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Clinicopathological study of 9 cases of megakaryocytes in pleural and peritoneal fluids

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Abstract

To systemically analyze megakaryocytes in pleural and peritoneal fluids and their clinical significance. We retrospectively examined 10,846 pleural, peritoneal, and pericardial fluid samples obtained from 3 hospitals over a 20-year period. Megakaryocytes were observed in the pleural fluid samples from 7 patients and peritoneal fluid samples from 2 patients, and the incidence was 0.83%. The clinical diagnoses of these 9 patients included myeloproliferative disorders, trauma, and tumors. The serous effusions in all 9 patients were bloody, and the megakaryocytes could be associated with trauma, bone marrow pollution, extramedullary hematopoiesis, or cancer. Additionally, differentiating between megakaryocytes and tumor cells or nuclear mesothelial cells in the pleural fluid is difficult. Therefore, megakaryocytes should be carefully observed and differentiated in pleural and peritoneal fluids because they can be confused with other cells in the clinic. Altogether, the megakaryocytes in the pleural and peritoneal fluids were mainly associated with contamination in the bone marrow or extramedullary hematopoiesis.

Abbreviations: CML = chronic myelocytic leukaemia, EMH = extramedullary hematopoiesis.

Keywords: megakaryocytes, peritoneal fluids, pleural fluids

1. Introduction

Megakaryocytes are large polyploid cells that reside in the bone marrow and can produce platelets. Megakaryocytes are occasionally observed in the peripheral blood.[1] Megakaryocytes have also been observed in pleural and peritoneal fluids in patients with myeloproliferative disorders likely due to the development of extramedullary hematopoiesis (EMH) in the pleura or peritoneum,[2,3] where EMH underlies the trilineage formation of normal blood cells outside the bone marrow. This phenomenon rarely occurs in serous effusions.[4,5] Moreover, the presence of megakaryocytes in pleural and peritoneal fluids is rare and is often observed during necropsy.[3,6,7]

In the laboratory, megakaryocytes in pleural and peritoneal fluids from patients with solid tumors or lymphomas can be mistaken for metastatic cells, and reports have described the differences between megakaryocytes and malignant cells.[5] Thus, differentiating atypical megakaryocytes from malignant epithelial or mesenchymal cells in serous effusion is critical.[8] However, previous studies are rare, and reports of the prevalence and distribution of megakaryocytes in pleural and peritoneal fluids and their clinical significance are lacking.[3,9]

Here, megakaryocytes in 10,846 pleural, peritoneal, and pericardial fluids from patients at 3 different hospitals were retrospectively examined. The total incidence was calculated, and the clinical and cytological information of these patients were also collected. Furthermore, differentiating megakaryocytes from tumor cells or degenerated cells in these body fluids is difficult. Thus, the differences between megakaryocytes and tumor cells in pleural and peritoneal fluids were also summarized in this study.

2. Materials and methods

2.1. Patients

The records from the cytopathology laboratory in the Department of Clinical Laboratory of Zhejiang Provincial People's Hospital (Hospital A), People's Hospital of Mashan County (Hospital B), and People's Hospital of Songyang County (Hospital C) from 1997 to 2017 were examined for evidence of megakaryocytes in pleural, peritoneal, and pericardial fluid specimens (Table 1). The studies were approved by the review board at each hospital. The clinical diagnoses of the 9 patients included primary thrombocytosis, chronic myelogenous leukemia, polycythemia vera, fracture of the left rib with pleural fluid, multiple injuries (2 patients), lung cancer, and liver cancers (2 patients) (Table 2).

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Table 1

Characteristics of the 10,846 samples from the 3 hospitals.

	Pleural fluids	Peritoneal fluids	Pericardial fluids	Total	Megakaryocytes	Megakaryocyte rate
Hospital A	3998	3987	46	8031	6	0.75
Hospital B	1004	486	9	1499	2	1.33
Hospital C	823	486	7	1316	1	0.76
Total	4875	4079	61	10,846	9	0.83

2.2. Wright–Giemsa staining

Serous effusion was collected in an EDTA anticoagulant tube, mixed and centrifuged at 1500 rpm/min for 10 minutes. The supernatant was slowly drained, the sample was placed in a 5 μL sediment retention, and slides were prepared. The samples were dried and subsequently stained with Wright–Giemsa stain. The cells were observed under a microscope with a low power lens, and the suspicious giant nuclear cells under the oil microscope were confirmed by microscope photographs. Then, we generated a graphic picture using a digital camera. In addition, the number of megakaryocytes on each smear was carefully counted. The clinical information from the patients' medical records was also reviewed.

3. Results

3.1. Prevalence of megakaryocytes

We retrospectively examined 4875 pleural, 4079 peritoneal, and 61 pericardial fluid specimens collected at 3 hospitals over a 20-year period. Megakaryocytes were observed in 9 cases, the incidence was 0.83%, and the statistics from hospitals A, B, and C are displayed in Table 1. Thus, the incidence of megakaryocytes in serous fluid was relatively low. Megakaryocytes may be overlooked by pathologists, and a systematic analysis of megakaryocytes in serous fluid should be performed.

3.2. Clinical findings

As previously reported, EMH was the primary cause of megakaryocytes in pleural and peritoneal fluids. In our study, 3 cases displayed EMH (Table 2). Case 1 had primary thrombocytosis, and the platelet count was 1037×10^9/L. Thus, abnormal hematopoiesis in the pleura could be a reason for the presence of megakaryocytes in pleural fluid (Fig. 1A). Case 2 had chronic myelocytic leukemia (CML). In this case, many types of megakaryocytes and minor blasts were observed in the pleural and peritoneal fluids; thus, extensive EMH should be considered (Fig. 1B). Case 3 had polycythemia vera with myelofibrosis, and megakaryocytes were observed several times in the pleural and peritoneal fluids from this patient (Fig. 1C); erythroblast and immature granulocytes were also observed in the pleural fluids.

Additionally, 3 cases were trauma patients (Fig. 1, D–F, Table 2). Of these patients, case 4 had a rupture of the rib and a low pleural fluid volume. Cases 5 and 6 had multiple injuries resulting in the rupture of the ribs and a lumbar spine injury.

Finally, cases 7, 8, and 9 were tumor patients with liver and lung cancers (Fig. 1, G–I, Table 2). However, whether the megakaryocytes were associated with the tumor cells is unclear.

3.3. Cytological findings

All pleural and peritoneal fluids from the 9 patients were bloody, and the number of megakaryocytes in each case is listed in Table 3. The background cells included monocytes, lymphocytes, macrophages, and neutrophils. In certain cases, immature cells could be observed in the smear. In case 2, 8% of the cells on the slides were metamyelocytes, and the patient was diagnosed with CML. In case 6, 5% of the cells on the slides were myelocytes, representative picture about the myelocyte (Supplemental Figure 1A, arrowed, http://links.lww.com/MD/C408) in the same patient was shown, and the patient was diagnosed with multiple injuries. In case 7, 4% of the cells on the slides were metastatic cancer cells, and the patient was diagnosed with lung cancer, and the representative picture (Supplemental Figure 1B, http://links.lww.com/MD/C408) about the metastatic cancer cell in the same patient was shown. However, no immature cells were observed in the other cases.

Additionally, the megakaryocytes in the pleural and peritoneal fluids can be divided into the following 3 types: huge megakaryocytes containing multilobed nuclei with different sized cytoplasm, which are the most difficult to differentiate

Table 2

Clinical and pathological features of the 9 patients included in this study.

Patient	Age	Sex	Patient history	Type of effusion	Trauma	Splenomegaly	Diagnosis
1	70	M	Hypertension	Pleural	No	Yes	Primary thrombocytosis
2	31	F	CML	Peritoneal	No	Yes	CML
3	63	F	Rib fracture	Pleural	Yes	Yes	Polycythemia vera and myelofibrosis
4	51	F	None	Pleural	Fracture of the left rib	No	Fracture of the left rib with slight pleural fluid
5	61	M	Hypertension	Pleural	Fracture of L2 lumbar vertebra	No	Multiple injuries
6	50	M	None	Pleural	Multiple injuries	No	Fracture of the left rib and lumbar with hemopneumothorax
7	80	M	None	Pleural	No	No	Lung cancer
8	51	M	None	Peritoneal	No	No	Liver cancer
9	41	M	None	Peritoneal	No	No	Liver cancer

All patients were alive. Three patients had myeloproliferative diseases, 3 patients were trauma patients, and 3 patients were tumor patients.
F = female, M = male.

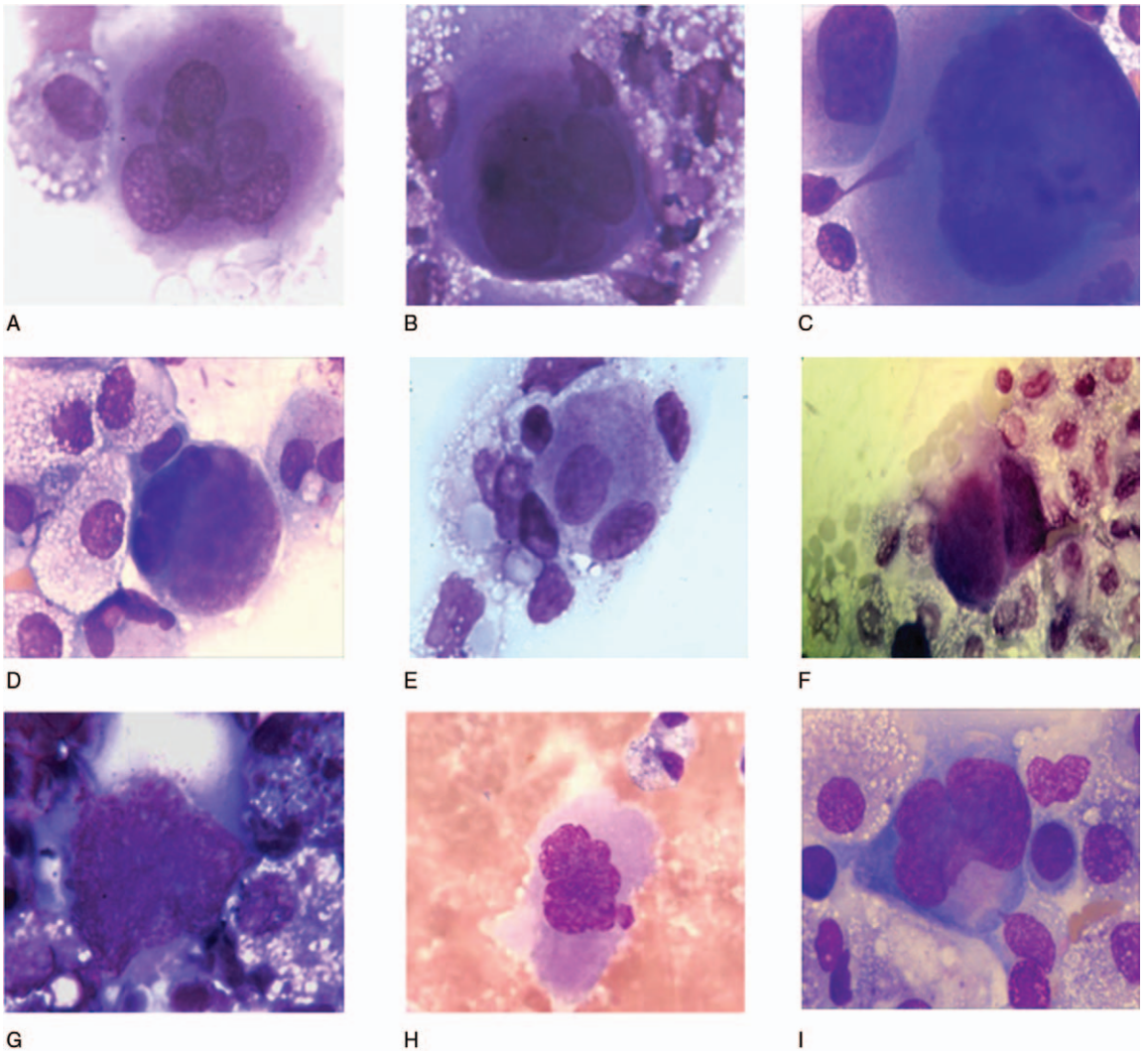

Figure 1. Wright–Giemsa staining (×1000). A, Case 1: Large megakaryocytes containing multilobed nuclei with different sized cytoplasm. These cells also had dense chromatin and a rich inner and outer cytoplasm, and the inner cytoplasm was filled with purplish red particles. B–D, Cases 2 to 4: Megakaryocytes containing multilobed nuclei and dense chromatin. E, Case 5: Megakaryocytes containing multilobed nuclei and dense chromatin surrounding a group of macrophages and lymphocytes (F, G). Cases 6 and 7: Megakaryocytes containing large nuclei and dark nuclear chromatin staining. H and I, Cases 8 and 9: Megakaryocytes containing lobed nuclei and dense chromatin.

Table 3

Megakaryocytes in pleural and peritoneal fluids and other cytological findings in these 9 patients.

Patient	Characteristics of effusions	No. of megakaryocytes	Background cells	Immature cells
1	Bloody	10	Monocyte	No
2	Bloody	12	Lymphocyte	Metamyelocyte (8%)
3	Bloody	6	Macrophage	No
4	Bloody	4	Macrophage	No
5	Bloody	7	Macrophage	No
6	Bloody	3	Neutrophil	Myelocyte (5%)
7	Bloody	1	Lymphocyte	Metastatic cancer cell (4%)
8	Bloody	2	Macrophage	No
9	Bloody	1	Neutrophil	No

from tumor cells (Fig. 1, A–E); small megakaryocytes containing large nuclei with dark nuclear chromatin staining, which are similar to dyskaryotic cells and can be observed in patients with multiple injuries (Fig. 1, F, G); and megakaryocytes with lobed nuclei. Similar to the mesothelial cells, denucleation was observed in several megakaryocytes in the pleural and peritoneal fluids (Fig. 1, H, I).

3.4. Differential diagnosis of tumor cells

In the clinic, the differential diagnosis of tumor cells in pleural and peritoneal fluids is critical. The following points should be emphasized. First, the nucleoli of carcinoma cells are very obvious, whereas the nucleoli of megakaryocytes are usually small and difficult to find (Table 4). Second, the nuclei of mature megakaryocytes are multilobed with condensed chromatin and

Table 4

Differences between megakaryocytes and tumor cells in pleural and peritoneal fluids in this study.

	Nucleolus	Nucleus	Distribution	Cytoplasm
Tumor cell	Large and prominent	Large or ununiform size, loose chromatin and light staining	In bulk and deranged	Cloudy and foamy; the boundary is unclear
Megakaryocyte	None	Large, multilobed nuclei, condensed chromatin and dark staining	Single and on the margins of the slides	Abundant amount of cytoplasm with rich particles, occasionally with platelet adhesion

dark staining. In contrast, carcinoma cells can have multiple nuclei and usually have loose chromatin and light staining. However, megakaryocytes with unlobed or only bilobed nuclei are more likely to be confused with malignant cells (Table 4). Therefore, careful attention should be paid to this cell type. Third, in contrast to tumor cells, most megakaryocytes do not adhere to each other and are usually on one of the margins of the slides (Table 4). Fourth, the cytoplasm of megakaryocytes is rich and surrounded by regular platelets, but the cytoplasm of tumor cells is often cloudy and foamy (Table 4).

4. Discussion

Megakaryocytes are derived from the bone marrow. Megakaryocytes are large cells that are rarely present in the blood and pleural and peritoneal fluids.[4] Kumar was the first to centrifuge pleural and peritoneal fluid and perform HE staining to observe these cells.[3] However, our methods are different because we centrifuged and concentrated the pleural and peritoneal fluids and performed Wright staining to observe these cells. Using these methods, the megakaryocytes appeared more clearly and were easily identified.

EMH undergoes trilineage formation from normal blood cells outside the bone marrow. Megakaryocytes are predominantly observed in the spleen and liver and rarely observed in serous effusions. An accurate diagnosis requires the presence of megakaryocytes and other precursor hematopoietic cells.[5,10] In this study, cases 1, 2, and 3 all showed EMH, revealing that EMH is an important cause of megakaryocytes in pleural and peritoneal fluids. Additionally, in trauma patients, the rupture of the ribs can tear the pleura. Thus, megakaryocytes in the bone marrow can enter the serous cavity, resulting in the presence of megakaryocytes in the pleural fluid. Moreover, myelocytes were observed in the pleural fluid in case 6, supporting that megakaryocytes are derived from the bone marrow. Thus, clinicians should carefully attend to other immature granulocytes in the pleural and peritoneal fluids that originally reside in the bone marrow. These findings support the hypothesis that megakaryocytes are derived from the bone marrow.[5] Additionally, benign multinucleated mesothelial cells resemble megakaryocytes and should also be examined. In contrast to megakaryocytes, benign multinucleated mesothelial cells contain distinctly multiple, rather than lobed, nuclei that are smooth and round to ovoid with sharply defined borders. Moreover, mesothelial cells frequently adhere to each other.[3,8]

In general, megakaryocytes are 10 to 15 times larger than typical red blood cells, which have, on average, a 50 to 100 μm diameter. The nuclei of megakaryocytes can become very large and lobulated and, under a light microscope, can lead to the false impression that there are several nuclei. An initial report by Calle 3[2] in 1968 described the presence of megakaryocytes in abdominal fluid from a patient with an eventual diagnosis of myelofibrosis. In another study, Kumar reviewed nearly 5000 cases of pleural and peritoneal effusions over a 22-year period and found only 5 cases with megakaryocytes.[11] The clinical diagnoses of these patients included myelofibrosis, CML, and lymphoma. Similarly, a retrospective study of 20,793 effusions found a total of 8 cases of EMH (7 pleural and 1 peritoneal) in 5 patients over a 21-year period. One study also reported the presence of megakaryocytes in ascitic fluid. This patient had myelofibrosis with myeloid metaplasia complicated by ascites.[12] Altogether, these few cases highlight the rarity of extramedullary hematopoietic effusions.[5]

The differentiation between megakaryocytes and malignant cells can be difficult in cytologic specimens. However, distinguishing mature megakaryocytes from tumor cells can be easily accomplished in serous fluid. Many megakaryocytes are extremely large and are much larger than typical carcinoma or lymphoma cells, but in contrast to tumor cells, they do not adhere to each other.[11] The following points should also be emphasized. First, megakaryocytes with abundant cytoplasm do not exhibit the marked vacuolization that is frequently observed in adenocarcinoma cells. Second, mature megakaryocytes have multilobed nuclei and nuclear membranes that are not very prominent. In contrast, carcinoma cells may have multiple nuclei, but these nuclei are not usually lobed. Third, the nuclei of carcinoma cells are usually angulated and sharp. Fourth, the nucleoli of carcinoma cells are often obvious, whereas the nucleoli of megakaryocytes are usually small or even difficult to find. However, megakaryocytes with unlobed or only bilobed nuclei are more likely to be confused with malignant cells.[3]

In summary, in this study, 10,846 pleural, peritoneal, and pericardial fluid samples from patients at 3 hospitals were retrospectively reviewed. Only 9 cases had megakaryocytes. These cases can be divided into the following 3 types: myeloproliferative disorders, trauma, and tumors. Therefore, the correct diagnosis of megakaryocytes in patients with myeloproliferative disorders, trauma, and tumors is important.

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