Myeloid sarcoma or lymphoblastic lymphoma? A closer look at the laboratory diagnosis

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Although a myeloid sarcoma is a rare form of extramedullary leukaemia, its early diagnosis has been proven to be of utmost importance. Its presence is strongly related to the onset or the presence of systemic bone marrow leukaemia. However, the diagnosis of myeloid sarcoma is not straightforward. In the existing literature, approximately half of the cases of myeloid sarcoma were initially misdiagnosed as lymphoma. The current case reports details on the laboratory diagnosis of myeloid sarcoma in a 25-year old male. The laboratory presentation of myeloid sarcoma and the consecutive steps in order to correctly diagnose myeloid sarcoma using a variety of laboratory techniques including microscopy, flow cytometry and cytogenetics are highlighted.
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Introduction

A myeloid sarcoma (MS) has been defined by the World Health Organization (WHO) 2008 as a tumour mass consisting of myeloid blasts (granulocytes, monocytes or both) with or without maturation occurring at any anatomical site other than the bone marrow.[1] It is a rare condition which can occur in three clinical settings. Firstly, in non-leukaemic patients often preceding the onset of acute myeloid leukaemia (AML).[2] Secondly, associated with acute blast transformation of myelodysplastic syndrome (MDS) or chronic myeloid leukaemia (CML) and thirdly, in association with known AML.[3,4] Its diagnosis and treatment should be considered equivalent to that of AML and thus prognosis benefits from early detection. However, myeloid sarcomas are often misdiagnosed as malignant lymphomas. Literature reports up to 50% of misdiagnosed cases, especially in those patients presenting with primary MS.[5,6] The current case study reports a 25 year-old male presenting with a mediastinal mass, suggestive for lymphoblastic lymphoma, but due to adequate laboratory screening diagnosed as MS with bone marrow involvement.

Clinical presentation

A 25-year old male student had been complaining of a persistent cough for several months despite antibiotic treatment and was referred to the hospital by his doctor for a CT scan of the thorax. Upon arrival, the patient had an overall healthy appearance and did not report any serious medical history except a minor weight loss of about one kilogram, which was considered negligible.

Laboratory diagnostics

Full blood analysis was performed which did not reveal any abnormal parameters, including a perfectly normal blood cell count. The CT scan showed a large mediastinal mass which was further explored by PET scan and mediastinoscopy. A biopsy was taken. The age of the patient combined with his clinical presentation predicted the presence of a lymphoma. The screening procedure of the

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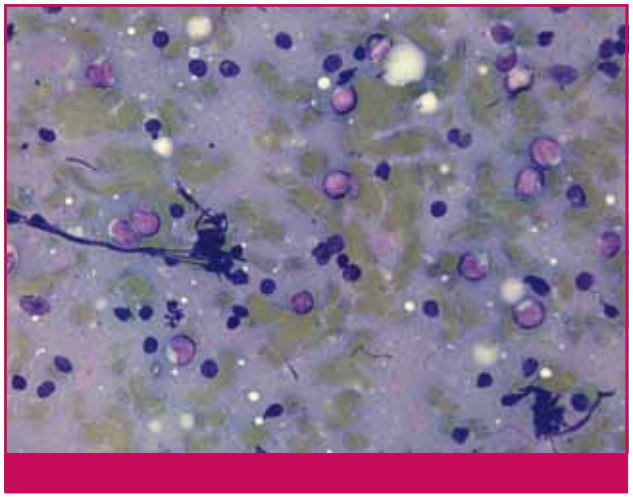

Figure 1A. Morphological analysis of the mediastinal mass showing normal lymphocytes and large blast-like cells.

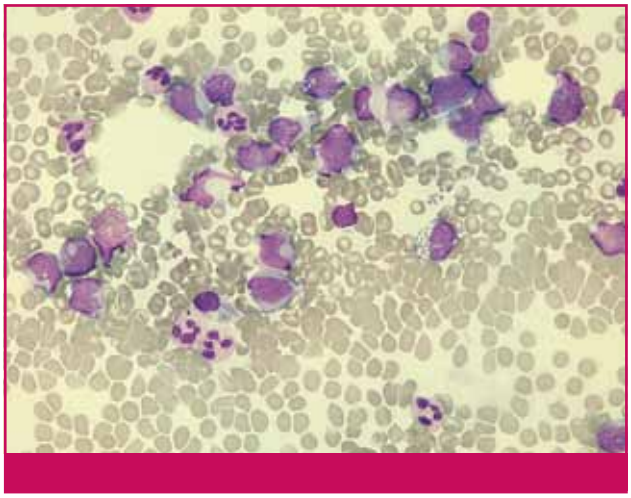

Figure 1B. Morphological analysis of the bone marrow with monoblasts and promonocytes.

Table 1. Screening and diagnostic panel for flow cytometric examination of extramedullar masses with the three chosen diagnostic tubes indicated

Screening panel					
	FITC	**PE**	**ECD**	**PE-Cy5**	**PE-Cy7**
T	CD4	CD8	CD45	CD3	CD5
B	kappa	lambda	CD10	CD19	CD20
Plasma cell	CD19	CD56	CD45	CD138	CD38
Diagnostic panel					
tube 1	CD13	CD56	CD34	CD7	CD45
tube 2	CD235a	CD11c	CD34	CD117	CD45
tube 3	CD16	CD36	CD34	CD14	CD45
tube 4	CD61	CD41	CD34	CD42	CD45
tube 5	CD45	CD34	HLA-DR	CD19	CD2
tube 6	MPO	CD34	/	CD19	CD45
tube 7	/	CD34	cytCD3	CD19	CD45
tube 8	TdT	CD34	/	CD19	CD45
tube 9	/	IgM	CD34	CD19	CD45
tube 10	/	CD79a	CD34	CD19	CD45

mass biopsy was two-fold. A thorough morphological screening was performed combined with a full characterisation of the white blood cells using flow cytometry. The morphological examination indicated the presence of two cell types; normal adult lymphocytes and large blast-like cells with little cytoplasm and no obvious granulation (*Figure 1a*), in accordance with the initial presumption of a lymphoblastic lymphoma. Next, the

Table 2. Screening and diagnostic panel for flow cytometric examination of bone marrow

	FITC	PE	ECD	PE-Cy5	PE-Cy7
Screening panel					
tube 1	CD71	CD33	CD34	CD15	CD45
tube 2	CD3	CD16+CD56	CD45	CD14	CD38
tube 3	CD4	CD8	CD45	CD3	CD5
tube 4	kappa	lambda	CD10	CD19	CD20
Diagnostic panel					
tube 1	CD13	CD56	CD34	CD7	CD45
tube 2	CD235a	CD11c	CD34	CD117	CD45
tube 3	CD16	CD36	CD34	CD14	CD45
tube 4	CD61	CD41	CD34	CD42	CD45
tube 5	CD45	CD34	HLA-DR	CD19	CD2
tube 6	/	MPO	CD34	CD19	CD45
tube 7	/	CD34	Cyt CD3	CD19	CD45
tube 8	TDT	CD34	/	CD19	CD45
tube 9	/	IgM	CD34	CD19	CD45
tube 10	/	CD79a	CD34	CD19	CD45
tube 11	CD19	CD34	CD45	CD20	CD10

mass biopsy was characterised using flow cytometry. Three cell populations were distinguished, including lymphocytes, granulocytes and a large abnormal population. A full flow cytometric analysis consists of a two-step procedure including a set of initial screening tubes which aim to separate the normal cell populations and detect any deviation from the normal pattern, followed by a larger set of diagnostic tubes, referred to as the diagnostic panel, to characterise the abnormal population in detail. The entire protocol for flow cytometric analysis of extra medullary masses is depicted in *Table 1*. According to the initial screening, the abnormal population of the mediastinal mass showed a CD45weak+, CD19partial+, CD38+, CD20- and CD10- phenotype. The full diagnostic panel includes a wide variety of markers (*Table 1*). However, due to the limited amount of biopsy material sent to the laboratory, only three diagnostic tubes could be run, which were chosen based on the data gathered so far. The phenotype was completed with the following markers: CD34partial+, HLA-DR+, TdT- and CD79a-. The final phenotype of the mediastinal mass did not disagree with the initial presumption of B-lymphoblastic lymphoma but some essential markers such as CD79a, CD10 and TdT were missing. Nevertheless, the positivity for CD19, combined with the morphological examination and the clinical presentation of the patient are suggestive for the presence of a B-lymphoblastic lymphoma. A bone marrow biopsy was taken and an identical laboratory approach was applied, covering a morphological examination and a flow cytometric analysis (*Table 2*) and cytogenetic analysis. Surprisingly, the morphological screening of the bone marrow did not confirm the presence of lymphoblasts as expected, but revealed numerous monoblasts and promonocytes (*Figure 1b*). Note that the percentage of monoblasts and promonocytes in the bone marrow did not exceed 20%, failing the demand to diagnose AML. Moreover, the initial flow cytometric screening showed

Key messages for clinical practice

- Morphological examination needs to be completed with flow cytometric data, especially to identify rare haematological malignancies such as myeloid sarcoma.
- The full flow cytometric screening should include markers to exclude myeloid malignancies such as myeloid sarcoma, either during screening or in the diagnostic panel.
- Be aware of cross-lineage markers such as CD19 which should always be interpreted with caution and should be considered combined with other flow cytometric data.

an abnormal population in the bone marrow with following phenotype: CD45weak+, CD34 partial+, CD19partial+ CD38+, in agreement with the phenotype of the mass biopsy, but also entirely positive for CD33, a myeloid marker. The excess of monoblasts and promonocytes combined with CD33 positivity overruled the initial presumption of B-lymphoblastic lymphoma. The full diagnostic panel led to the final immunophenotype of the abnormal population with CD45weak+, CD34partial+, CD19partial+, CD38+, CD20-, CD10- CD33+, HLA-DR+, TDT- CD79a-, CD11c+, CD13+ and MPO+, in full agreement with AML of the monocytic/monoblastic lineage according to the WHO 2008.

Genetic profiling included the analysis of thirteen metaphases. One cytogenetically abnormal clone was observed with multiple numerical and structural abnormalities. All metaphases showed derivative chromosomes two, five, ten and sixteen, a partial terminal deletion of the long arm of chromosome eleven and a marker chromosome. Numerical aberrations included monosomy of chromosomes three and fourteen. Sex chromosomes appeared normal. According to the International System for Human Cytogenetic Nomenclature (ISCN, 2009), the formula of the karyotype was 45,XY,der(2),-3,der(5),der(10),del(11)(q?),-14,der(16),+mar[13].

Due to the percentage of bone marrow blasts being <20% and the persistent complaint of a cough for months, it was considered highly unlikely that the bone marrow was the origin of the detected malignancy. Finally, this patient could be correctly diagnosed with an MS which showed significant staging in the bone marrow. The patient successfully completed induction and consolidation chemotherapy according to the EORTC AML12-standard protocol (etoposide, daunorubicin and cytarabine). Complete remission was obtained with normalisation of the karyotype. The patient recently underwent allogenic stem cell transplantation with his HLA-identical sister after full conditioning with total body irradiation and high dose cyclophosphamide.

Conclusion

In laboratory diagnostics the aim is to report a correct diagnosis, preferably as soon as possible. As techniques advance rapidly, several approaches are currently at the disposal of the laboratory workers. Nevertheless, the diagnosis of some haematological malignancies is not straightforward with overlapping phenotypes and aberrant expression of CD markers across cell lineages. Moreover, the rarity of some malignancies often contributes to their misdiagnosis, as is the case of MS. This case report highlights the importance of combining morphological examination with an extended flow cytometric screening. A full flow cytometric characterisation should include an initial screening which gives an overview of the normal blood cell populations and indicates any deviation from normality. In order to detect MS using flow cytometry of extramedullary masses, it is essential to either include a myeloid marker in the initial screening tubes or to include myeloid markers in the diagnostic panels.

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