Severity Scoring System to Predict the Necessity of Regular Transfusion among Patients with Hemoglobin H

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Abstract

Background: Hemoglobin H (HbH) is usually recognized as mild thalassemia. However, a wide range of clinical manifestations, from fatal hydrops fetalis to asymptomatic mild anemia, is observed. A severity scoring system to guide the management of patients with HbH is needed. Objective: To develop a scoring system to predict the necessity of regular transfusion among patients with HbH. Methods: Patients were classified into 2 groups according to transfusion requirement: severe among transfusion-dependent thalassemia (TDT) and nonsevere among nontransfusion-dependent thalassemia (NTDT). Clinical and hematological parameters associated with transfusion dependency were identified and β-coefficients of significant parameters from multiple logistic regression analysis were used to develop a scoring system. Results: A total of 247 pediatric patients (24 severe, 223 nonsevere) with a median age of 14.3 (IQR 9.9-18.4) years were included. Multiple logistic regression analysis revealed 3 significant parameters associated with regular transfusion requirement including 1) age at diagnosis <2 years, 2) spleen size [?]3 cm and 3) Hb at steady-state <8 g/dL. Coefficients of the respective parameters were used to define the scores as 1, 2 and 2, respectively. A total score of [?]3 was associated with regular transfusion requirement among severe HbH (sensitivity 88%, specificity 83%). The newly developed scoring system was validated in the second cohort of 134 pediatric patients with HbH treated at another center. The cut-off score [?]3 yielded comparable sensitivity and specificity for the prediction.

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Abbreviations

ARMS amplification refractory mutation system

AST aspartate aminotransferase

CE capillary electrophoresis

EPO erythropoietin

Hb hemoglobin

HbH Hemoglobin H

HPLC high performance liquid chromatography

LDH lactate dehydrogenase

NTDT nontransfusion-dependent thalassemia

PCR polymerase chain reaction

ROC receiver operating characteristic

TDT transfusion-dependent thalassemia

Abstract

Background: Hemoglobin H (HbH) is usually recognized as mild thalassemia. However, a wide range of clinical manifestations, from fatal hydrops fetalis to asymptomatic mild anemia, is observed. A severity scoring system to guide the management of patients with HbH is needed.

Objective: To develop a scoring system to predict the necessity of regular transfusion among patients with HbH.

Methods: Patients were classified into 2 groups according to transfusion requirement: severe among transfusion-dependent thalassemia (TDT) and nonsevere among nontransfusion-dependent thalassemia (NTDT). Clinical and hematological parameters associated with transfusion dependency were identified and β-coefficients of significant parameters from multiple logistic regression analysis were used to develop a scoring system.

Results: A total of 247 pediatric patients (24 severe, 223 nonsevere) with a median age of 14.3 (IQR 9.9-18.4) years were included. Multiple logistic regression analysis revealed 3 significant parameters associated with regular transfusion requirement including 1) age at diagnosis <2 years, 2) spleen size [?]3 cm and 3) Hb at steady-state <8 g/dL. Coefficients of the respective parameters were used to define the scores as 1, 2 and 2, respectively. A total score of [?]3 was associated with regular transfusion requirement among severe HbH (sensitivity 88%, specificity 83%). The newly developed scoring system was validated in the second cohort of 134 pediatric patients with HbH treated at another center. The cut-off score [?]3 yielded comparable sensitivity and specificity for the prediction.

Conclusion: This newly developed scoring system was practical and helpful to predict the necessity of regular transfusion among severe HbH.

1 INTRODUCTION

Thalassemia results from a defective α- or β- chain synthesis. Defective α-globin chain production in α-thalassemia results in excess β-liked globin chain and formation of hemoglobin (Hb) Bart's (γ_4) and HbH (β_4) starting in the intra-uterine fetal stage and immediately after birth, respectively. These tetramers precipitate on red cell membranes leading to ineffective erythropoiesis and extravascular hemolysis. Moreover, HbBart's and HbH are nonfunctional as they have high oxygen affinity. This results in tissue hypoxia among affected patients with α-thalassemia.[1]

HbH is predominantly found in Southeast Asian, the Mediterranean and Middle Eastern regions and is diagnosed when affected patients have only one remaining functional α-globin gene. In Thailand, HbH is the most common form of thalassemia found in 77% of patients.[2]Based on genotypes, it can be categorized as "deletional" (–/-α) and "nondeletional" (–/$\alpha^{T}\alpha$) forms. Co-inheritance with a common variant of β-globin gene, known as HbE (β^{E}), leads to AE Bart's (–/-α, $\beta^{E}/\beta\cdot$ –/α $^{T}\alpha$, β^{E}/β) or EF Bart's (–/-α, β^{E}/β^{E}; –/$\alpha^{T}\alpha$, β^{E}/β^{E}) which is most frequently found on the Southeast Asian subcontinent.[3] Clinical presentations of these diseases are similar to HbH with the corresponding form of genotypes.

HbH is generally thought to be a mild form of thalassemia. In fact, marked phenotypic variability was found ranging from hydrops fetalis[4] to asymptomatic mild anemia. Related studies have demonstrated that clinical severity of patients affected with HbH is mainly associated with their HbH genotypes. Those with nondeletional HbH presented anemia and nondeletional HbH is diagnosed at younger age as compared with those with deletional HbH.[3, 5-9]Thalassemic facies, icteric sclera and hepatosplenomegaly were more prominent among those affected with nondeletional HbH as compared with deletional HbH.[3, 5-10] Moreover, laboratory parameters indicating degree of hemolysis including decreased baseline Hb level, increased reticulocyte count, bilirubin and lactate dehydrogenase were more pronounced among patients with nondeletional HbH.[6-11] As a consequence, affected patients with nondeletional HbH were more likely to receive blood transfusion and occasionally become transfusion-dependent.[6-11]

Clinical phenotypes of the patients; however, vary even among those with the same form of HbH genotypes.[12, 13] A study from Greece demonstrated that 6 of 18 (33%) patients with nondeletional HbH had severe clinical phenotype, as classified by Hb <8 g/dL, age at diagnosis <2 years, requiring frequent or occasional transfusions, presence of severe bone change and pronounced splenomegaly. The remaining patients (67%); however, exhibited less severe clinical presentation.[12] Another study from Iran showed that 8 of 18 (44%) patients with HbH with nondeletion genotype had received blood transfusion, six patients of which (33%) were transfusion-dependent, whereas 11 of 15 patients with deletional HbH were nontransfused.[14] A recent report from a tertiary care center in Thailand also showed that 25 of 39 (64%) of nondeletional and 4 of 37 (11%) of deletional HbH and patients with AEBart's had received blood transfusion, while the remaining patients were transfusionfree.[8] This evidence suggested that other unidentified genetic and environmental factors are associated with degree of clinical severity among patients with HbH.

Unlike β-thalassemia,[15] systematic classification of the severity among HbH is unavailable to date. In related studies on genotype-phenotype correlation of HbH, patients were often classified as mild, moderate and severe according to expert opinions regarding patient history, physical examination and basic hematological parameters.[13] Moreover, management and monitoring of patients with HbH varied widely among institutions. As a consequence, a number of patients were undertreated and did not enjoy a good quality of life.

This study aimed to develop a simple severity scoring system for pediatric patients with HbH to be employed by hematologists and nonhematologists as a guide for counseling and providing optimal care for affected patients with HbH.

2 MATERIALS AND METHODS

2.1 Patients

The study cohort, conducted from January 2018 to September 2019, enrolled Thai pediatric patients with HbH, AE Bart's and EF Bart's, who had been diagnosed and followed-up at the Department of Pediatrics, Faculty of Medicine, Ramathibodi Hospital, Bangkok, Thailand for a minimum of two years. Patients with

immune hemolytic anemia or chronic illnesses were excluded. The study was approved by the Ethics Committee, Faculty of Medicine, Ramathibodi Hospital, Mahidol University, Bangkok (ID 03-61-42). Informed consent was obtained from patients and parents, and the study was conducted under the Declaration of Helsinki.

2.2 Clinical and laboratory data

Clinical data and routine laboratory screening were obtained from medical records. Patients or parents were also interviewed for missing data. Complete blood counts were determined using an automated blood cell analyzer (Beckman Coulter DXH 800). Alpha-thalassemia genotype was confirmed using gap-polymerase chain reaction (PCR) for deletional genotypes and amplification refractory mutation system (ARMS)-PCR or direct DNA sequencing for nondeletional genotypes as previously described.[16] Hemoglobin electrophoresis was performed using high performance liquid chromatography (HPLC) or capillary electrophoresis (CE). Serum ferritin was measured using a Vitros ferritin assay (Ortho Clinical Diagnostics Johnson & Johnson Company, UK). Additional blood samples at steady-state were collected once from each patient to determine biological parameters of erythropoietin (EPO), reflecting tissue hypoxia; and reticulocyte count, aspartate aminotransferase (AST) and lactate dehydrogenase (LDH) reflecting hemolysis**.**

2.3 Development of the novel scoring system

Enrolled patients were classified in two groups according to regular transfusion requirement: 1) severe among transfusion-dependent thalassemia (TDT, requiring transfusion [?]4 times yearly and 2) nonsevere among nontransfusion-dependent thalassemia (NTDT). Clinical and laboratory data of TDT and NTDT groups were compared and parameters associated with regular transfusion requirement were identified by univariate analysis. Further multiple logistic regression analysis was performed to determine the remaining significant parameters. β- coefficients of significant parameters were subsequently used to develop the scoring system. Cut-off score to predict the necessity of regular transfusion was determined using receiver operating characteristic (ROC) curve analysis.

2.4 Validation of the scoring system

The newly developed scoring system was validated in the second cohort of pediatric patients with HbH living in northern Thailand. All patients in this group had received a diagnosis and were followed-up at the Department of Pediatrics, Faculty of Medicine, Chiang Mai University, Chiang Mai, Thailand, for a minimum of two years. Clinical characteristics and essential laboratory findings were retrospectively reviewed from medical records. The severity scores for HbH were calculated and analyzed using ROC curve analysis. Comparison of ROC curve analysis acquired from the two patient cohorts were performed to validate the severity scoring system.

2.5 Statistical analysis

Statistical analysis was performed using IBM SPSS®Software, Version 22. Categorical data presented as number (percentage) were compared using Chi-square or Fisher's-exact test. Continuous data presented as median (interquartile range, IQR) were compared using the Mann-Whitney U test. Odd ratios of each significant parameter were obtained using univariate analysis. Multiple logistic regression analysis was used to identify the remaining significant parameters, and ROC curve analysis was then used to develop the scoring system.

3. RESULTS

In all 262 patients with HbH enrolled in the study. Fifteen patients were excluded: 3 patients had immune hemolytic anemia, 7 patients had other chronic illnesses and 5 patients denied to consent. The remaining 247 patients (113 males) were classified according to their transfusion requirement as TDT (n=24) and NTDT (n=223).

3.1 Genotypes

DNA analysis for α-globin mutations was available among 204 patients revealing 90 deletional and 114 nondeletional genotypes. An additional 33 and 10 patients were diagnosed as having deletional and nondeletional HbH solely by hemoglobin electrophoresis. Nondeletional HbH was found in 91.7% (22/24) of patients in TDT and 48.4% (108/223) of those in NTDT groups (p <0.001). A total of 50 patients co-inherited β- thalassemia as identified by hemoglobin electrophoresis or ARMS-PCR for common β-globin mutation (summarized in Table 1).

3.2 Patient characteristics

Median age at the last follow-up visit was 11.5 years (IQR 5.2-13.1) in TDT and 14.7 years (IQR 10.3-18.8) in NTDT groups. Median age at diagnosis of TDT of 1.4 (IQR 0.4-2.8) years was significantly lower than that of NTDT [3.4 (IQR 1.2-6.3) years (p <0.001). In the TDT group, regular transfusion was initiated at a median age of 4.5 (IQR 2.3-7.6) years and continued for a median duration of 5.2 (IQR 1.8-8.2) consecutive years. Packed red cell transfusion was provided at a median interval of 4 (IQR 4-6) weeks to yield a median pre-transfusion Hb level of 8.6 (IQR 8-9.2) g/dL. Median serum ferritin level of the patients in the TDT group was relatively well controlled at 1,025.9 (IQR 683.9-2,636.6) ng/mL while taking deferiprone or deferasirox monotherapy. However, it was unsurprisingly higher than that of the NTDT group [114.8 (IQR 63.9-173.2) ng/ml]. In the NTDT group, 96 (43%) patients had previously received red cell transfusion at least once. This group included 15 patients of deletional and 81 of nondeletional genotypes. The median height z-score for age in the TDT [-0.88 (IQR -3.31-0.28)] tended to be lower than that of the NTDT group [-0.57 (IQR -1.25-0.26)] at the time of diagnosis. However, regular packed red cell transfusion for those with more severe disease (TDT) tended to maintain optimal height (z-score [?]-2) at least up to the time of the report (Table 2).

In all, 7 patients in the cohort underwent surgical splenectomy and one received splenic embolization at the median age of 5.8 (IQR 4.1-7.7) years. Six of 8 patients in this group harbored the nondeletional genotype, two of whom were TDT. Splenectomy resulted in significant increment of median Hb level at steady-state from 6.1 g/dl (5.5-7.3) to 9.3 g/dl (7.9-11.0) (p=0.002). One patient with TDT became NTDT after the procedure, while the others remained transfusion-dependent.

3.3 Biochemical parameters associated with hemolysis and tissue hypoxia

To study further investigated whether biochemical parameters reflecting hemolysis and tissue hypoxia predicted the necessity of regular transfusion. Reticulocyte count, AST, LDH and EPO level were determined among 91 patients (TDT=18, NTDT=73). A significantly higher number of reticulocytes, AST LDH and EPO were found in the TDT as compared with the NTDT groups (Table 3). This suggested that severe HbH was associated with a more severe degree of hemolysis and higher degree of tissue hypoxia compared with the nonsevere group (Table 3).

Unlike β-thalassemia, total Hb routinely measured from red cells of the patients with HbH was not entirely "functional" Hb. Functional Hb, among those with optimal capacity of carrying and releasing oxygen to body tissue, was calculated from total Hb (g/dL) x [{1-(HbH% + HbBart's%)}]/100]. We initially hypothesized that functional Hb at steady-state may contribute at a higher magnitude to clinical severity of patients with HbH as compared with total Hb level. However, this study showed that the proportion of functional Hb in TDT group was comparable to that of NTDT (Table 3).

3.4 Development of the scoring system

Clinical and laboratory parameters associated with severe HbH; therefore became candidate factors for the scoring system, and were primarily identified using univariate analysis. These factors included nondeletional genotype (OR 11.7, 95%CI 2.7-51), age at diagnosis <2 years (OR 3.3, 95%CI 1.4-7.9), presence of thalassemic facies (OR 29.4, 95%CI 10.7-80.8) and icteric sclera (OR 3.8, 95%CI 1.5-9.7), liver size [?]3 cm below the right costal margin (OR 6.7, 95%CI 2.7-16.7), spleen size [?]3 cm below the left costal margin (OR 9.9, 95%CI 3.5-27.6) and Hb level at steady-state <8 g/dL (OR 14, 95%CI 5.4-36.3). Co-inheritance of β-thalassemia was not associated with severity of HbH (OR 0.38, 95%CI: 0.2-0.9, p=0.03). Nondeletional genotype and

thalassemic facies were found to be strongly associated with more severe HbH (TDT) and were excluded from subsequent analysis of the scoring system.

In addition, severe HbH was substantially associated with reticulocyte count >5% (OR 8.2, 95% CI 2.3-25.7), AST [?]40 U/L (OR 3.3, 95%CI 9.5-169), LDH [?]300 U/L (OR 29.4, 95%CI 8.3-143), and EPO level [?]50 mU/mL (OR 3.8, 95%CI 4.7-76.5). These laboratory results; however, were not calculated in subsequent multiple logistic regression analysis, owing to the limited number of studies.

Multiple logistic regression analysis revealed three remaining significant parameters associated with severe HbH, including age at diagnosis <2 years, spleen size [?]3 cm and Hb at steady-state <8 g/dL (Table 4). β-coefficients of the respective parameters were rounded up to whole numbers and used to define the severity scores of 1, 2 and 2, respectively (Table 5). Area under the curve (AUC) of total scores, analyzed using ROC curve, was 0.89 (95% CI: 0.81-0.96, p <0.001) (Figure 1A). An optimal cut-off score to predict severe HbH requiring regular transfusion was shown to be [?]3 with sensitivity of 87.5% and specificity of 83% (Figure 1B, Table 5).

3.5 Validation of the scoring system

Validation of the scoring system was performed in the second cohort of patients. These included 134 patients with HbH (71 males), who were followed-up at the Department of Pediatrics, Faculty of Medicine, Chiang Mai University in northern Thailand. Median age on last follow-up visit in this group was 16.7 (IQR 10.3-18.3) and 14.7 (IQR 9.8-20.8) years in TDT and NTDT groups, respectively. Seventeen patients (13%) were TDT, 16 of whom carried nondeletional HbH genotypes. Clinical characteristics of the patients in the validation cohort are summarized in Supplemental Table S1. Thalassemic facies were reported in 9 of 17 patients in the TDT group (52.9%) and 13 of 117 patients in the NTDT group (11.1%) (p <0.001). Ten of 134 patients were splenectomized at median age of 11.6 (8.9-15.3) years, all of whom carried nondeletional genotypes. All 8 patients, who were TDT presplenectomy became nontransfusion-dependent following the procedure with median pre- and postsurgery Hb levels of 6.5 (IQR 5.5-7) and 9 (IQR 8.6-10.3) g/dL, respectively. Growth of those in the TDT group was more impaired compared with that of the NTDT group, as demonstrated by weight and height z-score <-2 both at the time of diagnosis and time of report. However, initiating regular blood transfusion tended to improve height z-score from -3.5 (IQR -4.7, -2.3) to -2.3 (IQR -2.9, -1.2).

The severity scores were calculated, according to the newly developed scoring system, from 134 patients in the validation cohort. The total scores were further analyzed using ROC and showed AUC of 0.97 (95% CI: 0.94-1, p<0.001). Applying cut-off score [?]3 for this validation cohort, the AUC was 0.91 (95% CI: 0.86-0.96, p<0.001) with sensitivity of 100% and specificity of 81.4%. The comparison of cut-off score [?]3 AUC between the original and the validation cohorts revealed no statistical significant difference (p=0.19), suggesting the newly developed HbH disease severity scoring system remained valid while being applied among patients treated at different institutions.

4. DISCUSSION

HbH was generally described as a mild thalassemia disease, and affected patients were occasionally cared for by nonhematologist practitioners, especially in areas where thalassemia is prevalent like in Thailand. These patients sometimes received suboptimal disease monitoring and treatment to maintain normal growth and pubertal development. This study showed that 10% (24/247) of patients had relatively severe clinical manifestations resulting in transfusion-dependency at least during childhood. It also suggested that HbH is in fact diverse in clinical phenotypes. This study chose transfusion-dependency as an end-point to represent severe HbH disease as we thought the newly developed scoring system would also provide an appropriate family counseling and treatment guide to physicians.

The patients harboring nondeletional genotypes showed a wider range of clinical severity as compared with those harboring deletional genotypes. This was demonstrated when 19% (22/115) of patients with nondeletional genotypes became TDT, while only 2% (2/89) of those with deletional genotypes were TDT. One of the two patients with deletional HbH genotype who was in TDT group also harbored homozygous β^0-

thalassemia, which could well explain her disease severity. In addition, the vast majority of patients in both cohorts undergoing splenectomy were patients with nondeletional genotypes (6 of 8 patients in the first and all 10 patients in the second cohorts). This implied that genotype was a major determinant of severity among patients with HbH, similar to that demonstrated by a number of related studies.[3,7,11] Splenectomy was previously selected as a treatment option for patients having relatively low baseline Hb level and splenomegaly, regardless of transfusion-dependency. Although splenectomy resulted in significant increased Hb level in the vast majority of patients, it did not guarantee transfusion-free status as at least one of the patient in the first cohort remained transfusion-dependent after the procedure. In addition, risks of encapsulated bacterial infection and thrombo-embolic events should be extensively discussed with the patients and families before deciding on splenectomy.

Thalassemic facies was present at a significantly higher proportion in the TDT (70%) as compared with the NTDT group (8%,$p<0.001$) and was strongly associated with severe disease as identified by univariate analysis (OR 29.4, 95%CI 10.7-80.8). Therefore, close monitoring and early initiation of regular transfusion should be kept in mind once thalassemic facies was noted. However, we did not include the presence of thalassemic facies in our scoring system as it was rather a subjective measurement and might be difficult to evaluate for nonhematologist practitioners.

Three significant factors identified as the predictors of HbH severity and transfusion-dependency were age at diagnosis <2 years, spleen size [?]3cm and Hb at steady-state <8 g/dL. These parameters were simple and objective clinical presentations, physical measurement and basic laboratory investigation that could be evaluated by both hematologists and nonhematologists. Cut-off score of [?]3 yielded the highest AUC. In other words, according to the scores (Table 5), affected patients with presence of any two of the three significant factors had high chance of developing severe disease and to eventually become transfusion-dependent with sensitivity of 87.5% and specificity of 83%. However, higher scores were associated with higher specificity for prediction.

Biochemical parameters indicating higher degree of hemolysis (higher reticulocytes, AST and LDH) and tissue hypoxia (higher EPO level) were found to be associated with severe HbH. This suggested that genetic modifiers or cellular mechanisms underlying these processes should be further studied and could be candidate targets for novel treatment of patients affected with severe HbH. Nevertheless, these parameters could not be included with the severity scores owing to the limited number of blood samples. Monitoring of functional Hb level did not show additional benefit to total Hb level on monitoring of the disease severity. Whether regular transfusion for those with TDT to maintain normal growth and sexual development could be achieved by optimization of pre-transfusion functional Hb (rather than total Hb), similar to that performed in survivors of Hb Bart's hydrops fetalis syndrome,[17] is yet to be investigated.

To investigate whether the newly developed HbH disease severity scoring system could be generally applied to the patients coming from different regions and had been treated in different centers, validation of the score was performed in the second cohort of patients. Even though the patients in the second cohort had some different clinical and hematological characteristics from those of the patients in the first cohort, such as more impaired weight and height z-score in TDT groups, the newly developed scoring system and the cut-off score of [?]3 resulted in similarly high sensitivity and specificity to predict severe disease in both populations. This suggested that the newly developed scores could be widely used across different populations. Patients with severe HbH, among whom regular transfusion was initiated, would require the transfusion regimen for at least a period of time during childhood [median 5.2 (IQR 1.8-8.2) years]. It was also shown in the first cohort that when patients with severe HbH received regular transfusion from as early as 4.5 years of age, their growth would be maintained and comparable with that of NTDT. Although the patients with TDT in the second cohort had impaired weight and height z-score (<-2) from the time of diagnosis and the time of study, regular transfusion tended to improve height z-score to the point evaluated at the time of this study. These emphasized the role of initiating regular transfusion among pediatric patients with HbH with severe clinical phenotypes. Whether severe HbH patients would benefit from regular transfusion after final adult height and secondary sexual characteristics were achieved is yet to be investigated.

In comparison with previously reported scoring system for β-thalassemia,[15] the patients with higher scores by this newly developed scoring system, might be classified as only mild or moderate severity by the β-thalassemia scores. This suggested that it would be more suitable to classify severity of HbH based on a specific scoring system, rather than using the scores primarily designed for β-thalassemia. In addition, this study employed objective, simple and practical routine measurements for the severity scoring system, rather than other subjective evaluations, such as degree of bone change and growth impairment without availability of midparental height used in a number of related studies.[12-13]

In conclusion, the newly developed HbH diseases severity scoring system provided a simple and practical assessment for hematologists and nonhematologist caring for patients. This score would benefit counseling affected families, monitoring the disease and making decision to initiate regular transfusion to maintain optimal growth, especially among those with nondeletional HbH. Moreover, the novel scoring system can serve as a basis for future studies on other genetic modifiers affecting HbH disease severity, especially among those with nondeletion genotypes.

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CONFLICTS OF INTEREST

All authors declare they have no conflict of interests.

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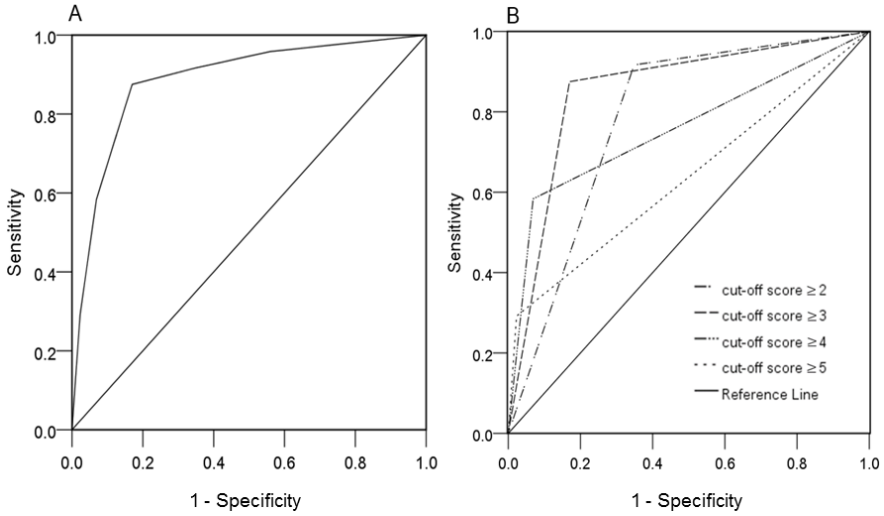
17. Amid A, Chen S, Brien W, et al. Optimizing chronic transfusion therapy for survivors of hemoglobin Barts hydrops fetalis. *Blood.*2016;127(9):1208-1211.**FIGURE LEGENDS**

FIGURE 1 Receiver operating curve analysis of the newly developed severity scoring system for patients with HbH

(A) Area under the curve of the newly developed scoring system was 0.89 (95%CI: 0.81-0.96, $p < 0.001$). **(B)**The cut-off scores [?]3 to predict regular transfusion requirements among patients with HbH yielded the best sensitivity of 87.5% and specificity of 83%.



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