DIAGNOSTIC DILEMMAS IN PAROXYSMAL NOCTURNAL HEMOGLOBINURIA

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ABSTRACT

Clinical and hematological parameters in six cases of paroxysmal nocturnal hemoglobinuria (PNH) are presented. The mean delay in diagnosis after onset of symptoms was 3.7 years. Initial diagnoses considered were: (a) hematuria; (b) iron-deficiency; hemolytic; megaloblastic or refractory anemia and (c) myelodysplastic syndrome. Clinical features included: reddish urine (5/6), unexplained abdominal pain (4/6) and pallor (6/6). Laboratory investigations showed anemia (6/6), leucopenia (3/6), thrombocytopenia (3/6), unconjugated hyperbilirubinemia at some stage (6/6), and bone marrow erythroid hyperplasia (6/6). Complications encountered were (a) gall stones needing cholecystectomy, hemosiderosis and proximal tubular acidosis in 1 case, (b) disseminated tuberculosis in 1 case and (c) abortion with congestive cardiac failure in one. PNH may present with atypical features and tests for hemosiderinuria, sucrose lysis test and HAM's test are required to establish the diagnosis.

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Introduction

Paroxysmal nocturnal hemoglobinuria (PNH) is an acquired clonal hematologic disorder characterised by hemoglobinuria, thrombosis, infection and a tendency towards bone marrow aplasia [1]. The central diagnostic feature of PNH is the increased sensitivity of the erythrocytes to complement mediated lysis [2]. Two main types of PNH may be distinguished on clinical grounds - 'classical hemolytic' PNH in which intravascular hemolysis is the main feature, or 'laboratory' PNH where the clone of PNH cells is identified by laboratory tests but where the hemolytic effect is trivial in relation to the general marrow disorder [3].

Material and methods

Six cases of PNH seen by the principal author from 1986 to 1992 are presented. Three cases were seen in Army Hospital Delhi (No.1 to 3), two cases (No.4 & 5) in CH (SC),

Pune, and one case (No.6) at Guy's Hospital London (during study leave). The last case (No.6) was an Asian Indian, and was followed up for 23 years after diagnosis. His clinical profile revealed some of the complications seen in the management of PNH if resources are not a constraint. This case has been included to highlight the long term course and complications of PNH which may be difficult to observe in the Armed Forces patients for administrative reasons.

The clinical profile of the cases is shown in Table 1 and salient hematological parameters in Table 2. All patients had undergone bone marrow aspiration and/or biopsy. Tests for hemolytic anemia i.e. Coomb's test, G6PD levels, osmotic fragility test, sickling test and Hb electrophoresis were done in all cases during early work up and no abnormality was detected. The HAM's test and sucrose lysis test were positive in all cases (Fig.1).

Summaries of the cases with the longest

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TABLE 1

Clinical Features - PNH

Case No.	Age* Sex	L (cm)	S (cm)	Abd. pain (No.)	Red urine (No.)	Initial diagnosis	Delay in final diagnosis	Complications Associations	Follow up
1	23M	1	-	+(1)	+(1)	Hookworms Hematuria	3 yr	-	1 yr
2	24M	-	-	+(1)	-	'Refractory' anemia	5 yr	-	6 mo
3	25F	2	-	+(3)	+(3)	Nutritional anemia	2 Mo	Abortion CCF	1 yr
4	33M	2	-	+(1)	+(4)	'Refractory anemia', Megaloblastic anemia, MDS	11 yr	Diss. TB	1 yr
5	20M	3	-	-	+(4)	Fe deficiency	2 yr	ASD	6 mo
6	31M	3	4	+++	+++	Hematuria	1 yr	Hemosiderosis Renal tubular acidosis, Gall stones	26 yr

L = palpable liver; S = palpable spleen; (No.) = (number of episodes), M = male; F = female; mo = months; Age* = age at diagnosis

TABLE 2

Laboratory data* - PNH

Case No.	Hb g/dl	TLC /mm ³	Platelet /mm ³	Neut %	Retic (%) Retic Index (%)	S.bil mg/dl	Hb uria	LAP score
1	4.6	4600	185000	28	8.0/1.7	4.2	-	ND
2	7	2800	140000	48	4.6/2.6	2.8	-	ND
3	2.8	4500	72000	48	3.0/0.69	3	+	ND
4	4	2600	76000	42	2.5/0.83	3.2	+	9
5	4.3	3500	82000	50	9.4/3.3	3.5	+	4
6	7	5000	150000	55	2.9/2.8	2.8	+	5

Neut = neutrophil; Retic = reticulocyte; Retic Index = reticulocyte % x patient's PCV/normal PCV

Hb uria = Hemoglobinuria; LAP = leucocyte alkaline phosphatase; ND = not done

* = Hemosiderinuria; Ham's test and Sucrose lysis test - positive in all cases

delay in diagnosis (No.4) and with the longest follow up (No.6) are given below, to emphasise the pitfalls in diagnosis and management.

CASE NO.4

A 33 year old soldier became symptomatic for the first time, 11 years ago when he developed fever, weakness and reddish urine for 2 days. On admission to a large service hospital his Hb was 5 g/dl. In the next 3 months he improved and was discharged with Hb 11.5 g/dl. Over the next 9 years he was admitted six times at large hospitals for severe anemia (with Hb ranging from 3.5 g/dl to 9 g/dl). Investigations for anemia, including bone marrow examination, were inconclusive and each time he seemed to respond well to treatment of anemia. During these episodes his urine remained overtly normal though the test for haemosiderinuria was not done. A year later on re-evaluation at a large service referral centre, he was

diagnosed to have megaloblastic anemia and discharged, but the anemia did not respond to B12 and folate.

He subsequently developed fever, pleural effusion, and a liver biopsy revealed granulomas. The fever and effusion subsided with antitubercular therapy and a revised diagnosis of disseminated TB with secondary myelodysplastic syndrome was thought of. After 6 months he reported to another large hospital for recategorisation with Hb 4g/dl and pancytopenia. During hospitalisation he developed severe colicky abdominal pain with a lump right lower abdomen which was thought to be acute appendicitis. With conservative management he recovered and the lump resolved. He also had epistaxis, secondary to thrombocytopenia.

He was transferred to CH (SC) for hematological assessment. Pancytopenia, mild unconjugated hyperbilirubinemia, and hemosiderinuria were detected. Sucrose lysis test and HAMs test were positive, confirming the diagnosis of PNH (Table II). Ultrasound abdomen

did not reveal any lump abdomen and colonoscopy was normal. Over the next 1 year he has had two episodes of fever with hemolysis, which resolved with washed red cells transfusions, glucocorticoids, and treatment of infection. The patient is on folic acid and hemoglobin is 10 g/dl.

CASE NO.6

A 30 year old Asian Indian immigrant initially developed red urine and weakness and was admitted to Guy's Hospital London as a case of hematuria. Detailed investigations for a calculus or neoplasm in the urinary tract were normal and he was discharged. One year later, the symptoms recurred, and moderate anemia was detected. He was referred to the hematology department with Hb 7g/dl, polychromasia and reticulocytosis. Tests for PNH were positive (Table 2). Over the next 16 years he received over 200 units washed packed cells (iron load > 50 g). He developed cardiac arrhythmias with cardiomegaly, serum ferritin was > 4000 µg/l and hemosiderosis was diagnosed. Liver biopsy confirmed increased iron content, but there was no cirrhosis and endocrinal profile was normal. He also developed features of metabolic acidosis and investigations revealed proximal renal tubular acidosis. Renal biopsy showed massive hemosiderin iron deposition in the proximal tubules. He was put on oral supplements of sodium bicarbonate and potassium chloride. In 1989 he developed pain abdomen and obstructive jaundice. Ultrasound abdomen and ERCP revealed cholecystitis and cholelithiasis. With special precautions and preoperative red cell transfusions, he underwent cholecystectomy successfully. When last seen (1990) he was apparently well, with no thrombotic complications or transformation to hypoplastic anemia or acute myeloid leukemia, though transfusion dependent.

Discussion

Paroxysmal nocturnal hemoglobinuria is a rare disorder. In AIIMS New Delhi, only 16 cases of PNH were detected over 9 years in the hematology clinic [4]. The 5 cases diagnosed in Army Hospital Delhi and CH (SC) are probably an under-estimation of the actual number present. PNH was not correctly diagnosed at presentation in all 6 cases, the mean delay in diagnosis being 3.7 years. As the confirmatory tests for PNH were positive in all, this delay can be attributed to lack of awareness and varied presentations. The classical nocturnal hemoglobinuria is present in only a minority of patients [2] and the diagnosis may be missed for months or years [1,3]. In most cases haemoglobinuria is irregular and transient. Hemoglobinuria was a prominent symptom in 2 of the 6 patients, but mistaken for hematuria initially. In 3 other

patients, it was mild or infrequent. Hemosiderinuria is one of the most reliable and regular features of diagnostic importance and was present in all the six patients.

A prominent symptom at some stage was abdominal pain without obvious cause, present in 4 cases. This has been attributed to mesenteric vein thrombosis [1] possibly due to activation of platelets by complement, or due to release of ADP from platelets leading to platelet aggregation [2]. There is a tendency for thrombosis to occur in splanchnic or hepatic veins. Occasionally cerebral or femoral thrombosis is noted. However, major thrombosis did not occur in our cases and this is also the experience of other workers in India [5]. Abdominal pain may also be due to pigment gall stones secondary to chronic hemolysis as in case No.6. Surgery if required in PNH needs special care to avoid complement activation in the perioperative period [6]. If pregnancy occurs it may be uneventful or can lead to abortion [7], as in case 3. Proximal tubular acidosis seen in Case No.6 is a rare complication, although marked hemosiderin deposition in proximal renal tubules is a common feature in biopsy and autopsy reports of PNH [8]. Various renal abnormalities including hematuria, have been described [2] but death from renal failure is rare [9].

PNH, although classified as a hemolytic anemia, can have atypical laboratory features. Reticulocytosis often tends to be lower than in other hemolytic anemias with a similar degree of anemia, due to associated bone marrow dysfunction. Thus in our cases, the mean reticulocyte count was 5.4% but the reticulocyte index was only 2%. The anemia of PNH may be associated with leucopenia, thrombocytopenia or both. There is an increased propensity to infections due to leukopenia or granulocyte functional abnormalities [3]. Bone marrow examination usually shows erythroid hyperplasia as in all our cases but may also be hypoplastic or aplastic. A syndrome called 'aplastic anemia - PNH syndrome' occurs when patients present like aplastic anemia initially and subsequently

develop PNH; the reverse can also occur [10].

The confirmatory tests for PNH are the sucrose lysis and Ham's test. The Ham's test is based on the observation of Dr. Thomas Hale Ham that PNH red cells incubated with serum acidified to pH 6.2 will lyse with both normal and autologous serum. This test is also positive in hereditary erythroblastic multinuclearity with positive acidified serum lysis (HEMPAS) but in HEMPAS lysis does not occur in autologous serum [11]. The Ham's test is highly specific but insensitive while the sucrose lysis test is much more sensitive but less specific. The limitation of the tests is that they only measure the sensitivity of erythrocytes to complement mediated lysis. When PNH cell production is low (as with a hypoplastic marrow) or when most PNH cells have been destroyed (after a lytic crises) the tests may not be positive [2]. Recently, using immunophenotyping and cytofluorometric assay, it has been possible to demonstrate deficiency of GPI linked membrane glycoproteins in erythrocytes, granulocytes, monocytes and even lymphocyte subsets in PNH [12-14]. Although immunophenotyping was not possible in our patients, deficiency of leucocyte alkaline phosphatase, a GPI linked membrane protein, was demonstrated in 3 cases.

Treatment of PNH is unsatisfactory and mainly supportive. In acute hemolysis glucocorticoids are helpful [2] by stabilising red cell membrane against complement lysis, as in case 4 and 5. Red cell transfusions not only raise the Hb but also suppress marrow production of PNH erythrocytes during crises. Washed red cells should be given, as whole blood transfusions at times exacerbate hemolysis. Erythropoiesis is enhanced with folic acid and androgen therapy. Iron should only be given if iron deficiency develops as it may exacerbate hemolysis in occasional patient due to increased production of PNH cells [2]. Infections should be vigorously treated to prevent complement activation, and established thrombosis requires anticoagulant therapy. In a small percentage of cases spontaneous remissions occur [3] but

more often the course is chronic. Some transform into aplastic anemia or acute myeloid leukemia.

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