Paroxysmal Nocturnal Hemoglobinuria Terminating in Acute Myeloblastic Leukemia

By David E. Jenkins, Jr. and Robert C. Hartmann

The underlying red cell defect in paroxysmal nocturnal hemoglobinuria (PNH) remains to be defined. The two most consistent abnormalities of the PNH erythrocyte are its increased susceptibility to hemolysis by complement and a reduction in the red cell enzyme acetylcholinesterase. Attempts to explain the red cell defect in terms of lipid or electron microscopic abnormalities have yielded variable and conflicting results.

Since PNH is an acquired disease associated with an abnormal red cell, it is logical to consider the role of marrow injury in the pathogenesis of this disorder. This question assumes even greater importance in view of reports of hypoplastic anemia occurring either prior to the onset or at some time during the course of PNH. This has led several authors to propose that, at least in certain patients, PNH results from repopulation of previously hypocellular marrow with abnormal, i.e., PNH erythrocytes.

The present report describes a case of PNH terminating in acute leukemia. Two similar reports are included in the current issue of this journal.

Because of the known association of aplastic anemia and acute leukemia with various forms of marrow injury, cases such as these raise an even stronger argument for the role of marrow injury in the pathogenesis of at least certain cases of PNH. At the same time caution is urged in reaching broad generalizations from such inferences. There are many pitfalls in the diagnosis of marrow aplasia as well as in the serologic tests for PNH, as will be discussed. Whether marrow injury and aplasia occur as a preliminary to all cases of PNH remains to be determined.

Methods

Laboratory studies during and after the patient's July 1965 admission to Vanderbilt University Hospital were performed by previously published methods. Studies prior to that time were done elsewhere with the exception of the acid hemolysis and thrombin tests and the erythrocyte acetylcholinesterase determination performed in Nashville in May 1965 on specimens sent from Memphis.
**Case Report**

A 31 year old white farm housewife (Vanderbilt PNH Case #9) first developed symptoms of anemia in April 1962, during the eighth month of her only pregnancy. At that time ankle edema was noted by her private physician and the hemoglobin concentration was 3.5 Gm. per cent. Physical examination was unremarkable as were the past and family histories except for diabetes mellitus on the maternal side. No significant history of toxic exposure was elicited. The only drugs received during pregnancy had been calcium and iron preparations. The patient's subsequent clinical course is depicted in Figure 1. Additional laboratory data are summarized in Table 1. Nine transfusions were given during the last month of pregnancy raising the hematocrit to greater than 30 per cent. At term a healthy, well-developed male infant was delivered by Caesarian section without complication. To date the child's growth and development have been entirely normal. A marrow aspiration was obtained following the above transfusions. Although the marrow smears were originally interpreted as hypocellular, subsequent review revealed them to technically inadequate for evaluation.

Six weeks postpartum jaundice was observed for the first time, and the hematocrit was 16 per cent. Shortly thereafter she was referred to Baptist Memorial Hospital in Memphis, Tennessee, for further study. Upon admission there were pallor and scleral icterus, but the spleen, liver, and lymph nodes were not palpable. Laboratory studies revealed: hematocrit 31 per cent; MCV 105, MCH 31, and MCHC 30; leucocytes, 2400 per cu. mm. with 99 per cent lymphocytes; platelets 90,000 per cu. mm.; serum urea nitrogen 11 mg. per cent; serum lactic dehydrogenase (LDH) 1,060 units; and serum bilirubin 1.2 mg. per cent unconjugated and 0.6 mg. per cent conjugated. The direct Coombs' test, L.E. cell test, L.E. fluorescent antibody test, and serologic test for

*We are indebted to Dr. John M. Bishop, Armstrong Clinic, Somerville, Tennessee and Dr. Alfred P. Kraus of the University of Tennessee School of Medicine, Memphis for referring this patient to us.*
Jenkins and Hartmann

Table 1.—Comparison of Laboratory Data Before and During Leukemic Phase

<table>
<thead>
<tr>
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<tbody>
<tr>
<td>Hematocrit (%)</td>
<td>23 (8-33)*</td>
<td>19 (9-34)</td>
</tr>
<tr>
<td>Transfusions/Months</td>
<td>39/42</td>
<td>61/10</td>
</tr>
<tr>
<td>Reticulocytes (%)</td>
<td>13 (1.5-40)</td>
<td>2 (0-12)</td>
</tr>
<tr>
<td>Leucocytes (x 10^6/cu. mm.)</td>
<td>2.7 (1.7-5.5)</td>
<td>10.9 (1.8-59.5)</td>
</tr>
<tr>
<td>Blood Leucocyte Differential:</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Segmented Neutrophils (%)</td>
<td>17 (0-24)</td>
<td>1.3 (0.5-3.0)</td>
</tr>
<tr>
<td>Lymphocytes (%)</td>
<td>84 (67-99)</td>
<td>17 (0-72)</td>
</tr>
<tr>
<td>Blasts (%)</td>
<td>0</td>
<td>77 (14-98)</td>
</tr>
<tr>
<td>Platelets (x 10^9/cu. mm.)</td>
<td>143 (89-245)</td>
<td>28 (2-115)</td>
</tr>
<tr>
<td>Marrow: Myeloblasts (%)</td>
<td>0.4 — 2.5</td>
<td>19 — 80</td>
</tr>
<tr>
<td>Normoblasts (%)</td>
<td>41 — 78</td>
<td>0 — 49</td>
</tr>
<tr>
<td>Hemoglobinuria:</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Gross — by History</td>
<td>Approx. 10 episodes</td>
<td>None</td>
</tr>
<tr>
<td>Occult — by Hemastix</td>
<td>None (7/65-10/65)</td>
<td>None (10/65-2/66)†</td>
</tr>
<tr>
<td>Plasma Hemoglobin (mg. %)</td>
<td>12 — 85</td>
<td>0 — 2</td>
</tr>
<tr>
<td>Bilirubin (mg. %)</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Unconjugated</td>
<td>0.3 — 4.6</td>
<td>0.2 — 0.8</td>
</tr>
<tr>
<td>Conjugated</td>
<td>0.2 — 0.6</td>
<td>0.2 — 0.8</td>
</tr>
<tr>
<td>PNH Serologic Tests: (% hemolysis)</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Acid Hemolysis</td>
<td>26 — 54</td>
<td>16</td>
</tr>
<tr>
<td>Thrombin Test</td>
<td>39 — 84</td>
<td>—</td>
</tr>
<tr>
<td>&quot;Heat&quot; Test</td>
<td>2.1</td>
<td>0</td>
</tr>
<tr>
<td>Sucrose Hemolysis</td>
<td>63 — 90</td>
<td>30 → 8 → 0</td>
</tr>
<tr>
<td>Serum LDH (units)</td>
<td>1060 — 2940</td>
<td>10/65 1200</td>
</tr>
<tr>
<td>Serum SGOT (units)</td>
<td>29 — 36</td>
<td>12 — 25</td>
</tr>
<tr>
<td>Fecal Urobilinogen (Ehrlich units/100 Gm.)</td>
<td>318 — 1335</td>
<td>52 — 117</td>
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</tbody>
</table>

* Mean in italics; range in parenthesis.
† After February 1966 periodic vaginal "spotting" gave positive Hemastix tests.

Syphilis were all negative. The osmotic fragility test, hemoglobin electrophoretic pattern, and an autohemolysis test, with and without glucose added, were all reported as normal.

The serum iron concentration was 225 μg. per cent with 100 per cent saturation of transferrin. Although a single reticulocyte count was 1.5 per cent, significant polychromatophilia of the erythrocytes was noted on smear. A marrow aspirate was cellular showing 41 per cent normoblasts and 2.5 per cent myeloblasts. Additional transfusions of whole blood were given in July and August 1962 without reaction.

From August 1962 through March 1965 the patient was treated with triamcinolone 8-16 mg. per day, and her hematocrit remained in the "low 20's." During this interval she experienced 4 to 5 episodes of voiding "Coca Cola" colored urine per year. Typically the urine would be dark in the morning with clearing later in the day. Each episode was followed by jaundice and increased weakness.

In April 1965 the patient experienced an episode of unusually severe hemoglobinuria and jaundice necessitating subsequent hospitalization. For the first and only time (except just prior to death in July 1966), the tip of the spleen was palpable. Again marked anemia and leukopenia with relative lymphocytosis (88 per cent) were present. A single reticulocyte count was 40 per cent. Serum bilirubin was 4.6 mg. per cent unconjugated and 0.5 mg. per cent conjugated, and the serum hemoglobin ranged from 48 to 85 mg.
The autohemolysis test was now positive both with and without the addition of glucose. Erythrocyte G-6-PD activity was normal. An oral cholecystogram revealed cholelithiasis, a finding confirmed on two subsequent examinations. Marrow aspiration smears were cellular, showing a striking increase in erythroid precursors (66 per cent) and 1 per cent myeloblasts. The direct Coombs' test was negative at this time and remained so through the rest of her course. A positive indirect Coombs' test was noted and antibodies of anti-Rh\(^{a}\) (anti-D) and anti-Rh\(^{b}\) (anti-C) specificity were subsequently identified. The patient's own cells were group A Rh negative.

At this time the diagnosis of PNH was strongly considered, and tests for urine hemoglobin and urine hemosiderin were positive. An initial acid hemolysis test was reported negative, but repeat acid hemolysis test in our laboratory showed 30 per cent hemolysis with 43 per cent hemolysis in the thrombin test. Erythrocyte acetylcholinesterase (AChE) activity at this time was 183 \(\mu\)M/hr./ml. red cells (25 per cent of normal).\(^{13}\) This value may have been influenced by the presence of transfused red cells since much lower values of 13 and 23 activity units were obtained in September and October 1965. At these times the patient had received no blood for a period of three to four months.

The patient experienced a stormy course in the spring of 1965, requiring three separate hospitalizations and many transfusions. All transfusions subsequent to the diagnosis of PNH were with saline-washed red cells although there had been no history of reactions to whole blood transfusions prior to that time. In April 1965 she received a short course of prednisone therapy. In May 1965 she was hospitalized with thrombophlebitis of the left leg which subsided following anticoagulation first with heparin and then with warfarin.\(^{9}\) In June 1965 she was hospitalized for somnolence and mental confusion. Neurosurgical evaluation revealed a left temporal mass, and at craniotomy an "old" intracerebral blood clot was evacuated from the region of the left temporal lobe. During the ensuing months she made a gradual but satisfactory recovery from the sequelae of her intracerebral hemorrhage. Postoperatively she received 5-5, diphenylhydantoin 100 mg. twice daily for a period of 6 months.

In July 1965 the patient was referred to Vanderbilt University Hospital for further study. Anemia, leukopenia with relative lymphocytosis (72–84 percent), and reticulocytosis (4.8 to 15 per cent) were again noted. Marrow aspiration smears were very cellular with 78 per cent erythroid precursors, a decrease in granulocytic precursors, and no increase in blast cells. Vim-Silverman needle biopsy was likewise cellular and showed erythroid hyperplasia. Additional pertinent studies revealed: platelets 113,000 to 227,000 per cu. mm.; LDH 1,080 units; SGOT 36 Karmen units; serum electrophoresis, normal; Arneth count, normal; and haptoglobins, absent by starch gel electrophoresis.\(^{10}\) Peripheral blood leucocyte alkaline phosphatase activity was absent, but the paucity of granulocytes made this test difficult to evaluate. Serologic tests for PNH were again positive (Table 1).

Metabolic studies were also carried out according to a previously described protocol.\(^{13}\) Continuous 12-hour urine collections for a period of 12 days showed no significant amount of hemoglobin by biochemical determination and gave negative tests by Hemastix testapes. Daily morning and evening urine Hemastix tests were continued on an outpatient basis and gave negative results until February 1966. Thereafter, this procedure was no longer useful as a test for occult hemoglobinuria, because recurrent episodes of vaginal bleeding produced contamination of urine specimens with whole blood. The average 24-hour urinary iron excretion over a 12 day period in July 1965 was 0.95 mg., a low value for PNH.\(^{13}\) Subsequent values determined during periods of hospitalization or estimated from single outpatient specimens\(^{13}\) ranged between 1.0 and 2.0 mg. urinary iron excretion per 24 hours. Serum iron concentration in July 1965 was 94 \(\mu\)g. per cent with 24 per cent saturation of transferrin. During the remainder of her course serum iron values ranged from 54 to 241 \(\mu\)g. per cent with iron saturation varying from 41 per cent to 98 per cent. The

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\(^{*}\) The serum haptoglobin determinations were performed by Dr. Walter E. Nance, Department of Medicine, Vanderbilt University School of Medicine.

\(^{†}\) Hemastix testapes were supplied by the Ames Company, Elkhart, Indiana.
marrow iron, determined by histochemical staining for marrow hemosiderin, was 3+ to studies performed in July 1965 by Dr. L. C. McKee, Jr., utilizing whole body counter. The plasma T% of Fe was 75 minutes, within the normal range. The plasma iron transport was 52 µg./100 ml./hr., a value approximately two times normal. The red cell Fe utilization was 100 percent by 7 days, falling to 45 percent by 12 days. These data suggest an active marrow with hemolysis of young red cells.

In August 1965 the patient was begun on fluoxymesterone* 10 mg. daily with progressive increase in the dosage to 60 mg. (Fig. 1). Because of concern that the large dose of androgen might be harmful to the fetus in the event of pregnancy, the patient was placed on Enovid®. Through August and September 1965 the patient's hematocrit values remained greater than 20 per cent, and she received no transfusions. In October her hematocrit fell to 14 per cent, following a week of fever and sore throat. Peripheral blood smear now revealed 14 per cent myeloblasts. Bone marrow aspirates were cellular revealing as before, an increase in erythroid precursors (49 per cent) but now showing also an increase in myeloblasts (19 per cent).

From October through December 1965 the patient again did reasonably well clinically despite the persistence of severe anemia necessitating additional transfusions. Beginning in January 1966, however, the course of her illness was steadily downhill and was characterized by repeated infections, purpura, and progressive anemia requiring large numbers of transfusions. This phase of the illness was characteristic of acute leukemia. Prominent among the infections were furunculoses and cutaneous abscesses, dental abscesses and repeated "flu-like" illnesses, all of which responded only temporarily to the best of a variety of antibiotics. Undoubtedly contributing to her skin infections were poor personal hygiene, acneiform lesions (aggravated by androgen therapy) plus the repeated episodes of purpura. At one point a lower facial lesion developed which did not appear infectious. A tentative diagnosis of leukemic infiltration was made, and the mass completely regressed following x-ray therapy. Although shotty axillary and inguinal nodes appeared, at no time was the liver palpated, and the spleen became palpable only on her last admission just four days prior to death.

Between October 1965 and January 1966 blasts became the dominant cell in both the marrow and peripheral blood (Fig. 1). Toward the end of her course the white cell count rose progressively and the reticulocyte count fell to virtually zero. From July to November 1965 the platelet count had ranged from 89,000 to 227,000 per cu. mm. Thereafter, there was a progressive fall to concentrations less than 10,000 per cu. mm. during the last three months of life.

Evidence for the type of hemolysis commonly seen in PNH also diminished during the leukemic phase of her illness. The serum bilirubin, plasma hemoglobin, serum LDH and fecal urobilinogen all decreased to normal or near-normal values (Table 1). Likewise the sucrose hemolysis test became progressively weaker and finally negative (Table 1). At no time during the course of her illness did the patient develop transfusion reactions. Tests for leuko-agglutinins† as late as January 1966 were negative.

The final brief admission in July 1966 was characterized by severe pseudomonas vulvitis and bilateral labial abscesses, purpura and terminal pulmonary edema. Permission for autopsy was not obtained.

DISCUSSION

The present report documents a case of PNH terminating in acute leukemia. Although the presence of PNH was not established until May 1965, the patient's previous course was consistent with a diagnosis of PNH. From the onset in 1962 her anemia appeared to be hemolytic. Furthermore, the patient

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* Halotestin (fluoxymesterone) was provided by Dr. Paul C. Schwallie, the Upjohn Company, Kalamazoo, Michigan.

† Dr. Thomas E. Brittingham, Department of Medicine, Vanderbilt University School of Medicine performed the leuko-agglutinin tests.
experienced four to five episodes yearly of voiding “Coca Cola” colored urine, very likely episodes of gross hemoglobinuria. When she was first seen at Vanderbilt in July 1965, serologic tests for PNH remained positive and the patient’s predominant hematologic picture was one of hemolytic anemia. By October 1965 the transition from PNH to acute leukemia was in progress. After January 1966 the course was typical for acute leukemia. Had the patient first been observed at this time without benefit of past history and studies, it is doubtful that a diagnosis of PNH would have been considered. Even the previously positive diagnostic tests, such as the sucrose hemolysis test, became progressively weaker and finally negative toward the end of the course. Although this may have been due in part to the leukemic transformation, the many transfusions required might alone have produced such changes since few of the patient’s own PNH cells were left in her circulation.

From the beginning of her illness until the development of leukemia our patient had marked leukopenia with a striking, relative lymphocytosis (Table 1). Although leukopenia is not unusual in PNH, such a persistent, selective reduction in neutrophils is uncommon.\(^2\)\(^,\)\(^13\) Only one of 10 other PNH patients studied at Vanderbilt University Hospital in recent years has demonstrated such a tendency toward neutropenia.\(^13\) In this latter patient (case 7) the neutropenia and leukopenia were neither so severe nor so persistent as in the present patient (case 9). Case 7 died of cerebral thrombosis after nine years of disease without evidence of leukemia either during life or at autopsy. Whether persistent, severe neutropenia in an individual with PNH indicates that the patient is more likely to develop leukemia, if survival is sufficiently prolonged, is a matter for future observation.

When the patient was first seen at Vanderbilt, she demonstrated several findings different from those we have usually observed in PNH. Although she had an increase in reticulocytes, plasma hemoglobin, serum LDH, and fecal urobilinogen, there was, on the other hand, minimal hemosiderinuria and bilirubin elevation and the serum SGOT was normal. Even occult hemoglobinuria (Hemastix) was absent (Table 1). Moreover, marrow iron stain demonstrated 3+ to 4+ iron throughout her Vanderbilt course (Fig. 1), a distinctly unusual feature in PNH in our experience.\(^13\) Whether these atypical features reflected the first manifestations of her leukemia, or were influenced by the many transfusions given in the spring of 1965, cannot be stated with certainty.

Another feature differing from the usual case of PNH was the absence of hemolytic reactions following repeated administration of whole blood. It is of interest that this patient failed to demonstrate leuko-agglutinins even after more than 50 transfusions. Among the five of our PNH patients who have received whole blood transfusions, she is the only one who developed neither leuko-agglutinins nor transfusion reactions. In this regard our experience agrees with that of others that the hemolytic transfusion reactions to whole blood seen in PNH may be conditioned, at least in part, by the presence of leuko-agglutinins.\(^2\)\(^,\)\(^17\)

Since this patient’s course terminated in acute leukemia, it is logical to consider whether medications may have played a role in the development
of her leukemia. Although 5-5' diphenylhydantoin is associated with certain hematopoietic toxicity\textsuperscript{18,19} and reticulo-endothelial abnormalities,\textsuperscript{20} we are not aware of any association of this drug with acute leukemia. Corticosteroids have at times seemed to accelerate the course in established acute myeloblastic leukemia,\textsuperscript{21} but we know of no evidence that these agents can cause leukemia. Moreover, corticoids have been given to many PNH patients over the past few decades without previous documentation of leukemic transformation. Shanbrom and Finch noted acute clinical relapses in some patients with established lymphomas receiving androgens,\textsuperscript{22} but again there is no evidence that these agents are de novo leukemogenic.

The most challenging and difficult point to consider is what information this and similar patients\textsuperscript{11,12} can provide concerning the relationship between marrow hypoplasia, acute leukemia, and PNH.\textsuperscript{10} Our patient illustrates certain problems in this type of analysis. For example, her initial marrow aspirate was reported as hypocellular. Review of this specimen, however, revealed it to be inadequate for interpretation, and repeat marrow examination within three weeks showed increased cellularity. This demonstrates the problem of establishing hypoplasia on the basis of marrow aspiration alone. In a patient with pancytopenia, as is frequently seen in PNH, it might be tempting to accept a hypocellular marrow aspirate as being representative of the entire marrow. However, marrow hypoplasia can only be documented with certainty by marrow biopsy. Even with biopsies technical problems can occur. For example, when a combined marrow aspiration and biopsy are performed with a Vim-Silverman needle, the marrow particles can be aspirated to such an extent that an erroneous impression of marrow hypoplasia results if the trephine biopsy is then obtained from the same site. This can be avoided by removing only the smallest amount of marrow by aspiration and then advancing the needle further into the marrow cavity before taking the biopsy. Not all individuals performing this combined procedure or interpreting specimens so obtained are aware of this potential problem.

A second problem in relating marrow hypoplasia and PNH concerns the vagaries of some of the diagnostic tests for PNH. Our patient provides a case in point. Her initial acid hemolysis test in April 1965 was reported negative. Yet repeat acid hemolysis and thrombin tests in our laboratory two weeks later were strongly positive. Although the acid hemolysis test is relatively simple in principle, there are many technical problems inherent in this test. These include pH of the serum, variation in potency for acid hemolysis between different normal sera, and lability of serum factors required for acid hemolysis upon conventional storage of serum at \(-20^\circ\). Although pH of the serum used in an acid hemolysis test is critical,\textsuperscript{2,23} it is frequently recommended that a predetermined amount of acid be added to serum, and the important point that pH should be checked following acidification is neglected.\textsuperscript{2,24} Additionally, the presence of a large proportion of transfused normal cells may be a problem. Thus, in a patient with a large number of transfused cells in circulation, performance of the acid hemolysis test with improperly acidified and improperly stored or weakly hemolytic sera might lead to a negative result. Early in our own experience with PNH we noted
at times much weaker acid hemolysis reactions than we have subsequently observed due to our appreciation of these simple but easily overlooked technical problems. Whether the sucrose hemolysis test for PNH\textsuperscript{14,15} will prove better in this regard is of considerable interest. In our studies to date the sucrose hemolysis test has been a sensitive and discriminating diagnostic test for PNH, provided certain precautions are observed.\textsuperscript{24} Standards for the performance and interpretation of the sucrose hemolysis test will be the subject of a separate communication.\textsuperscript{25}

Even with the recognition of the problems cited in the paragraphs above there exist well-documented cases of marrow hypoplasia preceding the onset of clinical PNH.\textsuperscript{2,6,7} Moreover, a certain percentage of patients with aplastic anemia show positive serologic tests for PNH without developing the total clinical picture.\textsuperscript{7} With the present report there now exists documentation of three separate cases of PNH terminating in acute leukemia.\textsuperscript{11,12} What information this provides concerning the etiology and pathogenesis of PNH in all patients, as opposed to only a portion of what has been termed the “PNH syndrome”\textsuperscript{26} is, of course, the crucial question.

Approached from the standpoint of acute leukemia it may be of interest to determine if rare cases might be preceded by unrecognized PNH. In our patient by the time the acute leukemia had advanced to the point that many transfusions were required (January 1966), there was little to suggest PNH clinically and diagnostic tests became weak and finally negative. Yet in the preceding three months manifestations of acute leukemia had been combined with the persistence of marrow erythroid hyperplasia and blood reticulocytosis. Therefore, we suggest serologic tests for PNH be performed in any case of acute leukemia showing erythroid hyperplasia or reticulocytosis or both.

\textbf{Summary}

A 34 year old white female with documented PNH developed acute myeloblastic leukemia. The details of this patient's clinical course are presented. Two similar cases are described in the same issue of this journal. The significance of these cases with respect to the potential role of marrow injury in the pathogenesis of PNH is discussed.

\textbf{SUMMARIO IN INTERLINGUA}

Un femina de racia blanc de 34 annos de etate con documentate paroxysmic hemoglobinuria nocturne disveloppava acute leucemia myeloblastic. Le detalios del curso clinic de iste paciente es presentate. Duo simile casos es describite in le mesme numero del presente periodico. Le significacion de iste casos con respecto al rolo potential de lesion del medulla in le pathogenese de paroxysmic hemoglobinuria nocturne es commentate.

\textbf{ACKNOWLEDGMENT}

The authors wish to thank Anita B. Arnold and Jeanne K. Jackson for their assistance.

\textbf{REFERENCES}


Paroxysmal Nocturnal Hemoglobinuria Terminating in Acute Myeloblastic Leukemia

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