

# Clinical signs and symptoms associated with increased risk for thrombosis in patients with paroxysmal nocturnal hemoglobinuria from a Korean Registry

Jong Wook Lee · Jun Ho Jang · Jin Seok Kim ·  
Sung-Soo Yoon · Je-Hwan Lee · Yeo-Kyeong Kim ·  
Deog-Yeon Jo · Jooseop Chung · Sang Kyun Sohn

Received: 18 January 2013/Revised: 15 April 2013/Accepted: 15 April 2013  
© The Japanese Society of Hematology 2013

**Abstract** Paroxysmal nocturnal hemoglobinuria (PNH) is characterized by chronic, complement-mediated hemolysis, frequently leading to debilitating clinical symptoms and life-threatening complications such as thromboembolism (TE). A retrospective analysis was performed on 301 patients from the South Korean National PNH Registry to describe disease burden and identify TE-associated risk factors. TE was identified in 18 % of patients and was associated with increased risk for mortality [odds ratio (OR), 6.85;  $P < 0.001$ ]. A multivariate analysis showed that PNH patients with elevated hemolysis [lactate dehydrogenase (LDH) levels  $\geq 1.5$  times the upper limit of normal (ULN)] at diagnosis were at significantly higher risk for TE than patients with LDH  $< 1.5 \times$  ULN (OR 7.0;  $P = 0.013$ ). The combination of LDH  $\geq 1.5 \times$  ULN with the clinical symp-

toms of abdominal pain, chest pain, dyspnea, or hemoglobinuria was associated with a greater increased risk for TE than elevated hemolysis or clinical symptoms alone. Continuous monitoring of these risk factors is critical for identifying PNH patients at risk for morbidities and mortality and allowing early intervention. (clinicaltrials.gov identifier: NCT01224483).

**Keywords** Risk factor · Hemolysis · Thrombosis · Mortality · Paroxysmal nocturnal hemoglobinuria

## Introduction

Paroxysmal nocturnal hemoglobinuria (PNH) is a progressive and life-threatening disease characterized by chronic complement-mediated hemolysis leading to severe

Jong Wook Lee and Jun Ho Jang contributed equally to this work.

J. W. Lee (✉)  
Division of Hematology, Seoul St. Mary's Hospital, College of Medicine, The Catholic University of Korea, 505 Banpo-dong, Seocho-gu, Seoul 137-701, Korea  
e-mail: jwlee@catholic.ac.kr

J. H. Jang  
Division of Hematology-Oncology, Samsung Medical Center, Sungkyunkwan University School of Medicine, Seoul, Korea

J. S. Kim  
Division of Hematology, Yonsei University College of Medicine, Seoul, Korea

S.-S. Yoon  
Division of Hematology-Oncology, Seoul National University College of Medicine, Seoul, Korea

J.-H. Lee  
Division of Hematology, University of Ulsan College of Medicine, Seoul, Korea

Y.-K. Kim  
Division of Hematology-Oncology, Chonnam National University Medical School, Gwangju, Korea

D.-Y. Jo  
Division of Hematology-Oncology, Chungnam National University School of Medicine, Daejeon, Korea

J. Chung  
Division of Hematology-Oncology, Pusan National University School of Medicine, Pusan, Korea

S. K. Sohn  
Division of Hematology-Oncology, Kyungpook National University School of Medicine, Daegu, Korea

morbidities and early mortality [1, 2]. PNH arises from a somatic mutation in the phosphatidylinositol glycan class A (*PIG-A*) gene [3], which prevents the synthesis of *N*-acetyl-D-glucosamine phosphatidylinositol, an essential component of glycosylphosphatidylinositol (GPI) anchor protein (GPI-AP). Deficiency of GPI-anchored complement inhibitor proteins CD55 (a decay-accelerating factor) and CD59 (a membrane inhibitor of reactive lysis) leads to chronic uncontrolled complement activation on the surface of blood cells. This results in increased red blood cell (RBC) hemolysis and direct activation of platelets and macrophages, causing chronic inflammation, thromboembolism (TE), ischemia, and end organ damage [4, 5].

PNH patients suffer from a range of frequently debilitating and distressing clinical symptoms due to chronic complement activity and hemolysis, including abdominal pain, chest pain, dyspnea, and hemoglobinuria, as well as life-threatening complications such as TE, pulmonary hypertension, and impaired renal function [2, 5–8]. Despite treatment with anticoagulants, corticosteroids, and transfusions, approximately 15–35 % of PNH patients die within 5 years of diagnosis [1, 6, 9, 10]. The most frequent cause of death in patients with PNH, despite best supportive care, is TE, accounting for 40–67 % of PNH-related deaths [2, 7].

To better understand the disease burden and the risk factors associated with TE in PNH, a retrospective analysis was performed on data from 301 PNH patients enrolled in the South Korean National Registry. This registry is a resource for long-term PNH disease observation, and it includes recently collected laboratory and clinical parameters. Data from this registry provided a representative profile of PNH disease course and outcomes. The aim of this study was to describe the disease burden and to systematically identify risk factors associated with TE in PNH patients.

## Design and methods

### Patients and diagnosis

The Aplastic Anemia (AA) Working Party of the Korean Society of Hematology established a nationwide registry of PNH patients. Nine institutions participate in the South Korean National PNH Registry, which includes approximately 96 % of all patients in the South Korean PNH population based on the number of PNH patients reported by the National Health Insurance Corporation. A retrospective chart review was performed on 301 patients diagnosed with PNH. All patients were enrolled in the registry in 2009, and the retrospective chart review included medical history data from the time of diagnosis to enrollment. Patient data included in this registry were captured using an electronic case report form that collected patient demographics,

medical history, and PNH-specific information, including RBC and granulocyte clone size, symptoms and complications, laboratory values, treatments, and, when applicable and available, cause of death.

When possible, PNH was confirmed at each individual site using a consistent flow cytometry protocol based on the analysis of expression of CD55 and CD59, as described by Kim et al. [11]. Briefly, phycoerythrin-labeled anti-CD55 or anti-CD59 antibody was added to samples of blood cells and, following incubation and washing, suspended in thiazole orange reagent prior to analysis. Approximately 50000 cells were analyzed in each sample. Minimum sensitivity varied over time and between institutions, depending upon the technology employed at each site. However, in the most recent assessments, the minimum clone size detection at most of the sites was 0.1 % for white blood cells and 3 % for RBCs. The percentage of GPI-AP-deficient cells was determined from assessments at diagnosis or at the closest time point to diagnosis.

In patients diagnosed before the establishment of flow cytometry, a positive Ham or sucrose-lysis test was used. The PNH granulocyte and RBC clone sizes were based on available data from chart reviews and were not limited to minimum clone size or flow methodology.

Elevated hemolysis was defined as lactate dehydrogenase (LDH) level  $\geq 1.5$  times the upper limit of normal (ULN) [7, 12–14]. LDH at diagnosis was chosen as a consistent point of reference for all analyses. In patients with elevated LDH at diagnosis, the occurrence of TE within 6 months of diagnosis was analyzed to determine the temporal relationship between LDH level and TE. In the presence of a zero frequency, it is not strictly possible to estimate the odds ratio (OR) and the 95 % confidence interval (CI); thus, in a sensitivity analysis any zero values were imputed as 0.5.

Clinical PNH symptoms, including abdominal pain, chest pain, dyspnea, and hemoglobinuria, were based on physician reporting in medical charts, but did not necessarily capture the symptom onset date. Dates of diagnosis, flow cytometric assessments, bone marrow transplant (BMT), TE, and death were accurately recorded. Incidence of TE was calculated in two ways: either as occurrence at a specific time point or as cumulative incidence over a specific time period. Incidence of TE was reported in clone size categories of <20, 20–50, and >50 %. The cumulative incidence of TE was collected for the time periods prior to and post diagnosis of PNH. This study was conducted in accordance with the Declaration of Helsinki and was reviewed and approved by the institutional review boards of participating hospitals.

### Statistical analysis

Binary variables were analyzed using univariate and multivariate logistic regression. Potential risk factors

included LDH  $\geq 1.5 \times$  ULN at diagnosis, abdominal pain, chest pain, dyspnea, hemoglobinuria, history of RBC transfusion, and granulocyte clone size. LDH values at diagnosis were used in all analyses to investigate their association with clinical outcomes.

For multivariate analyses, the statistical model included terms for all potential risk factors—age, gender, and bone marrow failure (BMF)—with nonsignificant factors being removed. The null hypothesis of no association between the occurrence of TE and a specific risk factor was tested using the log-likelihood ratio statistic, which follows a Chi-squared distribution. Results are presented as ORs and 95 % CIs.

To test whether the LDH threshold of  $\geq 1.5 \times$  ULN was an appropriate cutoff value for assessing risk of TE, receiver operating characteristic (ROC) analysis was used to investigate the effects of using cutoff points of LDH  $\geq 3.0 \times$  ULN and LDH  $\geq 5.0 \times$  ULN as compared with the LDH  $\geq 1.5 \times$  ULN cutoff point.

## Results

### Patient demographics and disease burden

A summary of patient demographics, medical history, and PNH-specific information for the 301 PNH patients is provided in Table 1. Median age at diagnosis was 37 years (range 8–88 years), median follow-up time from diagnosis was 6.6 years (range 0–28.8 years), and there was an approximately equal number of male and female patients. Diagnosis by flow cytometry of either granulocyte or RBC clone or both was reported for 236 patients (78.4 %), Ham and sucrose tests for 56 patients (18.6 %), Ham test only for 7 patients (2.3 %), and sucrose test only for 2 patients (<1 %). Further analysis showed that there were no significant differences in prevalence of TE between patients diagnosed using the different diagnostic methods (data not shown). Median granulocyte and erythrocyte clone sizes were 48.8 and 28.1 %, respectively.

At diagnosis, 171 of 224 patients (76.3 %) with recorded LDH levels had values  $\geq 1.5 \times$  ULN (Table 1). The most frequently reported clinical symptoms were hemoglobinuria (56 %), pain (56 %), and abdominal pain (47 %) (Table 1). The reports of hemoglobinuria were most likely self-reported macroscopic hemoglobinuria, as this symptom was not objectively assessed. Corticosteroids (77.4 %), transfusions (59.1 %), and nonsteroidal anti-inflammatory drugs (NSAIDs) (21.9 %) represented the most common supportive care provided (Table 1). Anticoagulation therapy was administered to 14.6 % of patients and immunosuppressive therapy to 20.6 %

**Table 1** Burden of disease in PNH

Patient demographics and disease characteristics	<i>n</i> = 301
Age, years	
Median (range)	37 (8–88)
Mean (SD)	39.3 (15.4)
Patients <40 years, <i>n</i> (%)	172 (57.1)
Gender, female, <i>n</i> (%)	149 (49.5)
Additional bone marrow disorder, <i>n</i> (%)	
Aplastic anemia	121 (40.2)
Myelodysplastic syndrome	19 (6.3)
PNH granulocyte clone size, % ( <i>n</i> = 195)	
Median (range)	48.8 (0–100)
Mean (SD)	49.5 (30.8)
PNH RBC clone size, % ( <i>n</i> = 199)	
Median	28.1 (0–99.8)
Mean (SD)	33.2 (27.8)
LDH, fold above ULN ( <i>n</i> = 224)	
Median (range)	4.1 (0.2–36.3)
Mean (SD)	5.6 (5.5)
$\geq 1.5 \times$ ULN, <i>n</i> (%)	171 (76.3)
Follow-up since diagnosis, years	
Median (range)	6.6 (0–28.8)
Mean (SD)	7.8 (6.0)
Complications of PNH, <i>n</i> (%)	
TE	54 (17.9)
Venous	37 (68.5 <sup>a</sup> )
Arterial	17 (31.5 <sup>a</sup> )
Hemoglobinuria	169 (56.1)
Abdominal pain	141 (46.8)
Dyspnea	111 (36.9)
Chest pain	39 (13.0)
Pain	169 (56.1)
Prior or concomitant therapies, <i>n</i> (%)	
Corticosteroids	233 (77.4)
RBC transfusions	178 (59.1)
NSAIDs	66 (21.9)
Immunosuppressive treatment	62 (20.6)
Anticoagulation	44 (14.6)
Opioids	39 (13.0)
Bone marrow transplant	37 (12.3)

SD standard deviation

<sup>a</sup> % of patients with TE

(Table 1). Medical intervention was required in 66 of 169 patients (39.1 %) reporting pain; the most common interventions were NSAIDs, administered to 37 of these 66 patients (56.1 %), and opioids, administered to 22 of them (33.3 %). No patient received treatment with the terminal complement inhibitor eculizumab.

## Characteristics of TE

TE was reported in 54 patients (17.9 %) (Table 1), of whom 19 (35.2 %) had multiple TE events. TE occurred at both venous (69.1 %) and arterial (30.9 %) sites. TE was found in typical sites, including deep vein, pulmonary vein, and renal vein, as well as in atypical sites, including cerebral vein and renal artery (Table 2). Fifty-three percent of the TE events were reported in patients with physician-reported classic PNH and 47 % in patients with physician-reported underlying BMF; there was no statistically significant difference in the prevalence of reported TE between these two patient populations ( $P = 0.981$ ). In patients with a reported TE and granulocyte clone ( $n = 37$ ), TE was recorded in all three clone size categories assessed (< 20, 20–50, and >50 %), with the prevalence of TE being 16, 19, and 20 %, respectively; there was no evidence of any association between clone size category and the risk of experiencing a TE ( $P = 0.843$ ; Fig. 1). As full details of RBC transfusions are not collected in the PNH registry, we were only able to determine if patients did or did not have a history of transfusions. Despite this limitation, our data did suggest that there was a statistically significant association between a history of transfusion and increased risk of TE ( $P < 0.05$ ).

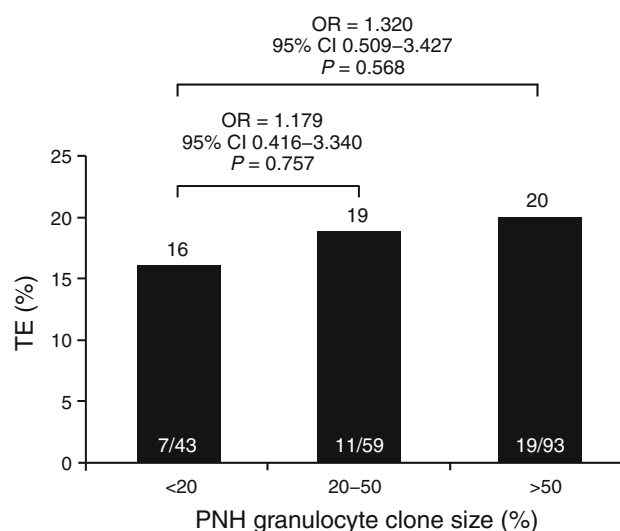
Five of 16 patients receiving prophylactic anticoagulant therapy (31.3 %) experienced a first thrombosis and 12 of 21 patients receiving therapeutic anticoagulant treatment (57.1 %) experienced subsequent TE events during

**Table 2** Location of TE

TE site	Events, <i>n</i>	% of total
Venous	56	69.1
Deep vein thrombosis (low extremity)	16	19.8
Pulmonary embolus	10	12.3
Renal vein thrombosis	9	11.1
Mesenteric, visceral vein thrombosis	9	11.1
Hepatic, portal vein thrombosis	7	8.6
Cerebral venous occlusion	2	2.5
Gangrene	2	2.5
Dermal thrombosis	1	1.2
Arterial	25	30.9
CVA	12	14.8
Mesenteric arterial thrombosis	5	6.2
Myocardial infarction	4	4.9
Unstable angina	3	3.7
Renal artery obstruction	1	1.2
Total	81 <sup>a</sup>	100

CVA cerebrovascular accident

<sup>a</sup> Occurred in 54 patients: 1 event in 35 patients, 2 events in 13 patients, 3 events in 4 patients, and 4 events in 2 patients



**Fig. 1** Incidence of TE in PNH granulocyte clone size categories. Overall test of association between clone size and TE:  $\chi^2 = 0.341$ , 2df,  $P = 0.843$

anticoagulant treatment. Based on a multivariate analysis, TE was strongly associated with an increased risk for mortality (OR 6.85; 95 % CI 2.90–16.18;  $P < 0.001$ ).

## Risk factors associated with TE

### Elevated LDH

Table 3 presents the demographics and disease characteristics at diagnosis in patients with and without TE. Median LDH at diagnosis was higher in patients with TE than in those without TE, with the difference between the two groups bordering on statistical significance ( $P = 0.05$ ). Age, history of bone marrow disorders, PNH granulocyte clone size, platelet count, and hemoglobin did not differ significantly in patients with or without TE (Table 3).

Univariate analysis showed patients with LDH  $\geq 1.5 \times$  ULN at diagnosis had a significantly increased incidence of TE [43 of 171 (25.1 %)] compared with patients with LDH  $< 1.5 \times$  ULN [2 of 53 (3.8 %); OR 8.57; 95 % CI 2.00–36.68;  $P < 0.001$ ]. Adjusting for age, gender, and BMF, multivariate analyses confirmed that elevated LDH  $\geq 1.5 \times$  ULN at diagnosis was independently associated with increased odds of experiencing TE (OR 7.0; 95 % CI 1.5–32;  $P = 0.013$ ; Fig. 2).

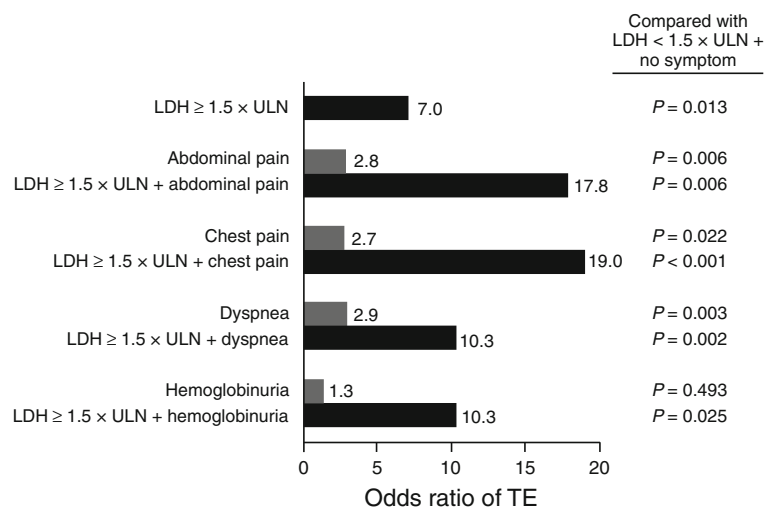
The occurrence of TE within 6 months of the LDH assessment was analyzed to determine if there was a temporal association between LDH level and TE. Eighteen of the 171 patients with LDH  $\geq 1.5 \times$  ULN (10.5 %) had a TE within the 6 months prior to or following LDH assessment, whereas no TE was reported within 6 months of a reported LDH  $< 1.5 \times$  ULN. After adjusting for the

**Table 3** Demographics and disease characteristics in patients with TE and without TE

Parameter	Total patients ( <i>n</i> = 301)	TE ( <i>n</i> = 54)	No TE ( <i>n</i> = 247)	<i>P</i> value
Age, median (range)	37 (8–88)	38 (19–88)	36 (8–88)	0.425
History of bone marrow disorders, %	47	47	47	0.981
Median granulocyte clone size, % (range) <sup>a</sup>	49 (0–100)	50 (1–100)	49 (0–100)	0.189
Granulocyte clone size $\geq 50$ %, %	48	51	47	0.621
White blood cell count, median, $\times 10^9/L$	3.5	3.7	3.45	0.604
Platelet count, median, $\times 10^9/L$	100	98	94	0.481
Hemoglobin, median, g/dL	7.8	7.5	8	0.111
LDH fold, median (range)	4.1 (0.2–36)	4.8 (1–17)	3.9 (0–36)	0.05
Patients with LDH $\geq 1.5 \times$ ULN at diagnosis, %	76.3	95.6	71.5	<0.001

<sup>a</sup> *n* = 195

**Fig. 2** Multivariate analysis of the effect of LDH  $\geq 1.5 \times$  ULN and clinical symptoms on associated risk of TE

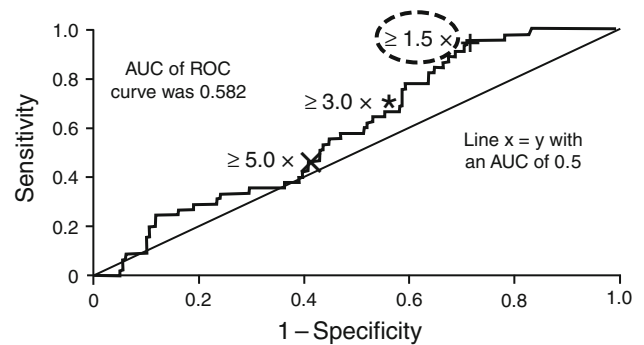


zero frequency of TE by imputing the value as 0.5, the odds of a TE within 6 months of an LDH assessment was 14.3 times higher with LDH  $\geq 1.5 \times$  ULN than with LDH  $< 1.5 \times$  ULN ( $P = 0.004$ ).

A ROC analysis was performed to determine which LDH value would represent the most sensitive threshold to detect TE (Fig. 3). This analysis demonstrated that LDH  $\geq 1.5 \times$  ULN as a threshold detected 96 % of the patients with TE and also showed that an LDH threshold of  $\geq 3.0 \times$  ULN or  $\geq 5.0 \times$  ULN at diagnosis detected only 67 or 47 % of the population with TE, respectively. Furthermore, logistic regression analysis demonstrated that neither LDH  $\geq 3.0 \times$  ULN nor LDH  $\geq 5.0 \times$  ULN was independently associated with risk of TE ( $\geq 3.0 \times$  ULN: OR 1.5, 95 % CI 0.78–3.07,  $P = 0.208$ ;  $\geq 5.0 \times$  ULN: OR 1.3, 95 % CI 0.66–2.45,  $P = 0.476$ ).

*Clinical symptoms*

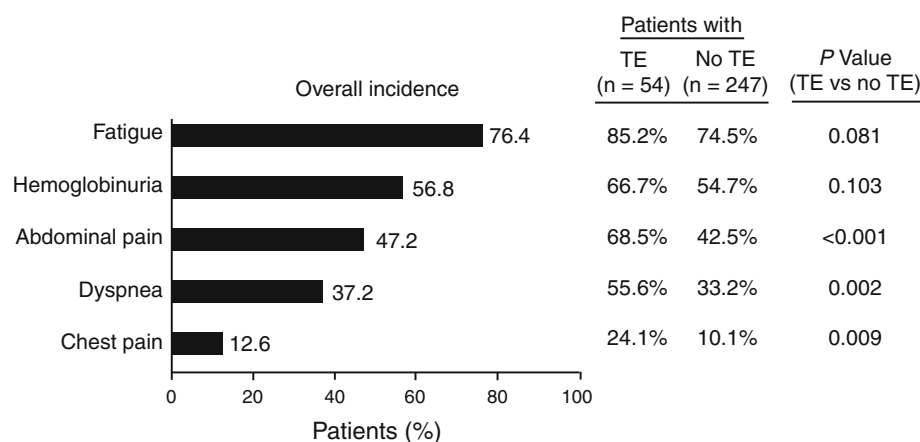
The incidences of abdominal pain, chest pain, and dyspnea were significantly increased in patients with TE compared with those without TE (Fig. 4). Multivariate analysis



**Fig. 3** Receiver operating characteristic curve of LDH cutoff for detecting TE

demonstrated that patients with abdominal pain, chest pain, and dyspnea had significantly increased odds of experiencing a TE compared with patients with no symptom (Fig. 2). Patients with LDH  $\geq 1.5 \times$  ULN at diagnosis were at increased risk of experiencing TE; the odds of a TE were further increased when patients presented with LDH  $\geq 1.5 \times$  ULN at diagnosis and any of the clinical symptoms of abdominal pain, chest pain, dyspnea, or

**Fig. 4** Incidence of clinical symptoms in the overall patient population and in patients with or without TE



hemoglobinuria (Fig. 2). This was particularly pronounced for abdominal pain (OR 17.79; 95 % CI 2.33–36.01) and chest pain (OR 19.04; 95 % CI 3.74–96.99) ( $P \leq 0.006$ ), but it was also seen in patients with dyspnea (OR 10.35; 95 % CI 2.31–46.45) and hemoglobinuria (OR 10.28; 95 % CI 1.34–79.02) ( $P \leq 0.025$ ).

## Discussion

This report describes the burden of disease, the clinical characteristics of TE, and the risk factors associated with TE in PNH patients from the South Korean National PNH Registry. PNH patients who had a history of TE exhibited a significantly increased incidence of elevated hemolysis ( $\text{LDH} \geq 1.5 \times \text{ULN}$ ) at diagnosis compared to patients with no history of TE. Abdominal pain, chest pain, and dyspnea—clinical symptoms associated with intravascular hemolysis—were significantly more prevalent in patients with TE. Age, history of bone marrow disorder, and platelet count did not differ significantly in patients with or without TE.

The prevalence of TE in this South Korean population (17.9 %) is similar to that reported in previous registry studies and historical reports on European patient populations. Studies with French and United Kingdom PNH patients have shown that approximately 28–39 % of PNH patients had thromboses over comparable observation times [1, 9, 10, 15, 16]. However, a large study by Nishimura et al. [6] in 209 Japanese PNH patients reported a much lower prevalence of TE among this patient population (approximately 5–10 %), which led to the perception that TE does not play a significant role in PNH-related death in the Asian population. This difference may be related to the fact that a larger percentage of patients in the Japanese population than in our South Korean population also had a diagnosis of AA (79 vs 40 %). Methodological differences in the gathering of prior TE events may also

have exacerbated this discrepancy; whereas we retrospectively and objectively reviewed all patient medical charts from electronic medical records, the Japanese study relied on physician questionnaires, which may have resulted in the underreporting of events, a known risk in survey-based studies. Finally, the low prevalence of TE reported by Nishimura et al. was not confirmed by a more recent study in Japanese patients with PNH [17], which reported that 17 % of patients entering the trial had a history of TE, which is consistent with the prevalence reported in the current study.

This is the first large study to identify chronic complement-mediated hemolysis, as measured by elevated LDH ( $\geq 1.5 \times \text{ULN}$ ) at diagnosis, as a significant and independent factor associated with increased risk of TE in PNH patients. Patients with elevated LDH at diagnosis had a sevenfold increase in the odds of experiencing a TE compared with patients with  $\text{LDH} < 1.5 \times \text{ULN}$ . The threshold of  $\text{LDH} \geq 1.5 \times \text{ULN}$  at diagnosis has been established in the literature and regulatory PNH clinical trials [5, 7, 8, 12–14, 18]. Our analysis shows that this LDH threshold more effectively identifies PNH patients with significantly increased risk of TE than the LDH thresholds of  $\geq 3.0 \times \text{ULN}$  or  $\geq 5.0 \times \text{ULN}$  at diagnosis. We establish that the risk of TE is significant and that patients should be monitored closely for TE and other poor outcomes when LDH levels exceed  $\geq 1.5 \times \text{ULN}$ .

Patients with  $\text{LDH} \geq 1.5 \times \text{ULN}$  who also presented with at least one of the four clinical symptoms associated with hemolysis had a further increased risk of TE compared with patients with  $\text{LDH} \geq 1.5 \times \text{ULN}$  alone. This increased TE risk may suggest that these symptoms are a result of chronic hemolysis or uncontrolled complement activity and reflect progressive end organ damage through chronic nitric oxide (NO) depletion, ischemia, and thrombosis. For example, pathological examination of intestinal biopsies reveals focal acute and chronic inflammation, fibrin thrombi, and ischemic necrosis in intra-abdominal



vessels in hemolytic PNH patients [19–21], and Hill et al. [22] reported evidence of myocardial scars suggestive of previous unsuspected ischemic damage in 2 of 10 hemolytic PNH patients (20 %) with no reported thrombosis.

In addition to being a potential risk factor for TE, symptoms such as abdominal pain, chest pain, and dyspnea contribute to the poor quality of life suffered by PNH patients [23–25]. Of note, our investigation showed that 33 % of patients reporting pain and requiring medical intervention were administered opioids for pain management, further demonstrating the significant burden of disease in PNH patients. These PNH-related symptoms have been linked to aberrant smooth muscle dystonia mediated by depletion of NO by increased levels of plasma-free hemoglobin released from lysed RBCs [5, 24]. Because these symptoms are significantly associated with risk of TE and possibly an indicator of organ damage, careful monitoring and early intervention are warranted when they are identified during the patient workup.

The relationship between clone size, symptoms, and outcomes of PNH remains, at present, unclear. Previous studies have suggested that patients with a larger granulocyte clone size (i.e., >50 %) are at an increased risk of TE [26, 27]. These reports provided a rationale for analyzing the prevalence of TE by clone size categories of <20, 20–50, or >50 % in our cohort. In contrast to the findings of these studies, our analysis did not indicate any significant relationship between clone size category and TE outcome. Although we did see a higher prevalence of TE in patients with clones >50 %, TE was also reported in patients with clone sizes of <50 %; in fact, 16 % of patients with a clone size <20 % reported a TE. Our results may differ from previous reports because of the large number of patients with smaller clone sizes who had hemolysis: 36 % of patients with a clone size of <20 % and 75 % of patients with a clone size of 20–50 % had  $\text{LDH} \geq 1.5 \times \text{ULN}$ . Unfortunately, directly comparative data are not presented in the previous studies. Hall et al. [26] did not report the median clone size or the prevalence of hemolysis in patients with clone sizes <50 %, and approximately half of the patient population in Moyo et al. [27] had concomitant AA and may, therefore, have been less likely to exhibit excessive hemolysis ( $\text{LDH} \geq 1.5 \times \text{ULN}$ ). Indeed, none of these latter patients reported symptoms of hemoglobinuria, esophageal spasm, or impotence, symptoms that are highly prevalent in PNH patients with hemolysis. Thus, the Hall and Moyo study populations may have included comparatively few patients with small PNH clones and hemolysis, which may in part account for their findings of an association between clone size and risk of TE.

Pu et al. [28] recently analyzed the association between clones and elevated LDH; their findings suggested a linear

relationship between LDH and granulocyte clone size, with a clone size of at least 23 % being a threshold predictor for laboratory evidence of elevated hemolysis. However, as noted, over a third of our population with a clone size below this threshold still had  $\text{LDH} \geq 1.5 \times \text{ULN}$ . Again, the difference between these results may be related to the differences in the patient populations, as Pu et al. focused on patients with AA or severe AA, with all patients having bone marrow cellularity of <20%. They reported that only 6 of 27 patients (22 %) had  $\text{LDH} \geq 1.5 \times \text{ULN}$  at diagnosis, and of the 15 patients with clone size <15 %, only 2 (13 %) had elevated LDH levels. In our study, by contrast, over 76 % of patients had  $\text{LDH} \geq 1.5 \times \text{ULN}$  at diagnosis, including 36 % of patients with a clone size of <20 %. This may explain why we observed a greater prevalence of  $\text{LDH} \geq 1.5 \times \text{ULN}$  and TE in patients with smaller clones and why the association between clone size and TE outcomes in our data was not statistically significant. Our data suggest that the risk of TE can potentially be more accurately assessed from LDH levels than from clone size alone. Clone size will inevitably be an important element in a patient's clinical risk profile, but a small clone size should not be considered to preclude the risk of TE, and management of TE in PNH should be independent of granulocyte clone size. However, given the limitations of a retrospective dataset, we acknowledge that further research needs to be carried out to elucidate the exact nature of the relationship between PNH clone size, hemolysis, and risk of TE.

Multivariate analysis demonstrated that TE was associated with a significantly increased risk of mortality. Previous observational PNH studies [1, 15] have also demonstrated a high mortality rate for PNH patients at risk for TE, suggesting that prevention and management of TE are critical for patients with PNH. More recent data from a French registry of PNH patients [9] also identified TE as a risk for mortality in a presumed mixed population of hemolytic and nonhemolytic PNH patients. Factors associated with risk of thrombosis included age >55 years, the use of transfusions, TE at diagnosis, and warfarin as primary prophylaxis. However, specific LDH values were not reported and were, therefore, not included in the evaluation.

It remains unclear whether all PNH patients at high risk for TE should receive primary warfarin prophylaxis. The etiology of hypercoagulability and the development of TE in PNH patients are multifactorial. Complement-mediated hemolysis can lead to NO depletion with subsequent arterial constriction, low blood flow, hypercoagulability of platelets, and formation of RBC prothrombotic microvesicles [8, 30]. Uncontrolled complement activation can also result in platelet vesiculation [31], C5a-induced granulocyte release of inflammatory cytokines, and tissue factor expression [32, 33], all precursors of thrombosis. Our data showed that anticoagulant therapy was ineffective in

addressing the prothrombotic sequelae of complement-mediated hemolysis, as 46 % of patients receiving either prophylactic or therapeutic anticoagulation therapy experienced a TE. This finding, along with previously published results, suggests that the use of anticoagulants may be ineffective in the management of TE in patients with PNH [1, 2, 7].

The database reported here retrospectively captured specific data related to hemolysis, including LDH values, as well as specific hemolysis-associated symptoms, providing a unique opportunity to evaluate association of presenting clinical symptoms and laboratory values to determine risk of TE and poor outcomes at diagnosis. However, while we identified abdominal pain, chest pain, and dyspnea as risk factors for TE, we acknowledge that more detailed information on frequency, intensity, and dates of symptom presentation was not captured. We believe that future prospective observations, perhaps from the international PNH registry, will further support the associations of hemolysis and other risk factors with TE.

In summary, our data show that PNH patients with LDH  $\geq 1.5 \times$  ULN at diagnosis have an increased risk of TE, the most frequent cause of death in PNH, compared with PNH patients with LDH  $< 1.5 \times$  ULN. The risk for TE is further increased with the additional presence of PNH-related symptoms. Age, gender, and clone size were not considered risk factors for TE in PNH patients. Physicians should, therefore, consider symptomatic PNH patients to be at particularly high risk for TE and mortality, as these clinical symptoms could represent continued progression and manifestation of uncontrolled complement activation and hemolysis. These results highlight the medical need for and urgency of early therapeutic intervention and monitoring of PNH patients with elevated LDH.

**Acknowledgments** The authors would like to thank G. Khursigara, PhD, of Alexion Pharmaceuticals; H. H. Cho of Handok Pharmaceuticals; K. Bonin, PhD, M. Hughes, PhD, and J. Safran of Infusion Communications for assistance in writing and editing; and P. McCloud, PhD, of McCloud Consulting for statistical analysis and interpretation. This work was supported by the Korean Society of Hematology.

**Conflict of interest** JWJ has received honoraria for lectures and advisory boards and has received consulting fees from Alexion Pharmaceuticals. JHJ, JSK, S-SY, J-HL, and D-YJ have received consulting fees from Alexion Pharmaceuticals. All other authors declare that they have no conflicts of interest.

## References

- Hillmen P, Lewis SM, Bessler M, Luzzatto L, Dacie JV. Natural history of paroxysmal nocturnal hemoglobinuria. *N Engl J Med*. 1995;333:1253–8.
- Parker C, Omine M, Richards S, Nishimura J, Bessler M, Ware R, et al. Diagnosis and management of paroxysmal nocturnal hemoglobinuria. *Blood*. 2005;106:3699–709.
- Takeda J, Miyata T, Kawagoe K, Iida Y, Endo Y, Fujita T, et al. Deficiency of the GPI anchor caused by a somatic mutation of the PIG-A gene in paroxysmal nocturnal hemoglobinuria. *Cell*. 1993;73:703–11.
- Tabbara IA. Hemolytic anemias. Diagnosis and management. *Med Clin North Am*. 1992;76:649–68.
- Rother RP, Bell L, Hillmen P, Gladwin MT. The clinical sequelae of intravascular hemolysis and extracellular plasma hemoglobin: a novel mechanism of human disease. *JAMA*. 2005;293:1653–62.
- Nishimura J, Kanakura Y, Ware RE, Shichishima T, Nakakuma H, Ninomiya H, et al. Clinical course and flow cytometric analysis of paroxysmal nocturnal hemoglobinuria in the United States and Japan. *Medicine (Baltimore)*. 2004;83:193–207.
- Hillmen P, Muus P, Dühsen U, Risitano AM, Schubert J, Luzzatto L, et al. Effect of the complement inhibitor eculizumab on thromboembolism in patients with paroxysmal nocturnal hemoglobinuria. *Blood*. 2007;110:4123–8.
- Hill A, Rother RP, Wang X, Morris SM Jr, Quinn-Senger K, Kelly R, et al. Effect of eculizumab on haemolysis-associated nitric oxide depletion, dyspnoea, and measures of pulmonary hypertension in patients with paroxysmal nocturnal haemoglobinuria. *Br J Haematol*. 2010;149:414–25.
- de Latour RP, Mary JY, Salanoubat C, Terriou L, Etienne G, Mohty M, et al. Paroxysmal nocturnal hemoglobinuria: natural history of disease subcategories. *Blood*. 2008;112:3099–106.
- Kelly RJ, Hill A, Arnold LM, Brooksbank GL, Richards SJ, Cullen M, et al. Long-term treatment with eculizumab in paroxysmal nocturnal hemoglobinuria: sustained efficacy and improved survival. *Blood*. 2011;117:5678–92.
- Kim Y, Lim J, Kim M, Kim Y, Lee JW, Han K. Quantitation of CD55 and CD59 expression on reticulocytes and mature erythrocytes in paroxysmal nocturnal hemoglobinuria, aplastic anemia, and healthy control subjects. *Ann Clin Lab Sci*. 2010;40:226–32.
- Hillmen P, Hall C, Marsh JC, Elebute M, Bombara MP, Petro BE, et al. Effect of eculizumab on hemolysis and transfusion requirements in patients with paroxysmal nocturnal hemoglobinuria. *N Engl J Med*. 2004;350:552–9.
- Hillmen P, Young NS, Schubert J, Brodsky RA, Socié G, Muus P, et al. The complement inhibitor eculizumab in paroxysmal nocturnal hemoglobinuria. *N Engl J Med*. 2006;355:1233–43.
- Brodsky RA, Young NS, Antonioli E, Risitano AM, Schrezenmeier H, Schubert J, et al. Multicenter phase 3 study of the complement inhibitor eculizumab for the treatment of patients with paroxysmal nocturnal hemoglobinuria. *Blood*. 2008;114:1840–7.
- Socié G, Mary JY, de Gramont A, Rio B, Leporrier M, Rose C, et al. Paroxysmal nocturnal haemoglobinuria: long-term follow-up and prognostic factors. *French Society of Haematology. Lancet*. 1996;348:573–7.
- Ziakas PD, Poulou LS, Rokas GI, Bartzoudis D, Voulgarelis M. Thrombosis in paroxysmal nocturnal hemoglobinuria: sites, risks, outcome: an overview. *J Thromb Haemost*. 2007;7:642–5.
- Kanakura Y, Ohyashiki K, Shichishima T, Okamoto S, Ando K, Ninomiya H, et al. Safety and efficacy of the terminal complement inhibitor eculizumab in Japanese patients with paroxysmal nocturnal hemoglobinuria: the AEGIS clinical trial. *Int J Hematol*. 2011;93:36–46.
- Hillmen P, Elebute M, Kelly R, Urbano-Ispizua A, Hill A, Rother RP, et al. Long-term effect of the complement inhibitor eculizumab on kidney function in patients with paroxysmal nocturnal hemoglobinuria. *Am J Hematol*. 2010;85:553–9.



19. Blum SF, Gardner FH. Intestinal infarction in paroxysmal nocturnal hemoglobinuria. *N Engl J Med.* 1966;274:1137.
20. Doukas MA, DiLorenzo PE, Mohler DN. Intestinal infarction caused by paroxysmal nocturnal hemoglobinuria. *Am J Hematol.* 1984;161:75–81.
21. Adams T, Fleischer D, Marino G, Rusnock E, Li L. Gastrointestinal involvement in paroxysmal nocturnal hemoglobinuria: first report of electron microscopic findings. *Dig Dis Sci.* 2002;471:58–64.
22. Hill A, Sapsford RJ, Scally A, Kelly R, Richards SJ, Khurisgara G, et al. Under-recognized complications in patients with paroxysmal nocturnal haemoglobinuria: raised pulmonary pressure and reduced right ventricular function. *Br J Haematol.* 2012; 1583:409–14.
23. Hill A, Hillmen P, Richards SJ, Elebute D, Marsh JC, Chan J, et al. Sustained response and long-term safety of eculizumab in paroxysmal nocturnal hemoglobinuria. *Blood.* 2005;1067: 2559–65.
24. Hill A, Rother RP, Hillmen P. Improvement in the symptoms of smooth muscle dystonia during eculizumab therapy in paroxysmal nocturnal haemoglobinuria. *Haematologica.* 2005;9012 (Suppl):40.
25. Meyers G, Weitz I, Lamy T, Cahn JY, Kroon HA, Severino B, et al. Disease-related symptoms reported across a broad population of patients with paroxysmal nocturnal hemoglobinuria. *Blood.* 2007;11011:3683.
26. Hall C, Richards S, Hillmen P. Primary prophylaxis with warfarin prevents thrombosis in paroxysmal nocturnal hemoglobinuria (PNH). *Blood.* 2003;102:3587–91.
27. Moyo VM, Mukhina GL, Garrett ES, Brodsky RA. Natural history of paroxysmal nocturnal haemoglobinuria using modern diagnostic assays. *Br J Haematol.* 2004;1261:133–8.
28. Pu JJ, Mukhina G, Wang H, Savage WJ, Brodsky RA. Natural history of paroxysmal nocturnal hemoglobinuria clones in patients presenting as aplastic anemia. *Eur J Haematol.* 2011; 871:37–45.
29. Risitano AM, Rotoli B. Paroxysmal nocturnal hemoglobinuria: pathophysiology, natural history and treatment options in the era of biological agents. *Biologics.* 2008;22:205–12.
30. Umans JG, Levi R. Nitric oxide in the regulation of blood flow and arterial pressure. *Annu Rev Physiol.* 1995;57:771–90.
31. Wiedmer T, Hall SE, Ortel TL, Kane WH, Rosse WF, Sims PJ. Complement-induced vesiculation and exposure of membrane prothrombinase sites in platelets of paroxysmal nocturnal hemoglobinuria. *Blood.* 1993;82:1192–6.
32. Weitz IC. Thrombosis in paroxysmal nocturnal hemoglobinuria—insights into the role of complement in thrombosis. *Thromb Res.* 2010;125(Suppl 2):S106–7.
33. Weitz IC. Thrombosis in patients with paroxysmal nocturnal hemoglobinuria. *Semin Thromb Hemost.* 2011;373:315–21.