High incidence of thrombosis in African-American and Latin-American patients with Paroxysmal Nocturnal Haemoglobinuria

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Summary

Paroxysmal Nocturnal Haemoglobinuria (PNH) results in a marked thrombophilic state by unknown mechanisms. Geographic differences in thrombosis incidence in PNH have been observed. We have reviewed 64 patients with "Classic PNH" from a single institution in order to determine the rate of thrombosis in different ethnic groups. When we compared African-Americans (n=11) and Latin-Americans (n=8) with other patients (n= 45), we found that African-American and Latin-American patients are at increased risk [Hazard ratio 3.66 (p=0.005) and 3.52, (p= 0.035) respectively by Cox regression]. Our data also suggest that this difference in the rate of thrombosis has an impact on length of survival. These findings demonstrate that ethnicity is a risk factor for thrombosis in PNH and have implications for decision-making regarding the management of these patients, including the prevention of thrombosis.

Keywords

Paroxysmal Nocturnal Hemoglobinuria (PNH), ethnicity, thrombosis, hypercoagulable states, deep venous thrombosis

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Introduction

Paroxysmal Nocturnal Haemoglobinuria (PNH) is an acquired clonal stem cell disorder characterized by complement-mediated haemolysis, hypercoagulability, and bone marrow failure, probably on an auto-immune basis (1). Patients with PNH are especially prone to thrombosis in abdominal veins, and with up to 40% incidence (2, 3), there are few conditions that confer as high a risk. Hypercoagulability may be a consequence of: (i) decreased fibrinolysis due to abnormal post-translational modification of the GPI-linked uPAR receptor (4); (ii) increased complement-mediated activation of CD59-negative platelets (5–10); (iii) increased thrombin generation on platelet-derived microparticles (11–13); (iv) abnormalities of the GPI-linked tissue factor pathway inhibitor (14); (v) abnormalities of endothelial cells secondary to the presence of a large PNH clone.

There is a marked geographical disparity in the incidence of thrombosis in PNH, with high rates reported to occur in the United States (15), Europe (2, 3, 16), and India (17), and much lower rates in Mexico, Japan, China, and Thailand (18–21). However, these differences may be due in part to publication bias. No single study has evaluated the effect of ethnicity within a certain district, and there are no data on African-American and Latin-American patients. Here we review a series of ethnically diverse patients referred to a single institution in New York City. We demonstrate that thrombosis is very common in all groups of patients with 'Classic PNH'-- and is particularly common in patients of African-American and Latin-American ancestry.

Methods

Ninety-two patients in this study were identified as having an abnormal flow cytometry test, among samples submitted from over 300 patients from 1995–2003. Samples were typically sent to further evaluate aplastic anemia, hemolytic anemia, unexplained thrombosis, or a prior diagnosis of PNH. Clinically significant haemolysis does not occur in those with very small PNH clones (and neither does thrombosis, with one exception mentioned below), who instead usually have features of acquired aplastic anemia. We have therefore designated 28 patients with ≤ 10%

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GPI-negative red cells and ≤ 25% GPI-negative granulocytes as "AA-PNH". We will consider henceforth the remaining 64 patients with large PNH clones as having "Classic PNH".

We performed flow cytometry studies as described (22) using a panel of anti-CD59, CD24, and CD16 antibodies. In our prior study, any sample with >0.1% GPI(-) red cells, or >2% GPI(-) granulocytes was considered abnormal (e.g., >7 SD above the mean of normal donors), and the finding could be reproducibly demonstrated.

To date the onset of PNH we have accepted the oldest abnormal flow cytometry study (or Ham test), even if performed elsewhere. Thrombosis was dated on the basis of available radiologic imaging. To our knowledge, none of the patients were treated with any anticoagulant prior to their first thrombotic episode: subsequently, all were given anticoagulants. In 4 patients there was evidence of thrombosis 0.3 to 2.4 years prior to the diagnosis of PNH: these patients were considered to have had PNH at the time of their thrombosis. Because BMT is expected to eradicate PNH clones (23), thus eliminating the risk of PNH-related thrombosis, in Kaplan-Meier analysis evaluating the outcome of thrombosis, patients were censored at the time of BMT. The Kaplan-Meier method was used to compute curves for time to thrombosis and overall survival. The log-rank test was used to assess the univariate statistical significance of covariates for predicting time to thrombosis or survival. The Cox proportional hazards regression model was used to estimate the combined predictive value of multiple covariates which were significant on univariate analysis ($p<0.05$). The occurrence of thrombosis and undergoing BMT were also treated as time-dependent covariates in a Cox regression model for overall survival. Of the 64 patients with "Classic PNH", 43 were White, 11 African-American, 8 Latin-American, and 2 Asian-American. Because of the small number of Asian-Americans, they were analyzed along with White patients.

Results and Discussion

Thrombosis is more common in African-American patients with PNH and this may have an impact on survival

The clinical features of the 3 groups of patients with "Classic PNH" were similar, although the African-American patients were, as a group, somewhat younger ($p=0.07$ for age by analysis of variance, NS for all other variables, Table 1)—-indeed African-Americans are known from census data to be, as a group, younger than the White population. Overall, 28 out of the 64 patients with "Classic PNH" developed thrombosis (Fig. 1A). 68% of thrombotic events were intra-abdominal, and 12% were in the intracranial dural venous sinuses. By contrast, of the 28 patients with "AA-PNH", only one (a 30-year-old man) developed thrombosis, and this was at an unusual site—- a retinal vein (Fig. 1A). Compared with White patients, the proportion of African-American patients who ever experienced thrombosis was twice as high (Table 1), and the hazard ratio for time to first thrombosis in this group was 3.66 ($p=0.005$). Similarly, the hazard ratio was elevated [3.52 ($p=0.035$)] in Latin-American patients (Fig. 1B).

As observed by others (24), the mean proportion of PNH granulocytes was higher in those who developed thrombosis (84%) versus those who never developed thrombosis (68%); this difference was observed in all three ethnic sub-groups. The only other potential covariates that predicted time to first thrombosis were increased PNH II counts and increased neutrophil counts. However, these risk factors appear to be independent of race and their inclusion in a Cox regression model had minimal effect on the magnitude of the hazard ratios for African-American and Latin-American patients.

In this small series of PNH patients the overall survival was significantly shorter in African-Americans than in White patients (Table 1 and Fig. 1C); survival was not significantly different between Latin-American patients and the other patients. Out

Table 1: Haematological parameters and outcome in patients with PNH based on ethnicity.

	African-American (n=11)	Latin-American (n=8)	Other patients (n=45)
Age at diagnosis	23.7 (12–49)	33.5 (13.3–56)	35.7 (15.3–72.1)
Absolute neutrophils x 10^3/µl	2.7 (0.2–8.6)	2.6 (1.3–4.1)	3.3 (0.3–15.9)
% Reticulocyte count	5.1 (0.3–15)	5.4 (3.4–7.8)	9.2 (0.4–39)
Haemoglobin (g/dl)	9.3 (5.3–13.3)	9.5 (8–11)	9.6 (5.5–13.9)
Platelet count x 10^3/µl	90.5 (15–227)	100 (22–207)	138.3 (10–708)
Lactate Dehydrogenase (Units/L)	1044 (228–3670)	903 (378–1683)	1536 (134–4400)
% PNH II red cells	5 (0–21)	6.1 (0–20)	7.2 (0–99)
% PNH III red cells	28.7 (10–83)	20.3 (3–46)	34.9 (0.2–88)
% PNH PMN's	78 (15–100)	75.3 (28–97)	74.5 (4.8–100)
Thrombosis # (%)	8 (73%)	4 (50%)	16 (36%)
Hazard ratio for thrombosis (95% CI)	3.66 (1.48–9.07)	3.52 (1.09–11.32)	1.00
Died (%)	4 (36%)	1 (13%)	5 (11%)
Hazard ratio for death (95 % CI)	4.62 (1.23–17.43)	5.18 (0.53–51.0)	1.00

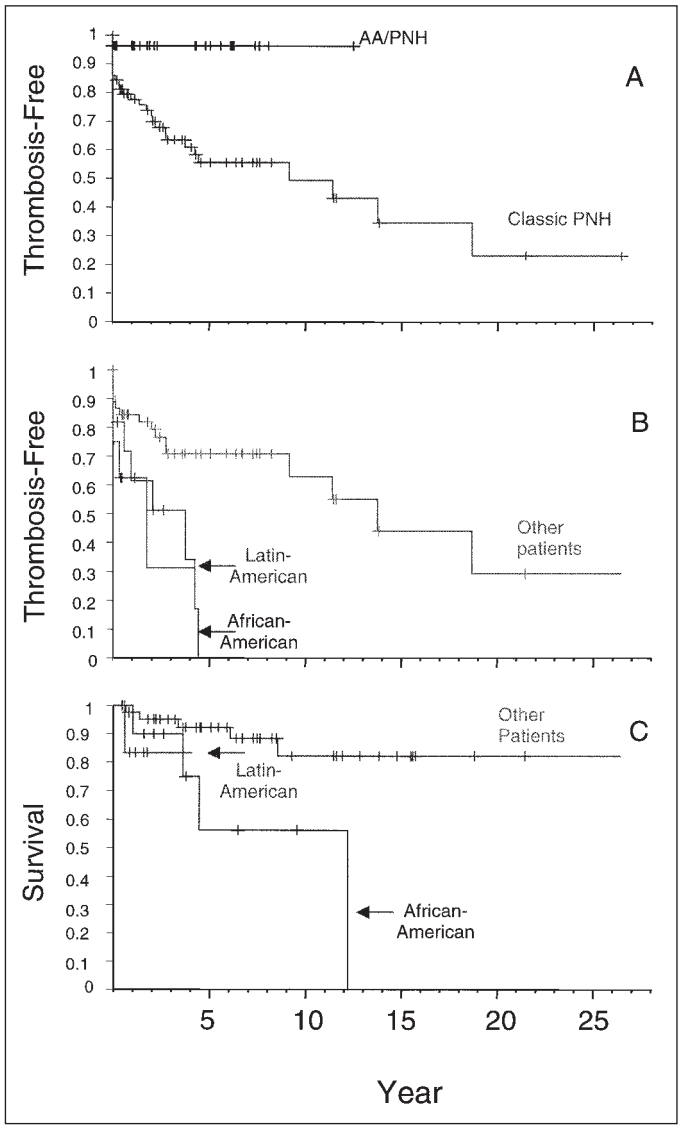

Figure 1: Outcome in patients with "Classic PNH" is influenced by ethnicity. (A) Thrombosis is a common complication in patients with "Classic PNH"—-affecting 44% of patients overall—-but is very rare in "AA-PNH". (B) African American and Latin-American patients are at increased risk of thrombosis [Hazard ratio 3.66 (p=0.005) and 3.52, (p = 0.035) respectively by Cox regression]. (C) African-American patients with PNH are at increased risk of mortality [Hazard ratio 4.62, 95% confidence interval 1.23–17.43]. In panel (A) and (B), patients were censored at the time of BMT. Since all of the patients who expired had previously had a thrombosis or had underwent BMT, thrombosis and thrombosis-free survival are equivalent.

of a total of 10 deaths, 3 occurred among the 6 patients who underwent BMT and were considered complications of the procedure. Of the remaining 7 deaths, 4 were directly attributable to thrombosis; and in 2 patients, although severe cytopenias were regarded as the primary cause of death, sequelae of thrombosis were regarded as contributory. Both BMT ($p<0.0001$) and occurrence of thrombosis ($p=0.003$) significantly increased subsequent mortality when entered as time-dependent covariates in a Cox regression model. Thrombosis has previously been associated with an increased risk of death (2).

Five patients were given tissue plasminogen activator to treat 6 episodes of diagnosed life-threatening intracranial or intra-abdominal thromboses. TPA was given within 1 month of the suspected onset of thrombosis as a 1 mg/kg slow intravenous infusion over 24 hours as previously described (25), resulting in resolution of thrombosis on 5 out of 6 occasions.

Hypotheses on the mechanism of increased risk in African-Americans

While differences in blood pressure, lipid profiles, and glucose tolerance may account for a higher risk of coronary disease in African-Americans, risk of venous thrombosis does not differ much in different ethnic groups in the United States (26, 27). Because the earliest pathologic abnormality in PNH is likely to be an autoimmune attack on hematopoietic stem cells (1), it is conceivable that there are differences in the target antigen or in the effector response among patient groups, that ultimately affect thrombosis risk. However, there was over-representation of patients with DR2-associated alleles in African-American patients (44%) and in White patients (23%), and autoimmune bone marrow failure by itself does not result in thrombosis (Fig. 1A). It is more likely that there are ethnic differences that relate to the mechanism of thrombosis specifically in PNH but not in idiopathic deep venous thrombosis. For example there might be interpersonal and inter-ethnic differences in the rate of complement deposition on platelets, which would be clinically significant only when blood cells are deficient in CD59, as is the case in PNH.

Prevention of thrombosis

For prevention of thrombosis, three approaches are available: (i) bone marrow transplantation; (ii) secondary prophylaxis with long-term anticoagulation for patients who have had thrombosis; and (iii) primary prophylaxis with anticoagulation for selected patients or for all patients who do not have a contraindication.

In a recent study, primary prophylaxis was effective in preventing an initial thrombosis, but at some risk of death from hemorrhage (24). If only selected patients are to be treated with primary prophylaxis, the decision should be based mainly on the co-existence, with the severely thrombophilic tendency of PNH itself, of known genetic and acquired thrombosis risk factors. Now we know that ethnic factors must be considered as well. Understanding the high risk of thrombosis in African-American and Latin-American patients may also influence decisions regarding bone marrow transplantation in PNH.

It should be noted that the standard of care for anti-coagulation in general is evolving, such that long-term anticoagulation is increasingly being considered acceptable to reduce the risk of recurrent *idiopathic* DVT (28) — a less serious threat than thrombosis in PNH. It is likely that these decisions will be facilitated with the availability of oral direct thrombin inhibitors--which have predictable effects on coagulation times and may render anticoagulation safer, more consistently effective, and more acceptable to the patient.

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