

Isolation of Peripheral Blood Nucleated Red Blood Cells from β -Thalassaemia Patients Using CD71 Magnetic Activated Cell Sorting

Haiyuni Mohd Yassim, Heba Ali, Rosline Hassan, Wan Zaidah Abdullah, Muhammad Farid Johan*

Department of Haematology, School of Medical Sciences, Universiti Sains Malaysia, 16150 Kubang Kerian, Kelantan, Malaysia

The molecular biology knowledge in β -thalassaemia is limited due to the involvement of various erythropoietic processes where the genetic information is lack due to nucleus ejection throughout the maturation of red blood cell activities concurrence with the accumulation of haemoglobin. Nucleated red blood cells (NRBCs) are typically found in peripheral blood (PB) of β -thalassaemia transfusion dependent patients and abundant in post splenectomy (Fig. 1A) [1]. The presence of NRBCs will provide further understanding on the molecular aspect of ineffective erythropoiesis in β -thalassaemia patients. Therefore, the objectives of this study were to isolate the NRBCs using CD71 magnetic beads from PB of β -thalassaemia patients and to compare the quantity of NRBCs enriched between non-splenectomised transfusion dependent and post-splenectomised transfusion dependent β -thalassaemia patients.

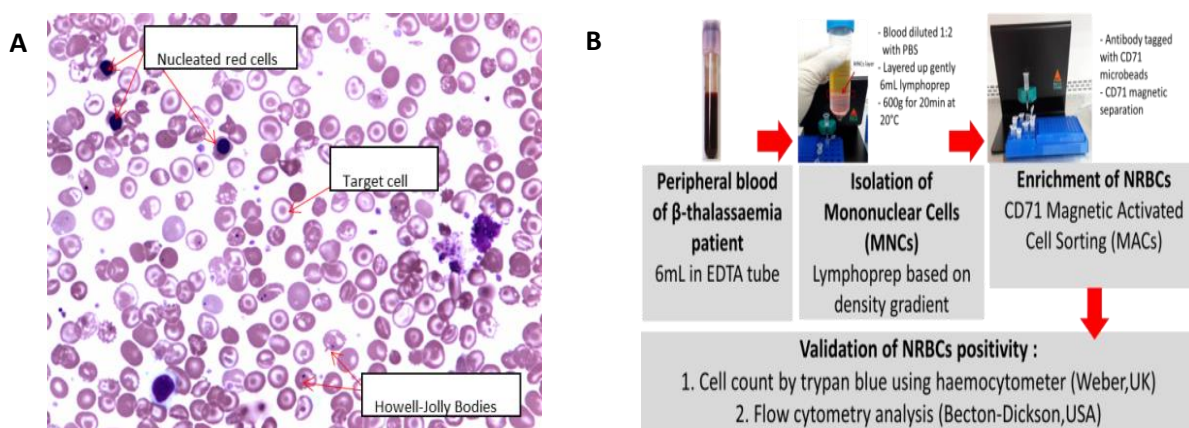


Fig. 1: A. NRBCs, nucleated red blood cell in peripheral blood film of β -thalassaemia patient. B. Magnetic activated cell sorting (MACs) with anti-CD71 for NRBCs enrichment.

NRBCs were isolated from 6mL PB of both groups after the isolation of mononuclear cells (MNCs) based on density gradient and Magnetic activated cell sorting (MACs) with anti-CD71 was used to enrich isolated NRBCs (Figure 1B). Cell count for NRBCs positive for

CD71 was determined by trypan blue using haemocytometer (Weber,UK) and flow cytometry analysis (Becton-Dickson,USA) was performed for method validation. NRBCs were successfully isolated from PB of 15 non-splenectomised transfusion dependent and 7 post-splenectomised β -thalassaemia patients with >90% specificity (Fig. 2). The median number of NRBCs ($\times 10^4$) enriched were 58.5 (283) in non-splenectomised transfusion dependent and 340 (338) in post-splenectomised transfusion β -thalassaemia patients (Table 1). Higher NRBCs were isolated from the post-splenectomised compared with non-splenectomised patients ($P = 0.012$).

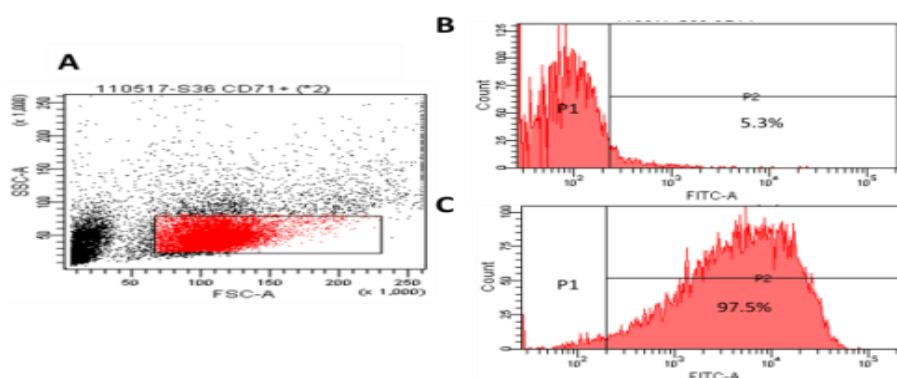


Fig. 2: Flow cytometry analysis of NRBCs positivity in β -thalassaemia patients using Fluorescein isothiocyanate (FITC). A. Gating strategy for NRBCs. B-C. P1 represents CD71- population in the 1st fraction whereas P2 represents CD71+ (NRBCs) after the 2nd fraction of NRBCs enrichment using MACS.

Table 1: Number of NRBCs recovered from peripheral blood of β -thalassaemia patients after MACS.

	Median (IQR)	
	β -thalassaemia	
	Non-splenectomised	Splenectomised
No. of MNCs ($\times 10^7$)	2.14 (2.3)	4.1 (5.3)
Total no. of cells after MACS ($\times 10^4$)	58.5 (283)	340 (338)

MNCs, mononuclear cells; MACS, magnetic activated cell sorting; NRBCs, nucleated red blood cells; IQR, interquartile range

This finding supports the idea of isolating the NRBCs from β -thalassaemia patients by using specific antibodies, CD71. This was consistent with the literature in which CD71 antibodies have been reported as the surface marker of NRBCs in cord blood [2]. It was important to isolate NRBCs from other haematopoietic cells as heterotopic interaction between cells could impact the molecular studies of this erythropoietic cell especially in term of epigenetic activity [3]. Epigenetic mechanisms such as methylation was reported to play role in regulating and maintaining the globin gene expression as the cells proliferate which starts in

the preproerythroblast and achieved high levels of transcription after the proerythroblast stage of development. This finding may provides the basis in understanding β -thalassaemia in term of epigenetics involvement which required a specific cells. In conclusions, MACs method is convenient, simple, and can be efficiently applied in isolating NRBCs from both non-splenectomised and post-splenectomised β -thalassaemia patients.

Keywords: NRBCs, CD71, MACs, β -thalassaemia

* **Correspondence:** faridjohan@usm.my

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