short report
Haematologica 1997; 82:695-697

DE NOVO SMOLDERING PAROXYSMAL NOCTURNAL HEMOGLOBINURIA: A FLOW CYTOMETRIC DIAGNOSIS

RAFAEL FORÉS, GUIOMAR BAUTISTA, JUAN LUIS STEEGMANN, F. JAVIER PEÑALVER, RAFAEL CABRERA
MANUEL N. FERNÁNDEZ
Department of Hematology, Clínica Puerta de Hierro, Universidad Autónoma de Madrid, Spain

ABSTRACT

An asymptomatic 26-year-old woman with mild macrocytic anemia (11.6 g Hb/dl) was studied. All biochemical parameters, bone marrow histology and cytogenetics were normal. The Ham's and sucrose tests were negative. A flow cytometric analysis revealed that CD55 and CD59 staining was absent in 20% and 21% of the granulocytes, but erythrocytes and CD34-positive bone marrow cells were CD55 and CD59 positive. Seven months after the initial study, the patient suffered an episode of hemoglobinuria, with mild anemia, moderate thrombocytopenia and a weak positive sucrose lysis test. A new flow cytometric analysis disclosed an increased percentage of CD55 and CD59 negative granulocytes and a 25% of erythrocytes with an intermediate pattern of fluorescence after CD59 labelling. At fourteen months, a population of CD55-deficient erythrocytes was detected and the Ham's test became positive. The present report is, to our knowledge, the first case of smoldering paroxysmal nocturnal hemoglobinuria in a patient with no previous aplastic anemia, or evident pancytopenia. The diagnosis was established by flow cytometry of peripheral blood granulocytes, with apparently phenotipically normal progenitor cells in an early stage of the disease. Flow cytometry appears to be a useful tool in our knowledge of paroxysmal nocturnal hemoglobinuria evolution.

Keywords: CD55, CD59, flow cytometry, GPI-linked molecules, paroxysmal nocturnal hemoglobinuria

Recently, Nakakuma *et al.*[1] proposed a classification of the clinical stages of paroxysmal nocturnal hemoglobinuria (PNH) based on flow cytometric analysis (FCA). They define *smoldering PNH* as a stage where the glycosyl-phosphatidylinositol (GPI) anchorage defect may be detected by FCA in bone marrow (BM) cells and peripheral blood leukocytes, but the Ham's test is negative.

This phenomenon has been recognized by us[2] and others[3] in patients with aplastic anemia or pancytopenia, where the GPI defect can be detected in peripheral blood leukocytes without expressing a full picture of PNH. However, smoldering PNH has not been described in patients with no previous bone marrow hypoplasia or evident pancytopenia. We herein present the clinical evolution of a patient with mild macrocytic anemia and mild thrombocytopenia in whom the FCA disclosed a smoldering PNH.

Case Report

A 26-year-old woman was sent to our hospital in February 1996, after an incidental finding of macrocytosis. She was asymptomatic with no abnormal clinical findings in the physical exam. The blood counts disclosed WBC 4.6×10^9/L, with a normal differential, and platelets 114×10^9/L. There was mild anemia with 11.6 g Hb/dL, MCV 106 fL and 1.1% reticulocytes, and the cytological examination was normal. All biochemical parameters, including LDH, ferritin, B12 and folates were normal. The Ham's and sucrose tests were negative. Bone marrow histology and cytogenetics were normal. The clinical and analytical data are shown in Table 1.

FCA was performed on peripheral blood granulocytes and erythrocytes. CD55 (clone NaM16-4D3, Bioatlantic®) and CD59 (clone NaM77-1E5, Bioatlantic®) staining was absent in 20% and 21% of the granulocytes. FCA of the erythrocytes was normal. Bone marrow FCA demostrated a defect of GPI-linked proteins in mature myeloid cells (defined by their light scatter properties), being more prominent on CD55 typing although lacking a clear separation between positive and negative populations. Double labelling CD55-FITC/CD34-PE (CD34: clone 8G12, Becton Dickinson®) and CD59-FITC/CD34-PE was accomplished in BM cells. An acquisition gate was placed in the CD34-

Correspondence: Dr. Rafael Forés Cachón, Servicio de Hematología, Clínica Puerta de Hierro, C/San Martin de Porres 4, 28035 Madrid, Spain. Fax. international +34.1.3730535.
Acknowledgements: the authors wish to thank M. Carmen Lago for technical assistance.
Received May 19, 1997; accepted September 22, 1997.

positive, low SSC, and the fluorescence intensity was analyzed (1,200 events were recorded), with the result of all CD34-positive cells being positive for both CD55 and CD59.

The patient was treated with folic acid, and one month later the hemoglobin rose to 12.2 g/dL. Seven months after the initial study, the patient returned complaining of dark urine after an astragalous fracture and hemoglobinuria was detected. Laboratory evaluation is shown in Table 1. The main abnormalities were mild anemia, moderate thrombocytopenia and a weak positive sucrose lysis test. The FCA of peripheral blood revealed an increased percentage of GPI-deficient granulocytes and BM myeloid cells. A minor (25%) population of erythrocytes showed an intermediate pattern of fluorescence after CD59 labelling (Figure 1).

Fourteen months after the initial study, a new FCA was performed which revealed a PNH population of erythrocytes in CD55 labelling in addition to

Table 1 Clinical and analytical course of the patient.

	At diagnosis	*7 months*	*14 months*
Clinical findings	None	Hemoglobinuria	None
PB leukocytes (x 10^9/L)	4.6	4.3	3.6
Platelets (x 10^9/L)	114	89	81
Ham's test	Negative	Negative	Positive
Sucrose lysis test	Negative	Positive	Positive
% CD55-ve granulocytes	20	33	41
% CD59-ve granulocytes	21*	33*	39*
% CD55-ve erythrocytes	0	0	26**
% CD59-ve erythrocytes	0	21**	37**
% CD55-ve BM myeloid cells	49**	65*	—
% CD59-ve BM myeloid cells	12*	39*	—
% CD34+ve/CD55-ve	0	—	—
% CD34+ve/CD59-ve	0	—	—

** trimodal histogram. ** population with intermediate pattern of fluorescence*

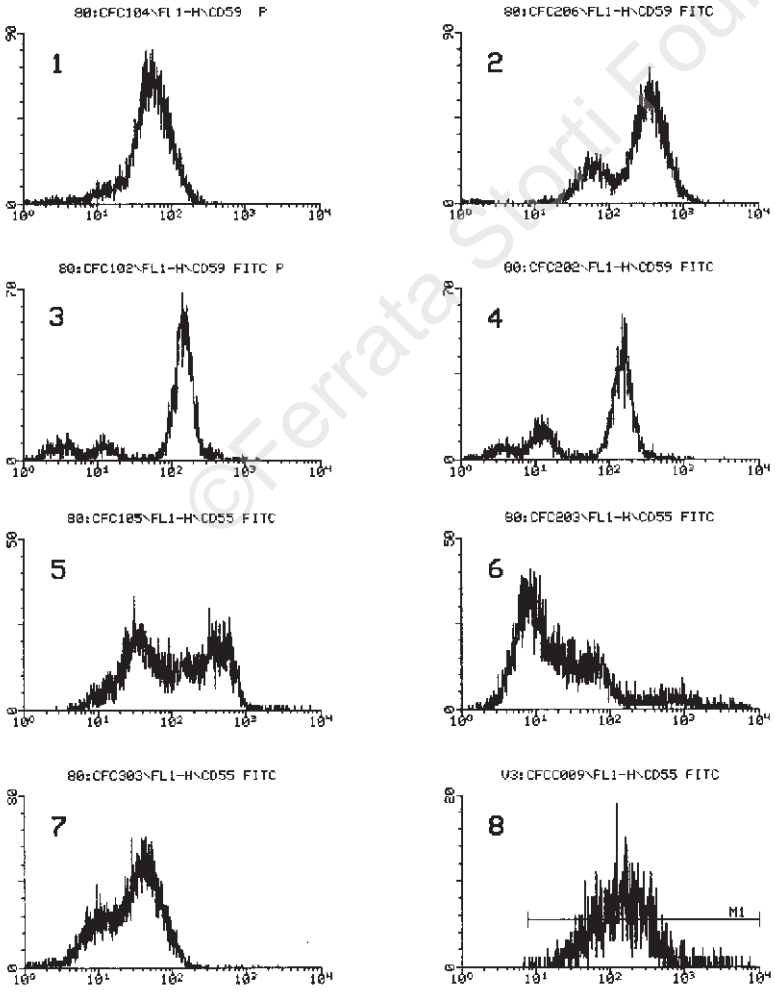

Figure 1. Representative flow cytometric histograms from the patient, at diagnosis (1,3,5) and seven months later (2,4,6). CD59 on erythrocytes (1,2) and granulocytes (3,4). CD55 on BM myeloid cells (5,6) and $CD34^+$ve cells (8)(the marker M1 indicates the population $CD34^+$ve/$CD55^+$ve). Histogram #7 shows CD55 on erythrocytes after diagnosis, with an incipient PNH population.

previous defects. At this time the Ham's test became positive.

Discussion

The present report shows that FCA can detect smoldering PNH in patients without previous aplastic anemia. In our patient, CD55 and CD59 analysis of peripheral blood granulocytes revealed the GPI anchorage defect, but this was not detected in CD34-positive cells.

Our findings are not in contradiction with those of Nakakuma *et al.*,[3] and the differences can be explained by methodological differences. This author analyzed bone marrow mononuclear cells after a lymphocyte exclusion procedure; we also performed the study on CD34-positive cells because of some authors[4] reported a downregulation of GPI linked proteins during normal hematopoietic development that could complicate the analysis. However, it is known that it is difficult to collect a sufficient number of CD34-positive cells in diseases like PNH. It can be argued that in some patients with smoldering PNH the molecular defect is present in progenitor cells, it cannot be revealed by FCA in this population, whereas it is easier to detect in peripheral blood granulocytes, as in our patient.

The presence of affected granulocytes and BM cells preceded the cytofluorometric detection of PNH erythrocytes, that appear insidiously in peripheral blood and accumulate in quantities sufficient to be detected by Ham's test.

In our opinion, the classification proposed by Nakakuma *et al.*[1] appears to be a useful tool in the knowledge of PNH evolution, as a result of the progressive expansion of an abnormal clone. Our understanding of the evolution of PNH may be increased by fluorescent cell analysis of peripheral blood at early stages of PNH, such as the present case seems to represent. Further studies are warranted to assess the true spectrum of this entity, as recently reviewed in this Journal,[5] and to explain puzzling manifestations such as the thrombotic ones.[6]

References

1. Nakakuma H, Kawaguchi T, Horikawa K, et al. Proposal for a clinical classification of the clinical stages of paroxysmal nocturnal haemoglobinuria. Blood 1995; 86:2051-2.
2. Forés R, Alcocer M, Díez-Martin JL, Fernández MN. Flow cytometric analysis of decay-accelerating factor (CD55) on neutrophils from aplastic anaemia patients. Br J Haematol 1995; 90:728-30.
3. Nakakuma H, Nakagura S, Iwamoto N, et al. Paroxysmal nocturnal hemoglobinuria clone in bone marrow of patients with pancytopenia. Blood 1995; 85:1371-6.
4. Terstappen LWMM, Nguyen M, Huang S, Lazarus HM, Medof ME. Defective and normal haematopoietic stem cells in paroxysmal nocturnal haemoglobinuria. Br J Haematol 1993; 84:504-14.
5. Rotoli B, Boccuni P. The PIG-A gene somatic mutation responsible for paroxysmal nocturnal hemoglobinuria. Haematologica 1995; 80:539-45.
6. Nafa K, Bessler M, Mason P, et al. Factor V Leiden mutation investigated by amplification created restriction enzyme site (ACRES) in PNH patients with and without thrombosis. Haematologica 1996; 81:540-2.