factor VIII(%)
Type 3							
3-1	Lymphoma-like	–	Monotonous	12.3	Lymph	20	5
3-2	DLBCL-like with mgk-blastic cells	+	Polymorphous	14	Lymph, PMN	40	5
3-3	Lymphoma-like	–	Polymorphous	12.2	Plasma cell, lymph	10	1
3-4	Many mgk-blastic cells with small cells	++	Monotonous	24.3	Lymph	50	ND
3-5	Lymphoma-like, prominent nucleoli	–	Polymorphous	10.4	Lymph, eo	5	3
3-6	DLBCL-like, prominent nucleoli	–	Monotonous	12.6	Lymph	80	1
3-7	DLBCL-like with mgk-blastic cells	+	Polymorphous	20.5	Lymph	1	ND
Type 4							
4-1	DLBCL-like	–	Polymorphous	12	Lymph	50	10
4-2	Lymphoma-like	–	Monotonous	8.7	Very few	40	ND
4-3	DLBCL-like	–	Monotonous	8.7	Lymph	60	1
Type 1							
1-1	DLBCL-like	–	Polymorphous	17.2	Few lymph	5	ND

Case no.	CD3	CD20	CD34	CD13	CD33	MPO	CD68	Spectrin	CD71	Glyco-phorin A	Other IHC
Type 3											
3-1	–	–	+	ND	+	–	–	ND	–	–	PAX5–
3-2	–	–	+	ND	ND	f+	+	–	+	–	TdT–
3-3	–	–	–	ND	ND	–	f+	–	ND	ND	AE1/3–, CD30–
3-4	–	–	f+	ND	ND	ND	ND	ND	ND	ND	
3-5	–	–	f+	ND	ND	–	ND	–	–	–	TdT–
3-6	–	–	–	ND	ND	–	ND	ND	+	–	
3-7	f+	–	f+	ND	ND	+	+	ND	ND	ND	CD138+(f)
Type 4											
4-1	ND	–	+	–	–	ND	f+	–	–	–	CD123–, CD163–, CD45RO–, TdT–
4-2	ND	–	–	+	+	–	+	ND	ND	ND	CD45RO–, CD79a–
4-3	–	–	ND	+	+	f+	f+	ND	–	–	CD8–, CD4–, TdT–, TIA1–
Type 1											
1-1	–	–	+	ND	ND	f+	+	–	ND	ND	CD56–, TdT–

DLBCL, diffuse large B-cell lymphoma; eo, eosinophils; f, focal; lymph, lymphocytes; mgk, megakaryo; ND, not done; PMN, polymorphonuclear cells.

The aforementioned clinical characteristics of frequent association with MPN and MDS and male predilection have also been documented in AMgkL.[12–14] This is not a surprising finding, considering that both neoplasms consist of morphologically and immunophenotypically similar tumour cells. The mechanisms of leukaemic cell migration and aggregation to form a mass lesion in extramedullary sites are still unclear, but the similar clinical characteristics we present here confirm that AMgkL and MSmgk are closely related diseases.

In terms of tumour site, MS is reported to occur in almost any part of the body. Frequent anatomical sites are reported to be skin/soft tissue, lymph node, and bone. These sites were also observed in the present study, although no skin involvement cases were observed. The number of mass lesions in MS, in general, is multiple only in <10% of cases.[5,15] In contrast, the majority of our cases (8/11, 73%) presented with multiple extramedullary mass lesions. The propensity for multiplicity seems to be a striking characteristic of MSmgk compared to other MS.

It is difficult to determine the frequency of MSmgk. Pileri *et al.* reported one of 92 MS cases (1.1%) displayed megakaryoblastic histology.[5] We found 16 case reports of MSmgk through a literature search with decent clinical and histological descriptions (Table 5).[16–30] The male:female ratio was 1.29, and the median age of the patients was 51 years. Eleven cases (69%) were Type 3 and two cases (13%) were Type 1. Extramedullary mass lesions were solitary in nine cases (56%) and multiple in seven cases (44%). The characteristic MSmgk morphology of scattered large multinucleated megakaryoblast-like cells, observed in three of 11 cases in the present study, was documented in nine of 16 cases.[16–30] These reference cases featured very similar characteristics to those of the present study, with a frequent association with MDS or MPN and multiple lesions.

Table 3 Flow cytometry results

Case	CD2	CD3	CD4	CD5	CD7	CD8	CD10	CD19	CD20	CD23	κ	λ	CD11c	CD13	CD14	CD25	CD30	CD33	CD34	CD56
3-1	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	+	–
3-2	–	–	–	–	+	–	–	–	–	–	–	–	–	–	–	–	–	–	+	–
3-7	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–
4-1	–	–	–	–	+	–	–	–	–	–	–	–	+	–	–	–	–	–	+	–
4-2	–	–	–	–	–	–	–	–	–	–	–	–	+	–	–	–	–	–	+	+
4-3	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	+	+

Flow cytometry was successfully performed in 6 of 11 cases, but CD41 was not included in any of the tests performed.

Table 4 Cytogenetic analysis (G-banding)

Case	Cytogenetics
3-1	46XY, add (14) (q22), add (16) (q11.2)[1]
4-1	75XXY,+Y-5-7-9+12+16+18+19+20+21+22 +mar1 [1]/72, idem,-Y-Y,add (1) (p36.1),+2 -4+5+5-8-9+12-13-14-15-18+2mar [1]

The OS of the present cases was significantly shorter than that of MS without megakaryocytic differentiation. Among the previous case reports (Table 5), follow-up data were available for 12 cases. The mean survival after the diagnosis was only 2.2 months, except for a single case where the patient was alive 12 months after the diagnosis. In contrast, the 5-year OS in MS has been reported to be 20–30%.[31,32] Although these data cannot be directly compared, they are compatible with our findings that MSmgk might have a worse prognosis.

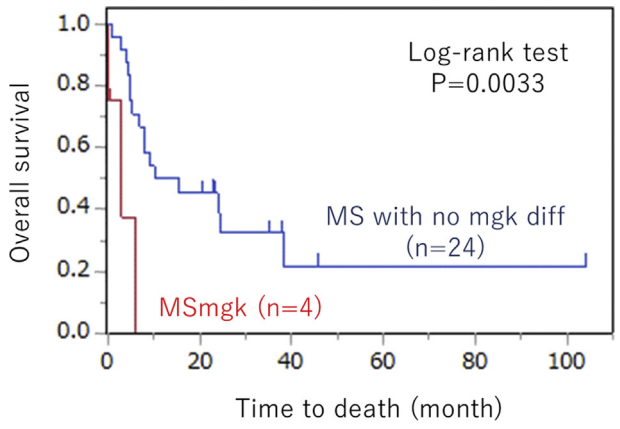

Fig. 2 Overall survival (OS) from the time of diagnosis of myeloid sarcoma (MS). When compared with MS with no megakaryocytic differentiation (MS with no mgk diff, *n*=24), the OS of the present cases (MSmgk, *n*=4) was significantly shorter (*p*=0.0033).

Many cytogenetic abnormalities have been reported in MS, including inv (16), t (8; 21) (q22; q22), 11q23, and +8. Of these, none have been proven to have prognostic significance. Tsimberidou *et al.* reported that chromosome 8 abnormalities in non-leukaemic MS cases were associated with shorter survival.[33] In the present study, cytogenetic analysis was successful in only two cases. Cytogenetic abnormalities are complex and inconsistent. Inv(3) (q21.3q26.2) and t (3; 3) (q21.3q26.2) have been reported to be associated with MDS with megakaryoblastic/megakaryocytic differentiation.[34,35] These were not identified in the present study. Further studies are needed to elucidate the cytogenetic characteristics of MSmgk.

The definition of megakaryocytic differentiation is controversial. The WHO classification 2017 revised 4th edition vaguely defined megakaryocytic differentiation in MS as 'tumours predominantly composed of megakaryoblasts'.[1] In the acute megakaryoblastic leukaemia section, megakaryoblasts are described as 'medium-sized to large blasts' immunophenotypically positive for one or more of the platelet glycoproteins.[1] Incorporating these definitions, in the present study we used blastic morphological features and CD41 IHC expression as a criteria for megakaryocytic differentiation.

In two cases (Cases 3-2 and 3-6), tumour cells showed coexpression of CD41 and CD71. We included these cases based on the above criteria, clinical data including prior history of AMgkL, and negativity with glycophorin A. Moreover, unlike glycophorin A, CD71 is expressed early in haematopoiesis before the progenitor cells differentiate into unilineage erythroid/megakaryocytic precursors. Recent studies have suggested there may be bipotent megakaryocytic-erythroid progenitors, some of which co-express CD41 and CD71.[36,37] We concluded that CD71 expression, with negative glycophorin A, would not exclude megakaryocytic differentiation.

Table 5 Myeloid sarcoma with megakaryocytic differentiation reported in the literature

Case	Age	Location	No. lesions	Past medical history	Type	Follow-up	Immunohistochemical stains of tumour cells				Ref
							Mgk differentiation	CD34	MPO	CD68	
1	23 F	Bone, soft tissue	Multiple	CGL × 4.2y	3	Died 1.5 m	CD41, F8	ND	ND	ND	16
2	23 M	Bone, soft tissue	Multiple	CGL × 1.8y	3	Died 1.2 m	N/A	ND	ND	ND	16
3	2 M	Bone, temporal	Solitary	None	1	Alive 1 y	CD41 (FC)	ND	ND	ND	17
4	64 M	R groin mass	Solitary	CIMF × 8y	3	Died 3 m	CD61	ND	ND	ND	18
5	36 M	Spine	Solitary	MDS	3	N/A	F8	+	+	+	19
6	59 F	Inguinal LN	Solitary	CIMF × 16y	3	Died 2 m	CD41, F8 (weak)	–	ND	–	20
7	68 M	Bone, iliac + multiple LN	Multiple	ET × 5y	3	Died 1 m	CD61, F8(f)	+	–	f+	21
8	22 F	LN, skin	Multiple	None	1	N/A	F8	–	+	–	22
9	68 M	LN, soft tissue	Multiple	MF × 4y	3	Died 8 m	CD42b	+	–	–	23
10	57 M	Intracranial	Solitary	MPN × 10y, MF × 3y	3	Died 2 m	CD42b, CD61	+	–	–	24
11	1 F	Liver	Solitary	M7, post-transplant day 50	4	Died 1 m	CD42b	ND	ND	ND	25
12	45 F	Bone	Multiple	vWD × 10y (concurrent AMgkL)	2	N/A	F8(f), CD61(f)	+	f+	ND	26
13	1 M	Left iliac bone	Solitary	Concurrent AMgkL	2	Died 1 m	CD42b	+	ND	ND	27
14	58 F	Breast	Solitary	ET × 10y	3	Died 0.25 m	CD61, F8	+	–	ND	28
15	87 F	Conjunctiva	Solitary	Concurrent MDS	2	Died 3 m	CD61, F8	–	f+	–	29
16	72 M	LN	Multiple	ET	3	Died 'shortly after'	CD61, F8	+	ND	ND	30

AMgkL, acute megakaryoblastic leukaemia; CGL, chronic granulocytic leukaemia; CIMF, chronic idiopathic myelofibrosis; ET, essential thrombocythaemia; f+, focal positive; (f), focal positive; F, female; F8, factor VIII; LN, lymph node; m, months; M, male; MDS, myelodysplastic syndromes; MF, myelofibrosis; Mgk, megakaryocytic; Ref, reference; vWD, von Willebrand disease; y, years.

CONCLUSIONS

The present study identified the clinicopathological characteristics of MSmgk; namely, the frequent association of prior histories of MDS/MPN, multiple lesions, and dismal prognosis. More detailed studies, including genomic or gene expression analyses, are required to elucidate this disease entity and improve patient prognosis.

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