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CASE REPORT

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Granulocytic sarcoma of the thymus in a nonleukaemic patient

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Abstract We report a case of granulocytic sarcoma arising from the thymus in a 17-year-old nonleukaemic patient. The patient presented with an anterior mediastinal tumour and underwent surgical resection. Histological examination showed a diffuse infiltrate of immature round cells in the thymus. Tumour cells were diffusely peroxidase positive, but naphthol AS-D chloroacetate esterase negative. Immunohistochemical staining revealed expression of CD34 and terminal deoxynucleotidyl transferase (TdT), but not of CD13 and CD33. Ultrastructurally, electron-dense or medium-density granules were present in the cytoplasm. Four months after successful autogenic bone marrow transplantation, pleural and pericardial fluid contained tumour cells with azurophilic granules, which expressed CD13 and CD33, but not CD34 and TdT. The patient died of the disease 18 months after clinical manifestation, but still without developing leukaemia. The granulocytic sarcoma in the present case may have originated from myeloid precursors in the thymus and remained within the extramedullary site despite the differentiation into a more committed myeloid lineage at the relapse.

Key words Granulocytic sarcoma · Thymus

Introduction

Granulocytic sarcoma is a heterogeneous neoplasm with regard to clinical behaviour. It usually occurs in association with acute myelogenous leukaemia (AML), chronic myeloproliferative disorder, and myelodysplastic syndrome [14]. While there is usually evidence of these haematological diseases in either the blood or the bone marrow at the time of diagnosis, in some cases the granulocytic sarcoma may precede them [3, 12, 14]. The common sites of occurrence are skin, lymph node, bone, orbit, and nasal fossa, but any site may be affected [3, 12, 14]. The lesions may also be present as anterior mediastinal masses in patients with symptoms of leukaemia, whereas they occur extremely rarely in nonleukaemic patients [2, 8, 11]: only three cases, which terminated in AML, have been reported in detail in the literature [8, 11].

We report here an additional case of granulocytic sarcoma in the anterior mediastinum, probably originating from the thymus, in a patient who did not have prior haematological disease and who did not subsequently develop leukaemia. Our initial diagnosis of the tumour was lymphoblastic lymphoma, a haematological malignancy that is much more frequent in the thymus, but subsequent differentiation of the tumour cells to more committed cells along the myeloid lineage was observed at the relapse. Thus, both the morphological characteristics of the tumour and the difficulty in diagnosing granulocytic sarcoma are also worth reporting.

Clinical history

A 17-year-old man was admitted to Tokyo Metropolitan Komagome Hospital in September 1992 complaining of shortness of breath. Chest roentgenography showed slight mediastinal enlargement. Computed tomography (CT) of the chest disclosed a huge tumour in the anterior mediastinum, invading both lungs. The clinical diagnosis was invasive thymoma. Surgical excision of the tumour was carried out, but this was not complete because of infiltration of the pleura and the lung. A definite histological diagnosis could not be made because of the primitive morphology of neoplastic round cells and negative reactions for B/T-cell-associated antigens and naphthol AS-D chloroacetate esterase. Peroxidase staining was not performed at that time. A tentative diagnosis of lymphoblastic lymphoma was made. The patient was treated with standard chemotherapy for malignant lympho-

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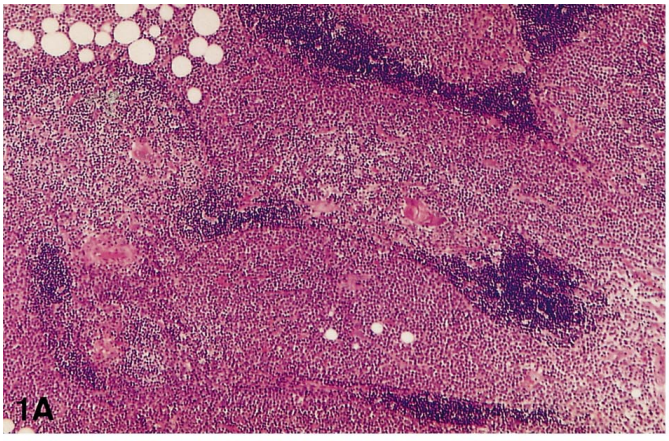
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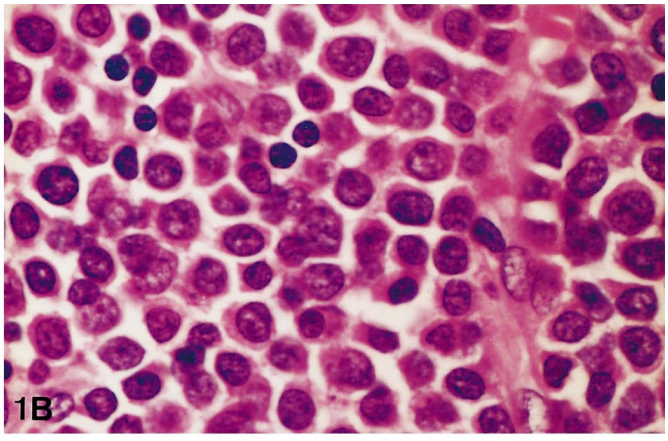
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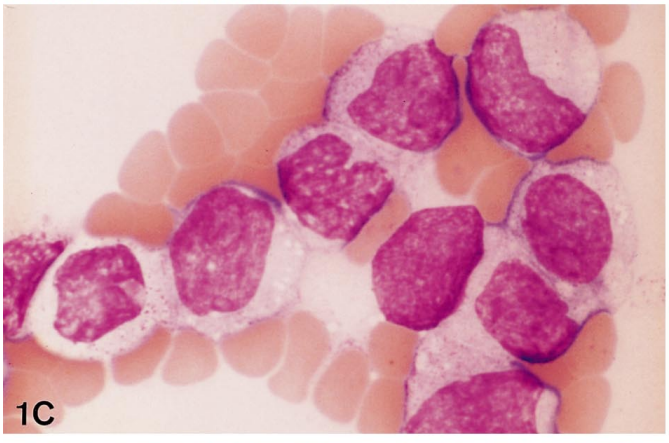
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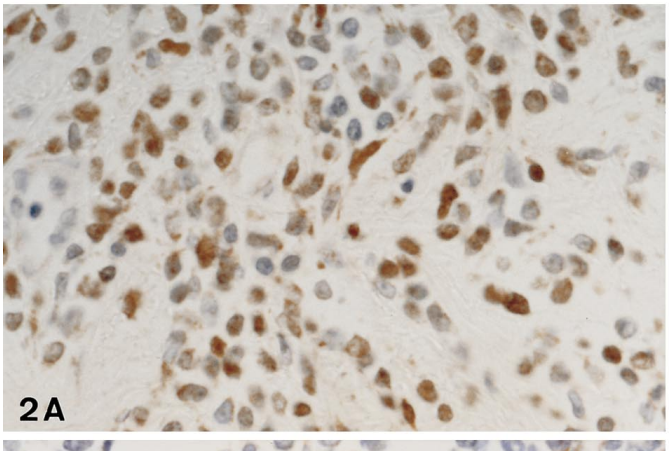
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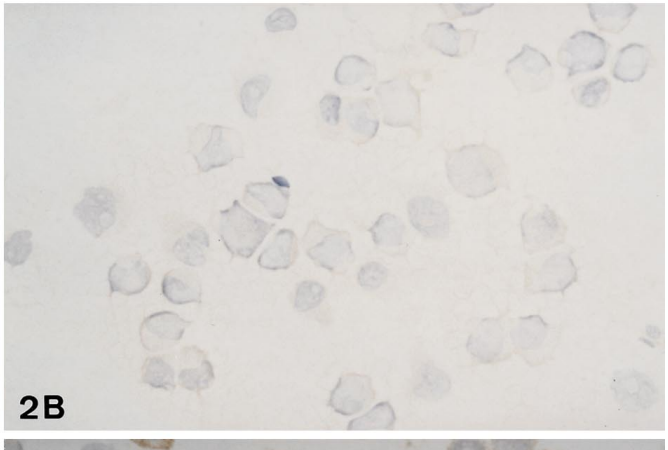
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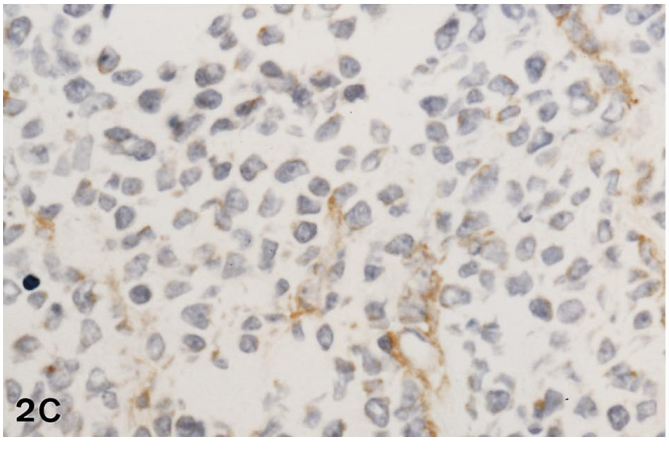
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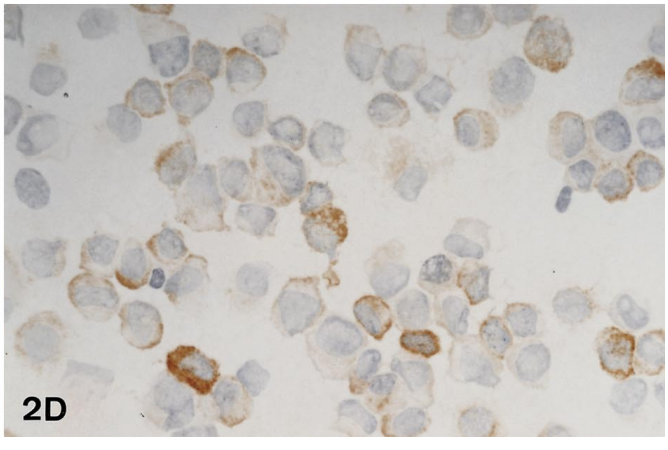
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Table 1 Immunohistochemical results (*TdT* terminal deoxynucleotidyl transferase)

Marker	Prototype	Source	Primary tumour	Recurrent tumour
Myelomonocytic markers				
CD13	MY7	Coulter Immunology, Hialeah, Fla.	–	+
CD15	LeuM1	Becton Dickinson, Mount View, Calif.	–	+
CD33	MY9	Coulter Immunology	–	+
B-cell markers				
CD10	J5	Coulter Immunology	–	–
CD19	B4	Coulter Immunology	–	–
CD20	L26	Dako, Glostrup, Denmark	–	–
	B1	Coulter Immunology	–	–
CD21	CR2	Becton Dickinson, Mount View, Calif.	–	–
T-cell markers				
CD1a	OKT6	Ortho Diagnostic Systems, Raritan, N.J.	–	–
CD2	Leu5b	Becton Dickinson, San Jose, Calif.	–	–
CD3	Leu4	Becton Dickinson, Mount View, Calif.	–	–
CD4	Leu3a+Leu3b	Becton Dickinson, Mount View, Calif.	–	–
CD5	Leu1	Becton Dickinson, San Jose, Calif.	–	–
CD7	Leu9	Becton Dickinson, San Jose, Calif.	–	–
CD8	Leu2a	Becton Dickinson, Mount View, Calif.	–	–
CD45RO	UCHL1	Dako Corporation, Carpinteria, Calif.	–	–
CD99	O13	Signet Laboratories, Dedham, Mass.	–	–
NK-cell markers				
CD56	NKH-1	Coulter Immunology	–	–
CD57	Leu7	Becton Dickinson, Mount View, Calif.	–	–
Miscellaneous				
CD34	NU-4A1	Nichirei, Tokyo, Japan	+	–
CD43	Leu22	Becton Dickinson, Mount View, Calif.	+	+
TdT	TdT	Life Science, St. Petersburg, Fla.	+	–
HLA-DR	HLA-DR	Becton Dickinson, Mount View, Calif.	+	+

Table 2 Histochemical results (*POX* peroxidase, *NASD* naphthol AS-D chloracetate esterase, *NE* not examined)

Stain	Primary tumour	Recurrent tumour
POX	+	+
NASD	–	NE

◀ **Fig. 1** Representative micrographs illustrating the histological and cytological features of **A**, **B** the primary tumour and **C** the recurrent tumour. **A** In the primary tumour, the thymic lobular structure is replaced partially by a dense infiltrate of round cells. H&E, original magnification ×5). **B** The cells have round nuclei with finely dispersed nuclear chromatin and occasional prominent nucleoli. H&E, original magnification ×200. **C** The recurrent tumour cells in the pleural fluid exhibit myeloid features with azurophilic granules in the cytoplasm. Wright and Giemsa, original magnification ×250

Fig. 2 Immunoperoxidase stain with anti-TdT antibody and anti-CD13 antibody in **A**, **C** the primary tumour and **B**, **D** the recurrent tumour. Most cells in the primary tumour (**A**) show nuclear staining with anti-TdT antibody, whereas all of the recurrent tumour cells (**B**) are negative. The primary tumour cells (**C**) lack staining with anti-CD13 antibody, whereas a considerable number of the cells of the recurrent tumour (**D**) show positive immunoreactivity. Original magnification ×120

ma and radiation therapy of the mediastinum, followed by autogenic bone marrow transplantation. In October 1993, radiographic evaluation demonstrated regrowth of the residual tumour and massive pleural and pericardial effusion. The cytochemical and immunophenotypic analysis of the tumour cells in the fluid revealed a myeloid phenotype. The patient died of the disease 6 months after the relapse. There was no evidence of the development of AML in the frequent bone marrow and peripheral blood examinations during the follow-up period. An autopsy was not performed.

Pathological findings

The primary mediastinal mass was an elastic hard tumour with an irregular contour, 10.5×5.5×5.0 cm in size. The cut surface was white to grey, and there was no fibrous encapsulation or lobulation. The tumour invaded the right lung, and multiple pleural satellite nodules were found. Microscopically, the thymic lobules were entirely or partially replaced by monomorphous round cells, which also infiltrated into the interlobular septa with hyaline sclerosis (Fig. 1A). There were scattered reactive lymphoid follicles with germinal centres. The tumour cells were medium sized and had a blast-like appearance with a round configuration. The nuclei were round or slightly irregular, with finely dispersed chromatin and

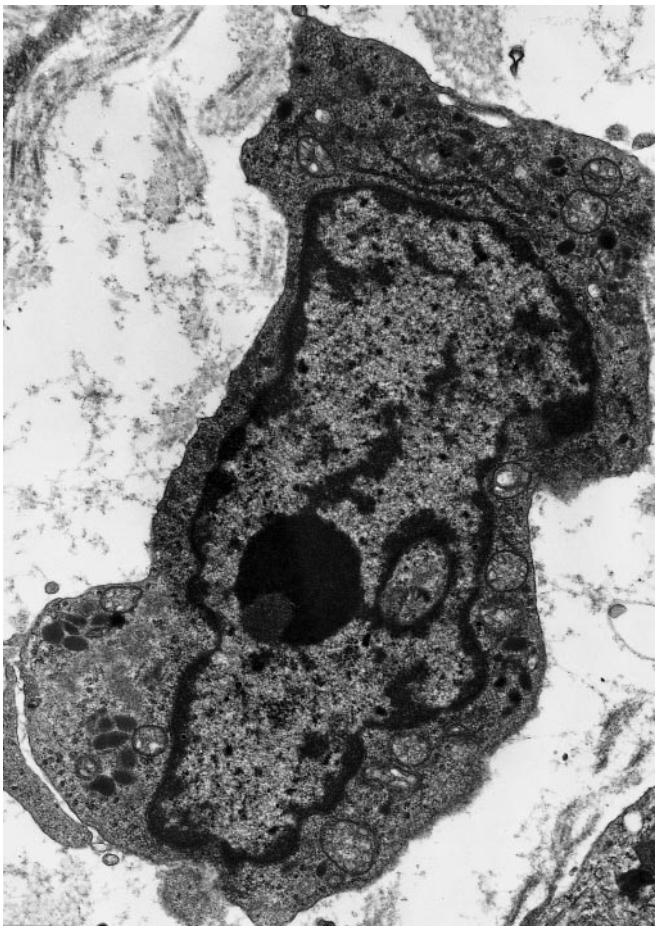

Fig. 3 Representative electron micrograph of a primary tumour cell. The cell contains scattered electron-dense or medium-density granules in the cytoplasm, compatible with myeloid differentiation. Original magnification ×10,000

occasional prominent nucleoli (Fig. 1B). A small number of lymphocytes were present in the tumour, but eosinophils and neutrophils were not observed.

The recurrent tumour cells in the pleural and pericardial fluid had fine dust-like azurophilic granules in the cytoplasm (Fig. 1C).

Specimens of the primary tumour were fixed for 10 h with periodate-lysine-paraformaldehyde (PLP), embedded in OCT compound, frozen in dry ice-hexane, and stored at –80°C. Cytological specimens obtained from the pleural and pericardial fluid at the time of relapse were prepared by standard techniques using cytospin or cell blocks. These sections or formalin-fixed, paraffin-embedded sections were stained by the avidin-biotin-peroxidase complex method for immunohistochemistry. The panel of antibodies used is listed in Table 1. Histochemical stains for naphthol AS-D chloroacetate esterase and peroxidase were also performed (Table 2).

The results of the histochemical and immunophenotypic studies are also summarized in Tables 1 and 2. The primary tumour cells were negative for naphthol AS-D chloroacetate esterase. Stains were positive for peroxidase in the primary and recurrent tumour. The primary tumour cells were positive for CD34 and TdT (Fig. 2A), and negative for CD13 and CD33 (Fig. 2C). In contrast, the cells in the recurrent tumour were positive for CD13 and CD33 (Fig. 2D), and negative for CD34 and TdT (Fig. 2B). In the primary and the recurrent tumour, expression of B-, T-, and NK-cell-associated antigens was not observed. The results of a flow cytometric analysis of the recurrent tumour cells in the pleural fluid were similar to the results of the immunohistochemical test.

Genotypic analysis of the primary tumour by Southern blot hybridization showed clonal rearrangement of the immunoglobulin heavy chain (IgH) gene, but not of the T-cell receptor (TCR)-β or -γ genes.

A retrospective ultrastructural study was carried out for the primary tumour. Electron-dense and medium-density granules were scattered in the cytoplasm, but Auer rods were not observed (Fig. 3).

Discussion

The thymic tumour in the present case was initially misinterpreted as a lymphoma, since neoplastic cells showed the morphology of primitive blast cells with the expression of TdT and clonal rearrangement of the IgH gene. However, we could have made a correct diagnosis of granulocytic sarcoma by a touch preparation had this been available. Positive peroxidase staining and electron microscopic findings displaying primary granules might have led to a correct diagnosis if the possibility of granulocytic sarcoma had been consciously taken into consideration.

The present case illustrates some of the pitfalls involved in the pathological diagnosis of unexpected granulocytic sarcoma in the clinical setting. First, this tumour occurred in a nonleukaemic patient, who did not subsequently develop acute leukaemia. Byrd et al. reported that nearly half (47%) of the patients with primary granulocytic sarcoma were misdiagnosed at the initial presentation [3]: the most frequent incorrect diagnosis was lymphoma, but other types of sarcoma were also frequently assumed. Second, the location of this tumour was also exceptional. It has been recognized that the thymus may be involved in all types of leukaemia. According to Middleton et al., thymic involvement was present in five of ten patients with myeloid leukaemia [13]. However, only three patients with primary involvement of the anterior mediastinum have been documented in detail [8, 11]. Mediastinal germ cell tumours coexisting with haematological malignancies including AML and granulocytic sarcoma have been also reported [5, 15]. The tumour in the present case was distinguished by the absence of germ cell tumour components. In contrast, the thymus is involved more frequently by malignant lymphoma [Hodgkin's disease, precursor T-lymphoblastic lymphoma, primary mediastinal large B-cell lymphoma, and mucosa-associated lymphoid tissue (MALT) lymphoma]. In our case, the tumour cells were similar to

those of lymphoblastic lymphoma in some features, such as the morphology and immunoreactivity for TdT. However, TdT may also be expressed in 14% of AML, and frequently in FAB M0 and M1 [10]. Although clonal rearrangement of the IgH gene was found in the present case, as in 20% of precursor T-lymphoblastic lymphoma/leukaemia [7], approximately 14% of cases of AML also demonstrate clonal IgH gene rearrangement [1]. Third, the primary tumour cells were not stained for naphthol AS-D chloroacetate esterase. Naphthol AS-D chloroacetate esterase stain is useful for demonstrating myeloid lineage of the tumour cells in paraffin-embedded tissue sections, but is not highly sensitive or specific. According to Davey et al., 3 of 15 extramedullary myeloid cell tumours (20%) were negative for naphthol AS-D chloroacetate esterase stain [4]. Enzyme histochemical staining for peroxidase and immunohistochemical staining for myeloperoxidase are more sensitive methods for establishing a correct diagnosis.

In the present case, the tumour regrew at the original site and without entering the bone marrow, excluding the possibility of the development of a second, independent neoplasm. Tumour cells initially lacked myeloid-associated antigens, CD13 and CD33, whereas they later acquired both these markers and lost the initially observed CD34 expression and TdT positivity, which are characteristics of progenitor cells [10], later at the relapse. It is possible that tumour cells may differentiate into cells more committed to the myeloid lineage. Although all the published cases of primary mediastinal granulocytic sarcoma have relapsed, immunophenotypic differences between the primary and recurrent tumour have not been documented [8, 11]. Eosiniphils and other cells of the myeloid series can be regularly seen in the human fetal and neonatal thymus, and in the connective tissue of the septa and around the blood vessels [6]. Furthermore, $CD34^+$, $CD4^-$, $CD8^-$, and surface $CD3^-$ immature thymocytes retain the capacity to differentiate into clonal myeloid lineage when influenced by cytokines from thymic epithelial cells [9]. Along these lines, this immature tumour may have arisen directly from myeloid precursors in the thymus.

In summary, we have reported an unusual case of granulocytic sarcoma of the thymus. Cases diagnosed as thymic lymphoblastic lymphomas should be studied carefully to ascertain the possible presence of granulocytic sarcoma.

References

1. Adriaansen HJ, Soeting PWC, Wolvers-Tettero ILM, von Dongen JJ (1991) Immunoglobulin and T-cell receptor gene rearrangements in acute non-lymphocytic leukaemias. Analysis of 54 cases and a review of the literature. Leukaemia 5:744–751
2. Brunning RD, Mckenna RW (eds) (1993) Acute myeloid leukaemias. In: Tumors of the bone marrow. (Atlas of tumor pathology) Armed Forces Institute of Pathology, Washington DC, pp19–142
3. Byrd JC, Edenfield WJ, Shields DJ, Dawson NA (1995) Extramedullary myeloid cell tumours in acute nonlymphocytic leukaemia: a clinical review. J Clin Oncol 13:1800–1816
4. Davey FR, Olson S, Kurec AS, Eastman-Abaya R, Gottlieb AJ, Mason DY (1988) The immunophenotyping of extramedullary myeloid cell tumors in paraffin-embedded tissue sections. Am J Surg Pathol 12:699–707
5. DeMent SH (1990) Association between mediastinal germ cell tumours and hematologic malignancies: an update. Hum Pathol 21:699–703
6. Henry K (1992) The thymus gland In: Henry K, Symmers WS (eds) Systemic pathology. Thymus, lymph nodes, spleen and lymphatics. Churchill Livingstone, Edinburgh, pp 27–140
7. Kichingman GR, Rovigatti U, Mauer AM, Melvin S, Murphy SB, Stass S (1985) Rearrangement of immunoglobulin heavy chain genes in T cell acute lymphoblastic leukaemia. Blood 65:725–729
8. Kubonishi I, Ohtsuki Y, Machida K, Agatsuma Y, Tokuoka H, Iwata K, Miyoshi I (1984) Granulocytic sarcoma presenting as a mediastinal tumor. Am J Clin Pathol 82:730–734
9. Kurtzberg J, Denning SM, Nycum LN, Singer KH, Haynes BF (1989) Immature human thymocytes can be driven to differentiate into nonlymphoid lineages by cytokines from thymic epithelial cells. Proc Natl Acad Sci USA 86:7575–7579
10. Lee EJ, Yang J, Leavitt RD, Testa JR, Clivin CI, Forrest A, Schiffer CA (1992) The significance of CD34 and TdT determinations in patients with untreated de novo acute myeloid leukaemia. Leukaemia 6:1203–1209
11. McCluggage WG, Boyd HK, Jones FGC, Mayne EE, Bharucha H (1998) Mediastinal granulocytic sarcoma. A report of two cases. Arch Pathol Lab Med 122:545–547
12. Meis JM, Butler JJ, Osborne BM, Manning JT (1986) Granulocytic sarcoma in nonleukaemic patients. Cancer 58:2697–2709
13. Middleton G (1966) Involvement of the thymus by metastatic neoplasms. Br J Cancer 20:41–46
14. Neiman RS, Barcos M, Berard C, Mann R, Rydell RE, Benett JM (1981) Granulocytic sarcoma: A clinicopathologic study of 61 biopsied cases. Cancer 48:1426–1437
15. Orazi A, Neiman RS, Ulbright TM, Heerema NA, John K, Nichols OR (1993) Hematopoietic precursor cells within the yolk sac tumor component are the source of secondary hematopoietic malignancies in patients with mediastinal germ cell tumours. Cancer 71:3873–3881