A Paroxysmal Nocturnal Haemoglobinuria Progress with Waldenström Macroglobulinemia Along with T Cell Monoclonal Expansion

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Received: 8 November 2013 / Accepted: 13 January 2014 / Published online: 24 January 2014
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Abstract Paroxysmal nocturnal hemoglobinuria (PNH) is an acquired hematopoietic stem cell clinical disease, which has been reported associated with T cell monoclonal expansion and plasma cell dyscrasias. There we reported a case with a 20-year clinical history of PNH. Lately diagnosis of Waldenström macroglobulinemia with the offered evidences of bone marrow examination, flow cytometry and immunofixation electrophoresis. T cell monoclonal expansion was established by polymerase chain reaction. Meanwhile the decreased expression of CD55 and CD59 on neutrophils and erythrocyte were obvious observed. Here we describe the diagnostic evaluation of this patient and provide a brief review of such clonal disorder.

Keywords Paroxysmal nocturnal hemoglobinuria · Waldenström macroglobulinemia · Monoclonal T cell proliferation

Paroxysmal nocturnal hemoglobinuria (PNH) is an acquired disease with the phosphatidylinositol glycan complementation group A (PIG-A) gene mutation of hematopoietic stem cell which result in complete or partial deficiency of several glycosylphosphatidylinositol (GPI)-linked proteins. Three cardinal features can be seen among these patients: intravascular hemolysis, thrombophilia and variable degrees of bone marrow failure [1–3].

Due to a very rare disease, large series information of PNH natural history is few. However, the estimated median survival may be over 10 years, with a quarter of patients surviving longer than 25 years. The major causes of death are thrombosis, infection and hemorrhage. Progression to malignant diseases has been occasionally reported. It has been estimated in the range of 2–3 % [1]. Reviewing literature, malignant transformation is mainly acute myeloid leukemia [4, 5]. Terminating B cell lymphoma even more seldom seen in literature [6]. Here, we report a PNH case terminating with Waldenström macroglobulinemia (WM) and T cell monoclonal expansion and review the literature.

Case Report

A 56-year-old man with a nearly 20-year medical history of PNH, presented initially symptoms fatigue, dizziness and typical dark urine. Intermittent glucocorticosteroid and stanozolol therapy was somewhat effective in his own words. 4 years before admission PNH was reconfirmed by flow cytometry with CD55 and CD59 deficient expression both on erythrocytes and granulocytes. The progression of fatigue dizziness and moderate fever brought him to our hospital lately. Physical examination on admission showed paled man with Cushing sign. His temperature was 38.4 °C. No lymphadenopathy was noted and finding on lung and cardiac examination were unremarkable except for tachycardia. Spleen was palpable 4 cm below the left cortical region. No liver enlargement was found. There were several palpable soft tissue masses scattered on the body. The masses were soft with slightly warm, tenderness and the size varied from 1 to 4 cm diameter. There was trace edema of the legs.

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Fig. 1 The CD55 and CD59 deficiency on granulocytes and erythrocytes

Laboratory studies yielded the following results: hemoglobin 29 g/L, MCV 88.9 fl, reticulocytes $0.0023 \times 10^{12}/L$, leukocyte count $1.55 \times 10^9/L$, with 29.7 % lymphocytes and 65.8 % of neutrophils, and platelet count $55 \times 10^9/L$. CD55 and CD59 deficient on erythrocytes were 2.8 and 2.9 %, corresponding on granulocytes were 50.6 and 50.3 % (Fig. 1). The indirect and direct Coombs test were both negative. Folate acid and Vitamin B₁₂ were both within the normal range. Lactate dehydrogenase (LDH) was 413 IU/L (References 110–220 IU/L). Serum protein electrophoresis showed monoclonal protein (M protein, Fig. 2) 22.2 %, and elevated γ -globulin proportion 27.3 %. Quantitation of immunoglobulin levels showed both IgG and IgA were decreased markedly (6.85 g/L and 260 mg/L), whereas IgM was 12,400 mg/L. Immunofixation electrophoresis showed a monoclonal IgM with the kappa light chain restriction (Fig. 3). Bone marrow smear showed markedly hypercellularity with myeloid to erythroid ratio 0.7:1, lymphoplasmacytic lymphocytes were about 44.5 % (Fig. 4). Bone marrow flow cytometry analysis gating on lymphocyte area showed B cells were about 58 % with expression of CD19, CD20, CD38, CD22 and surface kappa light chain restriction. There were no CD5, CD10 and CD103 detected on B cells (Fig. 5). The bone marrow sample γ gene V-J rearrangements amplified by PCR, using the TCR gene

Fig. 2 The monoclonal protein (M protein) caught by Serum protein electrophoresis

arrangement primers of BIOMED-2, picked up monoclonal stripe (Fig. 6) [7]. The peripheral blood sample for PIG-A gene mutation detected deletion mutation on exon 5 of chromosome X (Fig. 7). Blood and soft tissue mass drainage culture were both positive for cryptococcus, whereas the cerebrospinal fluid (CSF) was negative with cryptococcus by India-ink capsule staining. Routine test of CSF showed $10 \times 10^6/L$ nucleated cells, and biochemical tests showed glucose 8.44 mmol/L, chloridion 132.7 mmol/L, both

Fig. 3 Immunofixation electrophoresis showed a the monoclonal IgM and kappa light chain restriction stripes

elevated, and normal with microalbumin. Based on clinical and laboratory findings, diagnosis of PNH, Waldenström macroglobulinemia (WM) and sepsis of cryptococcosis were established.

Discussion

The high specificity and sensitivity of flow cytometry made a clearly PNH diagnosis, with a strong backbone of 20 years clinical history, *PIG-A* gene mutation and Coombs-negative hemolytic symptoms [8]. The assemblage of morphologic and immunophenotypic plasma lymphocytes in bone marrow, splenomegaly and the high level of serum monoclonal IgM consist of diagnosis of Waldenström macroglobulinemia (WM) [9]. The T cell monoclonal stripe of PCR slash a distinct mark for the T cell clonal expansion in our patient.

The terminate emergence of bone marrow failure affected most PNH patients [1, 10]. The underlying pathogenesis directly pointed to undefined immune dysregulation [11]. Lucia Gargiulo et al. [12] found highly homologous T-cell receptor beta sequences in most PNH patients which supported the hypothesis that autoreactive T cells selective damage the GPI-negative hematopoiesis. AM Risitano [13] definitely pointed out the large granular lymphocyte-like clonal expansion induced the antigen-

driven lymphocyte attack on hematopoietic progenitors in PNH. The monoclonal T cell expansion of our case might a testimony of the hypothesis that abnormal T-cell repertoire mediated the immune process in PNH.

It is already known that PNH cells have an increased tendency toward leukemogenesis and that the leukemic clone arises from the PNH clone itself. Régis Peffault [5] picked up on 8 Acute Leukemia out of 454 PNH cases with the 10 years cumulative incidence of 3.8 %. However the potential progress to B-cell malignant disease was rarely reported. The John Meletis [6] study about the information on the PNH phenotype in plasma cell dyscrasias (PCDs) found the two of seven patients with confirmed Waldenström macroglobulinemia (WM) can simultaneously present CD55⁺ and CD59⁺ PNH clone, indicating that B-cell malignancies may create an appropriate microenvironment, possibly due to an autoimmune mechanism, for the development and detection of the PNH clone. To our knowledge, there was no report of PNH patients terminating of Waldenström macroglobulinemia. Our case who had a 20 years history of PNH and progressed to Waldenström macroglobulinemia might be example of progression from PNH to B cell malignancy.

Except the only curable method, bone marrow transplantation, the treatment of PNH are pointing to anemia, thrombosis and hemolysis before Eculizumab era, which blocks terminal complement activation at C5². The employment of glucocorticosteroid is the strategy of controlling activated hemolysis, however augmenting the side effect of infection, as to this case of cryptococcal infection. So the usage of glucocorticosteroid should be deliberated.

This PNH and Waldenström macroglobulinemia case, accompanying the T-cell monoclonal expansion was very unusual to date. However the abnormal undefined underlying immune mechanism relationships among them was preonounced, and further research are need to clear the pathogenesis among such complicated clonal cases [14].

Fig. 4 Bone marrow smear

Fig. 5 The restrictively expresses kappa light (blue) of the CD19, CD20, CD38, CD30-positive/CD5, CD10-negative cell group (red). (Color figure online)

Fig. 6 The monoclonal rearrangement stripe of T cell receptor γ gene by PCR

Fig. 7 The deletion mutation of PIG-A gene (The deletion of T basic)

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