INTRODUCTION

Paroxysmal nocturnal hemoglobinuria (PNH) is a rare acquired hemolytic disorder caused by a stem cell mutation. It affects approximately 1.3 in 100,000 individuals and is characterized by chronic hemolysis, anemia, and thrombosis (1, 2). Thrombosis is the leading cause of death in PNH (3). The genetic basis of PNH are somatic mutations in the X-linked PIG-A gene (4). The mutation causes a partial or absolute deficiency of proteins linked to the cell membrane by a glycoporphathidylinositol (GPI) anchor (5).

The detection of low (or subclinical) PNH cells has potential diagnostic and therapeutic benefits. Low levels of PNH cells have been reported in myelodisplastic syndromes. Examples of this association include aplastic anemia and in some patients with refractory cytopenia with unilineage dysplasia (RCUD) (6-8). It has been shown that there is a better response to immunotherapy of patients when PNH cells are present (6, 9). However, other studies have shown no correlation (10-11).

The aim of this study was to quantify the number of PNH RBC in normal individuals and to determine if this is the case.

MATERIALS & METHODS

RED BLOOD CELL ANALYSIS

Wash 30 µl whole human blood.
Add 3 ml PBS. Centrifuge 5 mins at 300g, RT. Discard supernatant.
Resuspend in 1 ml PBS.
Stain 1 million/10 million RBC: 33/180 BC
1.64/5.6 CD59-PE
1.43/6.1 Glycophorin-A-FITC.
Negative control uses isotypes for both antibodies. Incubate 20 mins at 4°C.
Add 3 ml PBS. Centrifuge 5 mins at 300g, RT. Discard supernatant.
Resuspend 1 million RBC in 330 µl PBS. Resuspend 10 million RBC in 1800 µl PBS.
Acquire events at medium (60,1000 µl/min) flow rate.

WHITE BLOOD CELL ANALYSIS

Count whole blood.
Lyse 1 ml blood in 20 ml NHCl solution, 5 mins
Centrifuge 5 mins at 300g, RT Discard supernatant
Cold 5 ml PBS+0.1% (v/v) BSA. Centrifuge 5 mins at 300g, 4°C. Discard supernatant.
Resuspend in PBS+0.1% (v/v) BSA. 5×105 WBC/50 µl
Stain: 50 µl WBC
16.1 CD15-APC
2.5 µl CD33-PerCP Cy5.5
2.5 µl CD14-APC Cy7
2.5 µl CD24-PE
2.5 µl FLAER (Alexa 488)
Negative control uses only CD15 & CD33. Incubate 25 mins at 4°C.
Add cold 3 ml PBS+0.1% (v/v) BSA. Centrifuge 5 mins at 300g, 4°C. Discard supernatant.
Resuspend in 200 µl 1% (v/v) paraformaldehyde. Acquire 5000 CD15+ events at medium (60,1000 µl/min) flow rate.

ABBREVIATIONS

BSA- bovine serum albumin; FLAER- Fluorochrome (Alexa 488) conjugated non-lyzing pre-erythroid; PBS- phosphate buffered saline; RT- room temperature.

REFERENCES