CASE REPORT

Utilisation of CD99 Immunohistochemistry in the Diagnosis of CD34/CD117 Negative Acute Myeloid Leukaemia

Intan Noorliza Mohd Sopani[1,2], Sabariah Md Noor[1], Syirah Nazirah Mohd Tajuddin[2], Asmawiza Awang[3]

[1] Department of Pathology, Faculty of Medicine and Health Sciences, Universiti Putra Malaysia, 43400 Serdang, Selangor, Malaysia.
[2] Department of Pathology, Hospital Tuanku Ja'afar, Ministry of Health Malaysia, Jalan Rasah, Bukit Rasah, 70300 Seremban, Negeri Sembilan, Malaysia.
[3] Department of Pathology, Hospital Kuala Lumpur, Jalan Pahang, 50586 Kuala Lumpur, Malaysia.

ABSTRACT

Diagnostic and prognostic markers of acute myeloid leukaemia (AML) have already been well established. Latest advancements in molecular and cytogenetics play a substantial role in diagnosing and classifying AML according to the recent WHO classification 2022. However, due to some limitations, we still rely on immunophenotypic markers as the first-line method to diagnose AML as it is readily accessible in most centres and can provide an earlier diagnosis compared to molecular/cytogenetic analysis, which takes a longer time and requires transport logistics to a reference lab. We report an extraordinary case of CD34 and CD117 negative AML from a trephine biopsy and the role of CD99 in aiding the diagnosis. His diagnosis of AML is a diagnosis of exclusion after extensive immunohistochemistry was done to rule out other haemopoietic neoplasm and bone marrow infiltration by non-haemopoietic cells, as well as correlation with biochemical parameters, tumour markers, and radiological findings.

Malaysian Journal of Medicine and Health Sciences (2025) 21(SUPP12):118-121. doi:10.47836/mjmhs.21.s12.21

Keywords: Acute myeloid leukaemia, CD99, Immunohistochemistry, CD34, CD117

Corresponding Author:
Intan Noorliza Mohd Sopani, MB BCh BAO
Email: intannoorliza@gmail.com
Tel:+6011-15723676

INTRODUCTION

AML results from clonal expansion of the leukaemic stem cell (LSC) population which leads to the accumulation of leukaemic myeloid blasts in the peripheral blood, bone marrow or other tissues. Abnormal blood counts including leukocytosis and/or cytopenia, along with clinical symptoms such as fever, fatigue and easy bruising give a high suspicion of acute leukaemia. Full blood picture, bone marrow and trephine biopsy shall be performed for morphological assessment as well as further diagnostic tests including cytochemistry, immunohistochemistry, flow cytometry, molecular and cytogenetic analysis. The diagnosis of AML is confirmed by the presence of AML-defining genetic abnormalities according to the 5th edition of WHO Classification of Haematolymphoid Tumours or the presence of >20% myeloid blasts in the bone marrow. Myeloid blast is determined by the expression of myeloid lineage-specific markers, for example, MPO, CD117 as well as the demonstration of monocytic differentiation by the expression of CD64, CD36, CD14 or CD11c. Here, we present a rare case of CD34 and CD117 negative AML and further discuss CD99 expression in AML and its prognostic value.

CASE REPORT

A 60-year-old Chinese gentleman with no known medical illness, he first presented to the Emergency Department in February 2024 with a pre-syncopal attack and a one-week history of lethargy, feverish, giddiness, palpitation, reduced effort tolerance, loss of appetite and loss of weight roughly 20kg in two years duration. Otherwise, he denied any bleeding tendency, no history of recurrent infections or recent hospital admissions. On physical examination, he is cachexic-looking with

pallor and tachycardia. His blood pressure was normal with good perfusion and he was comfortable under room air. There was hepatosplenomegaly, however, no lymphadenopathy was noted. Rectal examination revealed prostatomegaly.

Initial full blood count at presentation showed moderate anaemia with haemoglobin 7.3 g/dL. His total white blood cells, including differential white cell counts and platelets, were within the normal limit. His renal profile and liver function test were normal. C-reactive protein (CRP) was markedly elevated >200mg/L. The patient came in with symptomatic anaemia associated with constitutional symptoms, hepatosplenomegaly and prostatomegaly. The initial differential diagnosis was to rule out underlying malignancy; however, all tumour markers, including CEA and PSA were normal.

CT scan of the thorax, abdomen and pelvis revealed hepatosplenomegaly and bony disruption of the left pubic bone, suspicious of bone metastasis/ haematological malignancy, i.e., multiple myeloma. Further tests for serum free light chain, urine, and serum protein electrophoresis were inconclusive. There was no evidence of renal impairment, hypercalcaemia or reverse albumin: globulin ratio to suggest multiple myeloma.

Full blood picture (Fig. 1) showed severe anaemia with poor reticulocyte response, dimorphic picture, no rouleaux formation or nucleated RBC seen. Lymphopenia and eosinophilia, with the presence of 1-2% suspicious large abnormal mononuclear cells exhibiting moderate amount of deeply basophilic cytoplasm, irregular nuclear outline, slightly coarse chromatin with eccentric nucleus and inconspicuous nucleoli. No Auer rod was seen.

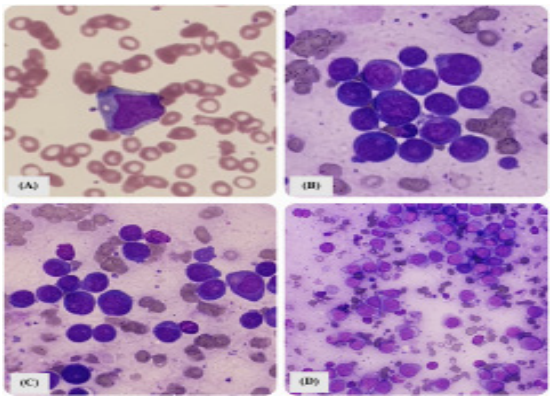

Fig. 1: Microscopic picture of (A) Peripheral blood smear (Wright stain, x 400); (B-C) Bone marrow aspirate (May-Grunwald-Giemsa stain, x 400); (D) Trephine imprint (May-Grunwald-Giemsa stain, x 400).

Bone marrow aspiration (BMA) and trephine biopsy were performed to rule out haematological malignancy or bone metastasis. BMA (Fig. 1) findings unfortunately showed a very limited sample with no marrow fragments available for assessment. However, there was presence of many abnormal cells (>60%) which are moderate to large in size, exhibit moderate amount of deeply basophilic cytoplasm and some with eccentric nuclei. Other haemopoietic cell lines (erythroid, granulocytes and megakaryocytes) were present but appear relatively reduced. Trephine rolls showed similar findings as bone marrow aspirate. Because of poor BMA sampling obtained, no further samples were sent for immunophenotyping, molecular and cytogenetics.

Trephine biopsy (Fig. 2 and Fig. 3) showed hypercellular marrow for age (100% cellularity) with diffuse infiltration by intermediate to large-sized cells (about 80% of all nucleated cells). These cells have round and moderately pleomorphic nuclei, dispersed chromatin, scanty to moderate amount of cytoplasm and some showed prominent nucleoli. Screening immunohistochemistry panel showed negative for immature markers (CD34, CD117, TdT), lymphoid markers (CD20, CD3) and plasma cell marker (CD138).

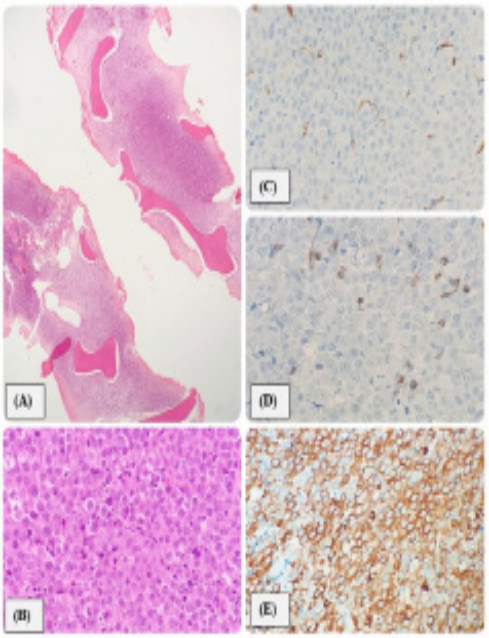

Fig. 2: Microscopic picture of trephine biopsy and immunohistochemistry staining (A) H&E x40; (B) H&E x 400; (C) CD34 x400; (D) CD117 x 400; (E) MPO x 400.

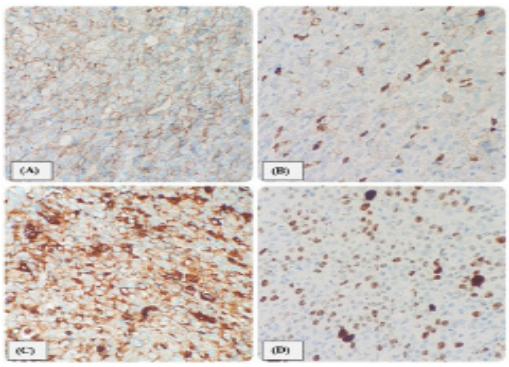

Fig.3: Microscopic picture of trephine biopsy and immunohistochemistry staining (A) CD99 x 400; (B) CD45 x 400; (C) CD68 x 400; (D) Ki67 x 400.

Extensive immunohistochemistry was subsequently performed to rule out other non-hematopoietic bone marrow infiltration, however was negative. In summary, these malignant cells were positive for CD99, MPO, CD68, CD4 and Vimentin with a weak heterogenous positivity for CD45. They frequently expressed Ki67. They were negative for lysozyme, CD34, CD117, CD163, CD61, Glycophorin-A, CD20, CD3, CD79a, PAX5, MUM-1, VS38c, CD138, TdT, CD30, ALK, EMA, Pan-CK, EMA, S-100, CK 8-18, CKCAM 5.2, P63, PAX8 and EP-CAM. Immunohistochemistry for CD13 and CD33 is not offered at our centre, therefore it was not performed. Granulopoiesis was observed in one marrow space, scattered megakaryocytes were noted, while erythroid lineage was nearly absent. Based on the positive immunohistochemistry findings, the trephine biopsy was concluded as AML. An outline of the algorithmic immunohistochemical approach for this case is illustrated in Fig. 4.

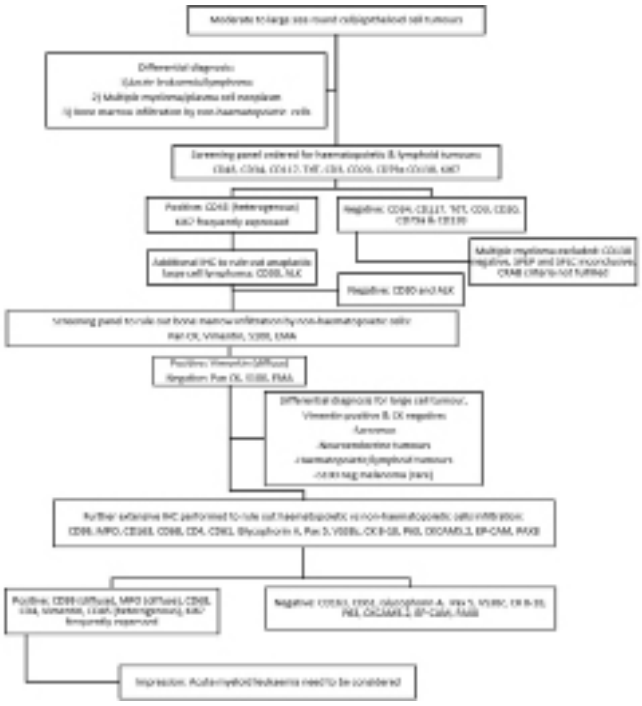

Fig. 4: Algorithmic immunohistochemical approach for large round cell/epithelioid cell morphology.

The diagnosis was delayed due to the complexity of the case. AML treatment was not commenced as the patient and his family members were keen on At-Own-Risk (AOR) discharge before the trephine report was validated. The clinical diagnosis at that time was non-haematopoietic malignancy Stage 4, and the patient was referred for palliative care prior to AOR discharge.

DISCUSSION

This case presents a diagnostic challenge for acute leukaemia as all markers of immaturity for both myeloid and lymphoid progenitors were negative. The blast morphology was not typical of AML therefore our differential diagnosis apart from acute leukaemia/lymphoma includes bone marrow infiltration by non-haematopoietic cells as well as multiple myeloma/plasma cell neoplasm. However, there was insufficient supporting evidence from immunohistochemistry, biochemical parameters and tumour markers to suggest metastatic bone marrow infiltration, and the origin of the primary cancer was not evident through radiological and clinical means. The patient also did not fulfil CRAB criteria and had inconclusive biochemical parameters including serum protein electrophoresis and serum free light chain, excluding the diagnosis of multiple myeloma/plasma cell neoplasm.

In routine practice, the diagnosis of AML should be supported by additional tests including immunophenotyping, molecular and cytogenetic analysis which were not available in this case due to inadequate marrow sampling. Using immunohistochemistry alone as a diagnostic tool has its limitations as it is difficult to simultaneously analyse multiple markers on a single tissue section, and there is a risk of false-positive and false-negative results owing to the quality of antibodies and staining. Conversely, immunophenotyping offers several advantages over immunohistochemistry, as it is more sensitive in detecting weakly expressed surface antigens and multiple antigens simultaneously. Nevertheless, cell loss is common during washing steps in the immunophenotyping procedure, therefore the presence of 1-2% blasts in the peripheral blood is not suitable for evaluation in this case, as it will not provide definitive results.

Due to these constraints, we depend solely on immunohistochemistry staining, whereby positive expression of CD99, MPO, CD68, CD4 and heterogenous CD45, along with the diffuse pattern of abnormal cell infiltrates, strongly indicated AML. While CD34 and CD117 are commonly used markers for AML, there is a subset of AML cases that are negative for both markers, for example, acute erythroid leukaemia and acute megakaryoblastic leukaemia. However, these are unlikely in this case due to a significantly reduced erythroid population, no increase in megakaryocytes and the presence of positive MPO expression. Negative expression of CD34 and CD117 in AML is rare, and the inability to prove genetic abnormalities by molecular and cytogenetic analysis further complicates the diagnosis.

In normal tissues, CD99 is mostly recognised for its expression in T lymphocytes. However, it is also identified as a tumour-associated antigen frequently overexpressed in haematological malignancies including AML, representing approximately 80% of cases reported in several population-based studies (1, 2, 3). In terms of the prognostic value, CD99 overexpression was positively linked to FLT3-ITD mutated AML while a negative correlation was observed with TP53 mutations;

additionally, patients with upregulated CD99 expression were associated with improved outcomes and longer overall survival (4). Another study by Zhang et al. (2000), also reported that the majority of relapsed AML cases were CD99 negative, with shorter median overall survival, thus proving that CD99 expression is a good prognostic factor in AML (5).

The latest technology on immunotherapies showed that CD99 has a good potential for targeted therapeutic strategy as it is often overexpressed on AML blasts and leukaemic stem cells, but is low or absent on normal hematopoietic stem cells (4). Anti-CD99 immunotherapy has a direct cytotoxicity effect on leukaemic blast in which there were significant reductions in leukaemic blast number after incubation with CD99 monoclonal antibody, HO36-1.1 among patients with AML and MDS (1).

CONCLUSION

Although CD99 expression is not specific, it has been widely used as a diagnostically useful marker, particularly in Ewing Sarcoma and acute lymphoblastic leukaemia/lymphoma. Our case report presents an insight into CD99 overexpression in AML, highlighting its potential as an additional diagnostic marker, prognostic indicator, and therapeutic target. Further research is required to clarify the mechanisms regulating CD99 expression and to explore its full clinical potential in haematological malignancies.

ACKNOWLEDGEMENT

We would like to thank the Director General of Health, Ministry of Health, Malaysia for permission to publish this article and all the laboratory staffs in Hospital Tuanku Ja'afar and Hospital Kuala Lumpur for their expertise.

REFERENCE

1. Tavakkoli M, Chung SS, Park CY. Do preclinical studies suggest that CD99 is a potential therapeutic target in acute myeloid leukemia and the myelodysplastic syndromes? Expert Opinion on Therapeutic Targets. 2018 Apr 20;22(5):381–3. DOI: 10.1080/14728222.2018.1464140
2. Omran AA, Osh S, Fouad, Elsharkawy AR. Role of CD99 in Adult Patients with Acute Myeloid Leukemia. The Egyptian Journal of Hospital Medicine. 2021 Oct 1;86(1):96–99. DOI: 10.21608/ejhm.2021.209990
3. Chung SS, Eng WS, Hu W, Khalaj M, Garrett-Bakelman FE, Tavakkoli M, et al. CD99 is a therapeutic target on disease stem cells in myeloid malignancies. Science Translational Medicine. 2017 Jan 25;9(374): eaaj2025. DOI: 10.1126/scitranslmed.aaj2025
4. Ali A, Vijaya Pooja Vaikari, Houda Alachkar. CD99 in malignant hematopoiesis. Experimental hematology. 2022 Feb 1;106:40–6. DOI: 10.1016/j.exphem.2021.12.363
5. Zhang PJ, Barcos M, Stewart CC, Block AW, Sait S, Brooks JJ. Immunoreactivity of MIC2 (CD99) in Acute Myelogenous Leukemia and Related Diseases. Modern pathology. 2000 Apr 1;13(4):452–8. DOI: 10.1038/modpathol.3880077