

Paroxysmal nocturnal haemoglobinuria

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Definition

Paroxysmal nocturnal haemoglobinuria (PNH) is an acquired chronic haemolytic anaemia in which intravascular haemolysis results from an intrinsic defect in the membrane of red cells that makes them highly susceptible to complement.

History and Epidemiology

The first description of a patient with PNH is usually credited to a classical paper published by Strubing^(21b) near Bonn in 1882, but in fact a good description was first given two centuries earlier (in Latin) by the Dutch physician W Schmidt.⁽²⁴⁾ Early in this century the clinical features were well characterized by Marchiafava and Micheli in Italy. In the 1930s Ham in the USA⁽⁷⁾ and Dacie in the UK⁽⁶⁾ developed the acidified serum test, which became the defining diagnostic test for PNH. Patients with this condition have been reported from all parts of the world, and there is no reason to think that the incidence of PNH varies significantly in different populations, although it may be more frequent in South East Asia. PNH is certainly a rare disease: although its true frequency has never been accurately measured, we can estimate that it is of the same order as that of aplastic anaemia (AA), with which it has a close relationship (see below). PNH is usually a disease of young adults, but it also occurs in children or it may present later in life.

Aetiology

In contrast to all other haemolytic anemias due to an intrinsic red cell abnormality, PNH is an acquired rather than an inherited disorder. This fact, together with the finding that normal cells co-exist in the patient's blood with those that are hypersensitive to complement, led some 30 years ago to the working hypothesis that PNH arises through a somatic mutation in a haemopoietic cell.⁽⁵⁾ The first evidence in support of this model was provided by showing that in women with PNH who were heterozygous at the X-linked locus for the enzyme glucose 6-phosphate dehydrogenase (G6PD), the patients' total red cell population showed expression of both G6PD alleles, but the fraction of red cells susceptible to lysis - which we shall call the PNH red cell population - showed expression of only one, suggesting that it was monoclonal in origin.⁽¹⁴⁾ Subsequently, it was shown that individual erythroid colonies from PNH patients had either the normal or the PNH phenotype, but they were never mixed.⁽¹⁹⁾ Quite recently a gene called PIG-A has been isolated by expression cloning, shown to correct the membrane abnormality of

PNH cells and mapped to the X chromosome on Xp21.3.^(2,22) Sequence analysis of this gene has revealed mutations (usually point mutations or small insertion-deletions) in the PNH cell population but not in the normal cell population of all PNH patients.^(3,13,25,26) Thus, the somatic mutation model can now be regarded as fully validated.

Clinical Features

In most cases the patient presents as a problem in the differential diagnosis of anaemia. The anaemia may have been discovered through an incidental blood count, or this may have been obtained because the patient complained of fatigue, or dysphagia, or abdominal pain, or other more vague symptoms. In many cases the passing of dark urine may have gone unnoticed by the patient, or its significance not appreciated by the physician, or the history of that telltale sign may be elicited only by specific questioning; but in some cases it is the abrupt onset of dark urine that brings the patient to the physician. The time course of the dark urine is extremely erratic, as it may be quite intense for a few days and then subside for weeks; and it may vary within the same day, being classically - but not always - darker in the morning (see Figure 1).



Figure 1: Consecutive urine samples from a PNH patient demonstrate variable intensity of haemoglobinuria over a 48 hour period.

The clinical consequences of the anaemia will depend of course on its time course and severity. In general the patient is relatively well adapted to the anaemia, in keeping with the fact that this is chronic; however, sudden severe exacerbation of anaemia may cause tachycardia and sometimes high output heart failure; in other cases intravascular haemolysis may be so abrupt that it leads to hypovolaemic shock.

In some patients PNH may present with typical signs and symptoms of thrombosis in a deep vein in one of the limbs. In others the thrombosis may be intra-abdominal, giving rise to one or more episodes of severe abdominal pain, the cause of which may be quite difficult to recognize. The hepatic veins are the most common site, causing the Budd-Chiari syndrome; mesenteric vessels may be also involved, but necrosis of the intestine is exceedingly rare.

Clinical Course and Relation to Aplastic Anaemia

The natural history of PNH is that of a very chronic disorder, which may afflict the patient continuously for decades. Without treatment the median survival is estimated to be about eight years; the most common causes of death are thrombosis or haemorrhage associated with severe thrombocytopenia.⁽⁹⁾ This latter complication signals a very

important feature of PNH, namely its two-way relationship to AA. On one hand, a patient who originally presented with the clinical picture described above - which we can refer as *florid PNH* - ally develops pancytopenia and is found to have a hypoplastic and eventually an aplastic bone marrow: we refer to this situation as *spent PNH*. On the other hand, it happens that a patient with AA, sometimes years since the original diagnosis, develops clinical and laboratory features of PNH. This development is estimated to take place in up to 30% of patients treated with antilymphocyte globulin (ALG),⁽²³⁾ but this does not at all mean that it is caused by ALG, since it may occur in patients who were treated otherwise (e.g., with androgens) or who had 'pontaneous remission' of AA. In fact, it appears that any patient who has survived AA without BMT is at risk of developing PNH. Thus, in terms of a temporal sequence PNH may be followed by AA, or AA may be followed by PNH. But on closer inspection we find, not surprisingly, that features of the two may also co-exist: for instance, a patient with florid PNH may have at the same time severe thrombocytopenia with very few megakaryocytes in the bone marrow. It is convenient to refer to these patients as having the *PNH-AA syndrome*.⁽²¹⁾

Another important disease association of PNH is the well-documented occurrence of acute myeloid leukaemia (AML). Although this is rare (estimated about 1% of all patients with PNH), it is certainly much more common than in the general population, and therefore PNH must be regarded as a potential pre-leukaemia condition.⁽¹¹⁾ The mechanism of this is not known, but it is reminiscent of AA itself terminating in AML.

Finally, and on a more optimistic note, full spontaneous recovery from PNH has been also well documented. In a recent analysis of long-term follow up of 80 patients with PNH, 10% of those who survived for 25 years or more evolved to spontaneous recovery.⁽⁹⁾

Laboratory Findings

In florid PNH the main blood finding is anaemia, which is usually normocytic or macrocytic. The blood film will often show considerable anisocytosis and polychromasia, but not many poikilocytes: overall, there are no morphological changes characteristic of PNH. The reticulocyte percentage may be increased to 10% or more, with absolute count values of 200,000/ml and above not uncommon. As in every chronic haemolytic anaemia there is an increased requirement for folic acid; also, and unlike in most other chronic haemolytic anaemias, there may be sufficient loss of iron through the urine to cause iron deficiency. Thus, the red cell parameters and morphology may be complicated by superimposed folate deficiency or iron deficiency, or both. The bone marrow shows normal or increased cellularity in florid PNH, with tremendous erythroid hyperplasia. The other important findings are in the urine, in which the presence of haemoglobin without red cells in the sediment is diagnostic of haemoglobinuria. Mild haemoglobinuria is easily missed by the patient, but it can be often demonstrated by collecting serial urine samples (see Figure 1). In the absence of this sign, it is useful to stain the sediment by the Prussian blue reaction, which will almost invariably demonstrate the presence of haemosiderin. Since most of the haemolysis is extravascular, the serum bilirubin level may be normal, but it is usually at least somewhat elevated when the haemolysis is severe.

Crucial for the diagnosis of PNH is a *positive acid haemolysis (Ham) test*.

(Author's note: The sucrose haemolysis or 'suger-water' test has been used extensively as an attractive simpler version of the Ham test. Unfortunately, it can give both false positive and false negative results; therefore, in my view, it is not very helpful.)

This test is based on the fact that the alternative pathway of complement is activated at mildly acid pH (about 6.7), which results in lysis of a proportion of the patient's red cells. This proportion (the *PNH red cells*) can be easily measured colorimetrically, and it yields an estimate of the size of the PNH clone (this is a minimum estimate because PNH red cells are selectively removed from the circulation - ough lysis - a much higher rate than normal red cells). In most patients with florid PNH, the percent lysis in the Ham test is of the order of 10-0%, but even higher values are found sometimes. In order to be sure of the diagnostic significance of values below 10% it is imperative to have rigorous controls in this test; when this is done, the background is less than 2%.⁽¹⁾

Over the past five years, flow cytometry has been employed extensively to demonstrate the deficiency in PNH red cells of GPI-linked proteins such as CD59, CD48 and CD55.^(1,16,20) This technique lends itself to accurate quantitation, as well as to testing for the PNH abnormality also in peripheral blood granulocytes (best with anti-CD14) and lymphocytes (both in B cells and in T cells). Granulocytes are always affected, and usually the proportion of PNH granulocytes is larger than that of PNH red cells (values of 90% or more are common), thus providing a more accurate measure of the size of the PNH clone. For this reason, if the clone is small, it may be undetectable by testing red cells; the Ham test may have been reported as negative, but it may be detectable by testing granulocytes. Thus, flow cytometry has a higher sensitivity than the Ham test in detecting a PNH clone: however, whether a patient without PNH red cells and without signs of haemolysis should be classified as having PNH because of the presence of a small fraction (say 10%) of PNH granulocytes becomes a matter of semantics.

Many patients with PNH have neutropenia or thrombocytopenia or both at some stage in the course of their disease. The combination of both these cytopenias along with persistently elevated reticulocytosis is highly suggestive of PNH, and it qualifies for the PNH-AA syndrome. With this form or this stage of the disease the marrow may become severely hypocellular, although the erythroid activity is the last to disappear before the marrow becomes frankly aplastic. At this time the patient may have become transfusion-dependent and the Ham test will be worthless. It is in such cases that flow cytometry of granulocytes may be the only way to demonstrate a PNH clone.

Differential Diagnosis

The diagnosis of PNH can be both easy and difficult. If the patient presents with the abrupt onset of haemoglobinuria, and this is recognized, the list of possible underlying causes is limited (see Table 1): a Ham test becomes imperative, and the diagnosis is quickly made. If it is recognized that the patient has a haemolytic anaemia, the range of possibilities is much wider, but by a process of elimination the right conclusion should be eventually reached.

Table 1. Differential diagnosis of hemoglobinuria.

Condition Approach	Circumstances	Diagnostic
G6PD deficiency activity	Exposure to trigger of hemolysis	Test for G6PD
Blackwater fever	Relatively rare complication of malaria	Blood slide for malaria parasites
Paroxysmal cold Donath-hemoglobinuria antibody	Usually associated with viral infection	Search for Landsteiner
Mismatched blood transfusion	Usually ABO incompatibility	Repeat cross-match
Paroxysmal nocturnal hemoglobinuria	Very rare in children	Ham's test
Clostridium welchii septicemia	Burns; severe open; traumatransfusion of contaminated	Culture of blood or appropriate patient blood material

However, experience shows that the interval between the first symptoms and the diagnosis is, on the average, between one and two years, and occasionally much longer. Indeed, PNH has been called a "reat impostor." Note the following examples:

- (1) Although the patient has severe haemolytic anaemia, this is missed because the patient is not clinically jaundiced, on account of the fact that the haemoglobin released intravascularly is lost through the urine and thus does not produce much hyperbilirubinaemia.
- (2) Because of chronic loss of iron through haemoglobinuria and haemosiderinuria the patient may be correctly regarded and treated as iron deficient, but this is not the primary diagnosis.
- (3) Deep vein thrombosis may dominate the clinical picture, and may lead to extensive work-up for coagulation abnormalities before the true cause for the thrombophilic state is appreciated.
- (4) The initial clinical picture may be dominated by attacks of severe abdominal pain, one of which is regarded as 'cute abdomen,' leading to surgical intervention. Such an attack may be due to hepatic vein thrombosis, heralding the Budd-Chiari syndrome: but in other cases it remains unexplained.

- (5) The patient may be subjected to extensive urological investigations that would be appropriate for a sign - roscopic haematuria - ch did not actually exist, because it was instead gross haemoglobinuria.

Pathogenesis and Pathophysiology

Ever since the introduction of the Ham test, the study of PNH has been centered on two main issues: (a) the nature of the red cell lesion which makes it susceptible to complement lysis, and (b) the basis for the long-term balance between the PNH blood cell population and an apparently normal cell population.

(a) Early studies of biochemical abnormalities in PNH red cells revealed that these were deficient in acetylcholinesterase (AChE), while PNH neutrophils were deficient in alkaline phosphatase. These findings did not seem immediately relevant to complement lysis, but because both of these enzymes are membrane-bound, they did point to a membrane defect. Over the past decade, the catalogue of proteins lacking from PNH cells has increased impressively in size.^(15,16) At first sight, this multitude of defects might not have seemed compatible with the notion of a single somatic mutation stated above. However, it quickly emerged that all these deficient proteins shared a distinctive structural feature. Indeed, they all belong to the class of proteins that are anchored to the membrane through a glycosyl phosphatidyl inositol (GPI) anchor. One of these proteins, the membrane inhibitor of reactive lysis (MIRL; also known as CD59), is of major importance in preventing attack to the membrane by the C5-C9 complement complex (see Figure 2).

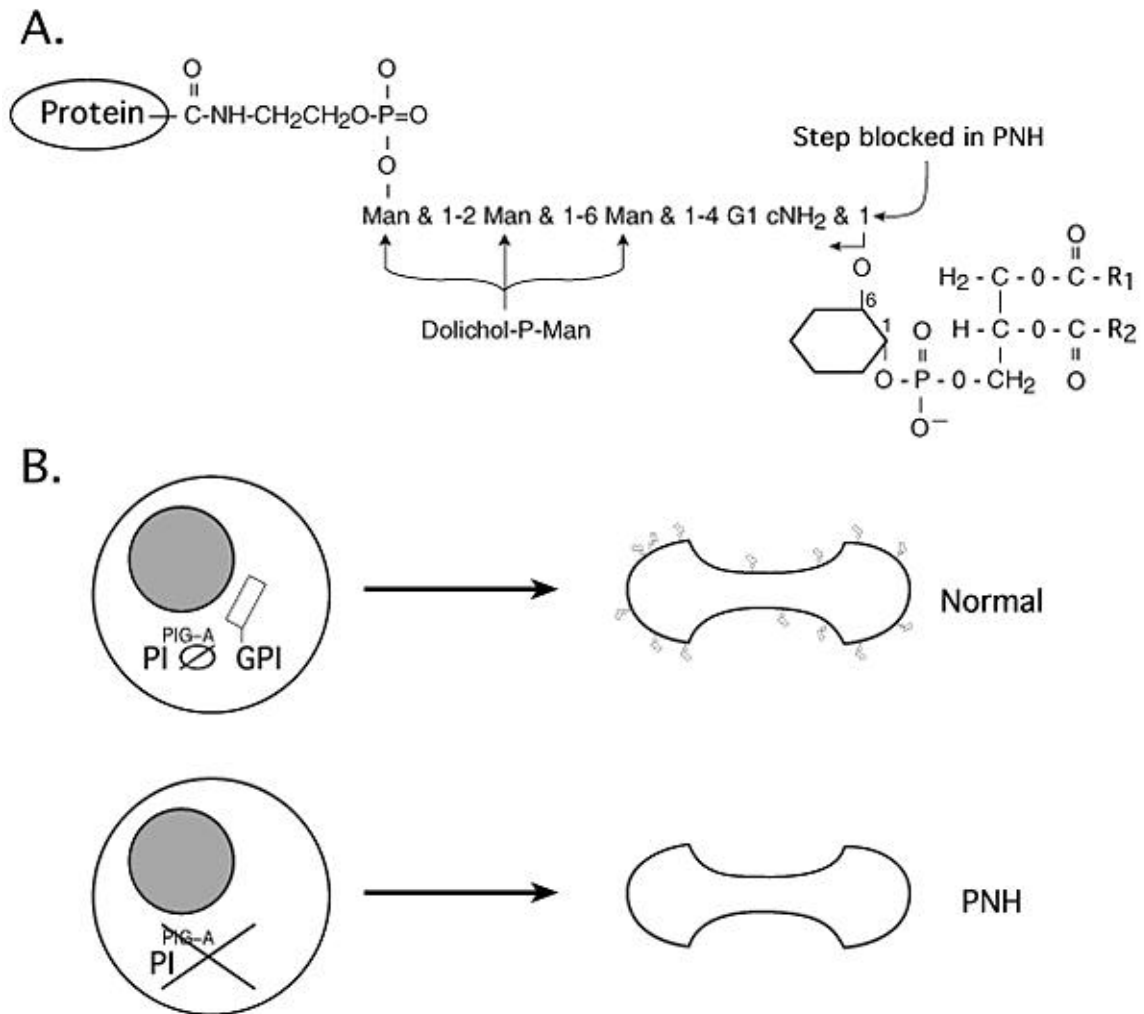


Figure 2. The primary lesion in PNH is a somatic mutation that decreases drastically or abolishes completely the protein product of the PIG-A gene. Top: the PIG-A protein in a normal stem cell or progenitor cell catalyzes an early step in the biosynthesis of a GPI anchor; the mature red cell derived from this normal progenitor is coated with GPI-anchored CD59 molecules. Bottom: since the PIG-A protein is missing or inactive GPI anchors cannot be synthesized in the stem cell-progenitor cell; the mature red cell derived from this mutated progenitor lacks CD59 molecules. As a result, the red cell is highly susceptible to complement-mediated lysis. In every patient with PNH the two types of red cells co-exist, but the ratio is highly variable (from 10 to 90% of red cells may have the PNH abnormality).

Inherited absence of this protein produces a phenotype very similar to PNH,⁽²⁷⁾ and therefore acquired CD59 deficiency offers a good explanation for intravascular hemolysis in PNH. It is now clear that the deficiency of GPI-linked proteins is the direct consequence of somatic mutations in the PIG-A gene (see above) (reviewed in 10b). Although the biochemical function of the PIG-A protein has not yet been elucidated, it is

almost certainly involved in the transfer of acetylglucosamine onto phosphatidylinositol, an early step in the biosynthesis of the GPI anchor. Since GPI-linked proteins are deficient on the membrane of cells belonging to the erythroid, myeloid, megakaryocytic and lymphoid lineages, we can infer that the somatic mutation responsible for the PNH phenotype has taken place in a totipotent cell, thus qualifying PNH as a stem cell disorder.

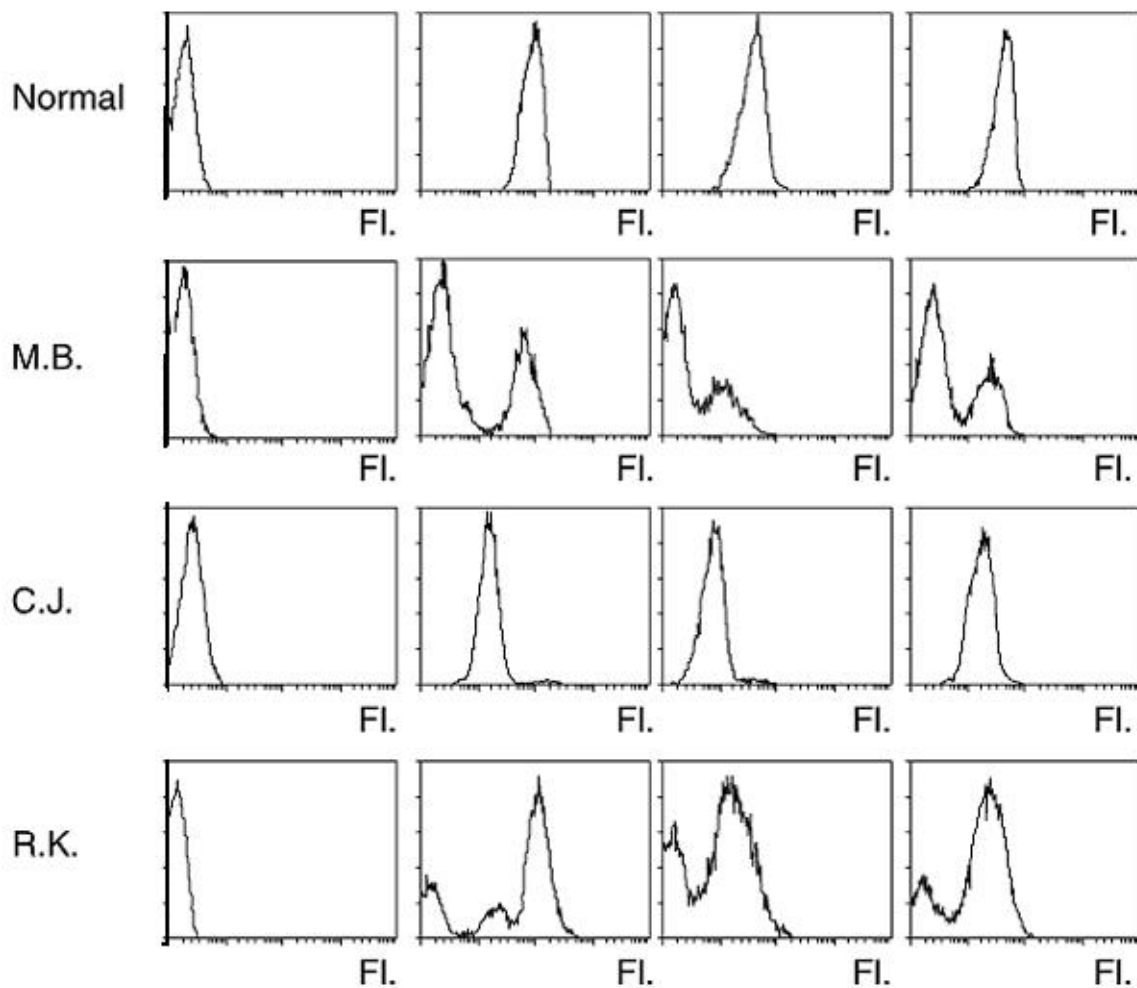


Figure 3. Normal and abnormal red cells in PNH patients can be displayed by flow cytometry. The top row of four panels is from a normal control, and the other three rows are from three different patients with PNH. The first column shows the patterns obtained by staining the cells with an irrelevant antibody (negative control); the second column shows the patterns obtained by staining the cells with anti-CD59; the third column shows the patterns obtained by staining the cells with anti-CD55; the fourth column shows the patterns obtained by staining the cells with anti-CD48. Normal cells show a unimodal distribution with all three antibodies; PNH patients usually show a bimodal pattern (clearly exemplified by patient MB), from which the size of the PNH population can be accurately measured (about 60% in the case of MB). Sometimes the PNH defect causes a complete lack of GPI-linked surface proteins (PNH III cells, as in MB), while sometimes there is only a decrease

in these proteins (PNH II cells, showing a shift in the modal value of fluorescence, as in patient CJ: in this rare patient, the PNH red cell population makes up over 95% of the total red cells, giving the impression of a unimodal distribution). In some cases, a PNH III population coexists with a PNH II population as well as with normal red cells, yielding a trimodal pattern, as seen with anti-CD59 in patient RK (second panel in the bottom row). In most laboratories, anti-CD59 has proven to be the antibody that gives the best resolution and quantitation not only of PNH red cells, but also of abnormal granulocytes in PNH patients.

The thrombotic features of PNH (see Figure 3) are probably the result of an intrinsic abnormality of the membrane of platelets, which causes them to become inappropriately activated within the circulating blood.

(b) Although the number of stem cells that contribute to haemopoiesis in the human bone marrow at any one time is not known, the progeny of a single mutated stem cell should not amount to more than a minute fraction of blood cells, perhaps less than 1%. Since the proportion of abnormal blood cells in PNH is much larger, we must infer that the PNH clone has undergone expansion; on the other hand, this expansion remains controlled, and residual normal cells are always present, as though, unlike in leukaemia, peaceful coexistence has been achieved between the two populations. From the hematological point of view, PNH often presents the unusual combination of pancytopenia and reticulocytosis in the peripheral blood with erythroid hyperplasia in the bone marrow. This finding, together with the close relationship between PNH and AA illustrated above, is consistent with the notion that an element of bone marrow failure may be present in every patient with PNH. Indeed, the proportion of PNH neutrophils in PNH patients who have normal or reduced neutrophil counts is often 90% or more, indicating an absolute decrease in normal neutrophils. In addition, markedly reduced numbers of erythroid and myeloid progenitors have been reported in the peripheral blood of patients with PNH, even when they had no overt AA.⁽¹⁶⁾

The data we have summarized indicate that there are two components in the pathogenesis and pathophysiology of PNH. On one hand, we have an abnormal clone that arises through a somatic mutation in the PIG-A gene in a haemopoietic stem cell. On the other hand, we have evidence of bone marrow failure (BMF). Since the PNH clone is often very florid, we infer that the BMF affects selectively the non-PNH stem cells.

In order to explain the coexistence of these two components, we can surmise in principle three possibilities: (i) The PNH clone and BMF co-exist by a sheer coincidence. (ii) The PNH clone causes BMF. (iii) BMF favors the development or the expansion of the PNH clone. Because AA and PNH are both rare diseases, the first possibility can be discarded on statistical grounds as being too improbable. The second possibility is unlikely, since PNH often develops in patients originally suffering from AA, who therefore already had established BMF at a time when no PNH clone could be demonstrated. Thus, the third possibility seems the most likely, and it has recently obtained support through the finding that more than one PNH clone, each with a different mutation, may coexist in PNH patients.⁽⁴⁾

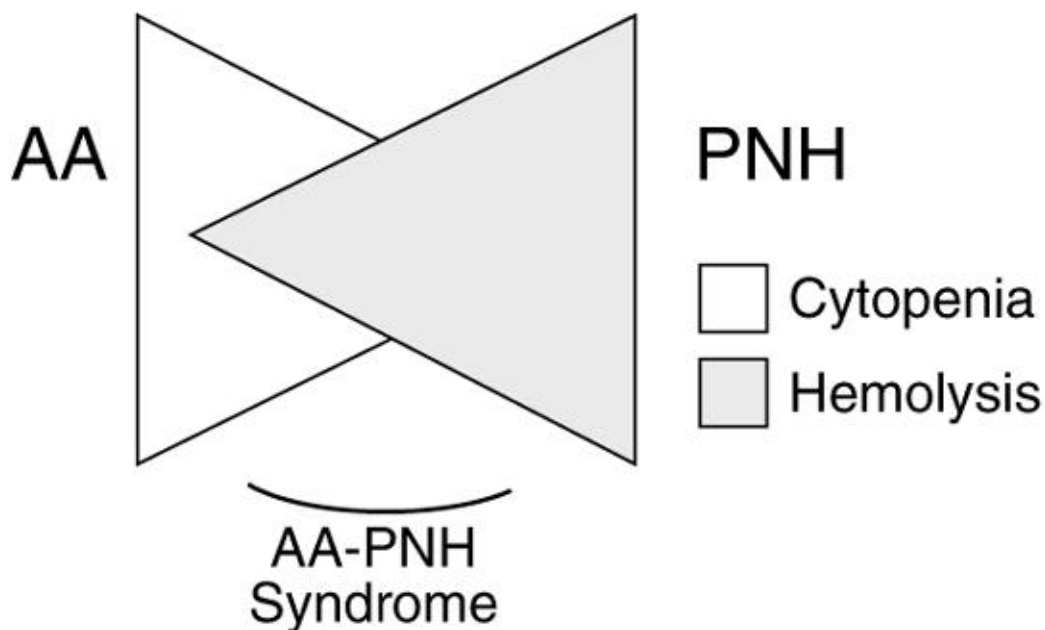


Figure 4. Possible relationship between PNH and aplastic anaemia (AA). The bone marrow contains a PNH clone (triangle) as a result of a somatic mutation in the PIG-A gene; but the expansion of the clone is favored by failure of the normal stem cells.

On the basis of these considerations, a model for the pathogenesis of PNH⁽¹⁷⁾ that explains most of its clinical and haematological features can be outlined as follows (see Figure 4):

- (1) PNH always co-exists with BMF.
- (2) BMF is clinically obvious in patients who initially present with AA and then develop PNH. In patients who initially present with PNH, BMF may not be obvious because by the time of diagnosis the PNH clone has expanded to the point where it provides a substantial proportion of the patient's hematopoiesis.
- (3) The PNH clone has a long but probably finite life span. If, by the time the PNH clone is exhausted, BMF has not recovered, the patient evolves clinically from PNH to AA. If, by the time the PNH clone is exhausted, BMF has recovered, the patient evolves to 'cured' PNH.
- (4) PNH clones arise through spontaneous somatic mutations in the PIG-A gene of haemopoietic stem cells. As long as the remaining stem cells are normal, clinical PNH will not develop. Thus, the development of PNH is conditional on a background of BMF.
- (5) The existence of a florid PNH clone while the rest of hematopoiesis is depressed suggests that the PNH clone can be spared selectively from the injury affecting the rest of the bone marrow.

- (6) In order to explain ⁽⁵⁾, we may surmise specifically that the damage to stem cells causing BMF is mediated through a GPI-linked surface molecule: in this case, the PNH cells lacking these molecules will survive.
- (7) Thus, the very defect of the PNH clone may endow it with a relative survival or growth advantage in a patient with BMF. If the patient has such a clone, he or she will present with PNH; otherwise he or she would present with overt AA. While PNH is often regarded as a further ‘complication’ of AA,⁽²³⁾ it is important to realize that a PNH clone can support substantially the patient’s haemopoiesis.⁽⁸⁾ The existence of two or more PNH clones in the same patient can be seen as a case of convergent evolution in the population genetics of hemopoietic cells.

Treatment

The only definitive treatment for PNH is bone marrow transplantation (BMT). In cases in which bone marrow failure has progressed to the stage of qualifying for severe aplastic anaemia, and if an HLA-identical sibling is available, BMT must be regarded therefore as the treatment of choice.⁽¹⁰⁾ In florid PNH without evidence of severe bone marrow failure, BMT has been attempted only in a handful of cases in which an identical twin was available and without myeloablative and immunosuppressive treatment: this type of BMT has not been successful. In view of the potentially life-threatening nature of the thrombotic complications of PNH, and in view of the currently improved results of conventional BMT from an HLA-identical sibling with full bone marrow ablation, this treatment must be now regarded as a valid option for all patients who have such a donor available and who are willing to accept the attending risks.

Table 2. Some items in the management of PNH.

- **Patience**
- **Folic acid**
- **Iron**
- **Blood transfusion**
- **Anticoagulants (tPA)**
- **Bone marrow transplantation**
- **(Anti-lymphocyte globulin)**
- **Patience**

For all other patients with florid PNH, and for all those who do not have a potential donor, treatment must be supportive (see Table 2). Once the diagnosis is firmly established it is very important to explain to patients that they can live with PNH, perhaps for many years. Without raising hopes too high, it is fair to mention that adequate support may see them through to spontaneous recovery. Blood transfusion is imperative when exacerbation of haemolysis threatens life, but it should not be used on a regular schedule. Rather, the (very variable) tolerance of the individual patient to anaemia should be

assessed, as blood transfusion is indicated only when the haemoglobin level falls below the tolerated level.

As in other chronic anaemias, some patients can lead a virtually normal active life with a steady-state haemoglobin level as low as 70 G/l, and in our experience, not infrequently patients are over-transfused. It is imperative to use on-line white cell filters for all transfusions. The use of this precaution has made it clear that the previously reported instances of haemoglobinuria triggered by blood transfusion result in fact from white cell reactions activating complement rather than from an increase in the haematocrit as such. A neglected cause of worsening anaemia is iron deficiency consequent to urinary iron loss. The MCHC and serum iron (rather than ferritin) should be monitored and iron deficiency corrected with oral iron whenever necessary. Again there is no documented evidence for the anecdotal contention that iron treatment can trigger off more haemoglobinuria.

Any patient with PNH who has experienced venous thrombosis, whether peripheral or hepatic, should be placed on prophylactic warfarin indefinitely, because there is serious risk of recurrence and venous thrombosis is one of the main causes of death. Although it may be regarded as perfectly rational to introduce warfarin in newly diagnosed patients even before they develop thrombosis, this has not been our policy thus far. For the treatment of hepatic venous thrombosis (Budd-Chiari), there is an immediate indication for thrombolytic therapy with tissue plasminogen activation.⁽¹²⁾ To our surprise, this has been effective in some patients even as late as four weeks after the onset of symptoms.

Although the haemolysis in PNH is complement-mediated, there is no evidence that corticosteroids will decrease the rate of haemolysis and there is no rationale for their use in PNH. However, immunosuppressive treatment has been sometimes used successfully.^(10a,21a)

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