

Factor Activity Levels and Bleeding Scores in Pediatric Hemophilia Carriers Enrolled in the ATHNdataset.

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Abstract

Background: Multiple studies have now shown that a significant proportion of hemophilia carriers meet criteria for having hemophilia and/or report abnormal bleeding. However, to date, investigations of hemophilia carriers have almost exclusively involved women over 18 years of age. Little is known about factor activity levels and bleeding scores in carriers during childhood. We queried a large deidentified database of subjects with bleeding disorders residing in the United States to determine factor activity levels and bleeding scores. **Procedures:** The ATHNdataset was queried for hemophilia carriers under 18 years of age. Collected information included demographics, factor activity levels, bleeding scores. **Results:** Over 700 carriers in the pediatric age group were identified of which 626 submitted factor activity levels. Nearly half had factor activity levels less than 40 IU/dL, thereby meeting criteria for having hemophilia. Of those reporting bleeding scores, only 13.5% reported an abnormal bleeding score for age. The proportion reporting abnormal bleeding scores was higher in those with factor levels less than 40 IU/dL (23%) than those greater than 40 IU/dL (9.7%). **Conclusions:** The proportion of pediatric carriers with hemophilia was double of that previously reported for adults. Of those with hemophilia reporting a bleeding score, the majority (77%) did not report an abnormal bleeding score for age. However, nearly 10% of pediatric carriers not meeting criteria for having hemophilia reported abnormal bleeding scores for age. Similar results are reported in adults suggesting that factor activity levels may not be predictive of bleeding symptoms in carriers.

Introduction

Hemophilia A and B are X-linked recessive conditions due to deficiencies of factors VIII and IX respectively. Affected males are prone to abnormal bleeding from trivial causes. Several studies over the past few decades have shown that hemophilia carriers also have a bleeding tendency.¹⁻³ Due to random X chromosome inactivation, female hemophilia carriers can have wide ranging factor activity levels.^{2,4} Approximately one-fourth of hemophilia carriers seen at hemophilia treatment centers (HTCs) have factor activity levels less than 40 IU/dL and are now classified as having hemophilia.^{1,4} Even amongst carriers seen at HTCs with factor activity levels greater than 40 IU/dL, about one-fourth report abnormal bleeding scores (BLS).¹ Thus, amongst hemophilia carriers, just less than one-half either have hemophilia and/or report abnormal BLS.

To date, studies of hemophilia carriers have almost exclusively focused on women over 18 years of age. Little is known about bleeding symptoms of hemophilia carriers during childhood. Only one study has included a significant sample size of children.¹ It showed that children were less likely than adults to have an abnormal BLS for age. Additional details such as the proportion of children who had factor levels less than 40 IU/dL, or the effects of race, ethnicity, type of hemophilia, or mutation on BLS were not included.

The American Thrombosis and Hemostasis Network (ATHN) is an organization whose purpose is to improve the lives of people with bleeding and clotting disorders. To that end, ATHN sponsors a HIPPA-compliant, de-identified dataset in which subjects agree to submit their health information. Over 140 Hemophilia Treatment

Centers (HTCs) across the United States have collected and submitted data into the ATHNdataset. Over 60,000 people with bleeding or clotting disorders have submitted data. Of this, approximately 3300 are hemophilia carriers, including 743 in the pediatric age group.

The purpose of this investigation is to characterize hemophilia carriers under 18 years of age enrolled in the ATHNdataset.

Methods

The ethical committees of participating HTCs authorized enrollment in the ATHNdataset. The parents or guardians of eligible children either opted in or provided informed consent to share their child's health information. Core data elements, including demographics, primary diagnosis, baseline factor activity levels, prescribed medications, inhibitor status, and insurance are submitted by each HTC.¹ The International Society on Thrombosis and Haemostasis Bleeding Assessment Tool Bleeding Score (ISTH-BAT BLS), factor activity level, and genotype were collected for participants in the *My Life Our Future (MLOF)* genotype initiative. Additional data elements such as bleeding events, including detailed menstrual bleeding, medication usage, and joint range of motion could also be submitted. Core data elements are audited by ATHN for consistency. Many other data element's collection and submission are left to the discretion of participating HTCs and are not audited. Because data elements such as bleeding events, medication usage, or detailed menstrual bleeding are likely underreported, this type of data was not subjected to further analysis.

The ATHNdataset was queried in June 2022 for hemophilia carriers under 18 years of age. Collected data included age, race, ethnicity, type of hemophilia (A or B), baseline factor activity level, genotype, and ISTH-BAT BLS. The ISTH-BAT BLS has been validated for use in children, and normal ranges are established for adults and children.⁵ An ISTH-BAT BLS of 3 or higher was determined to be abnormal for children under 18 years of age by Elbatarny et al.⁵ Although Elbatarny et al. initially established an ISTH-BAT score of 6 or higher as abnormal in adult women, subsequent analysis has shown a ISTH-BAT score of 5 or higher was abnormal for women aged 18-30.⁶ Since adolescents are biologically more similar to women age 18-30 (menstruating and at risk for post-partum hemorrhage) than children, we felt the revised definition of an abnormal ISTH-BAT score was more appropriate to use in adolescents aged 11-17. Doherty et al. did not revise the abnormal BLS for adolescents due to sample size limitations.⁶ Additional support for this methodology is found in Jain et al.⁷ This study showed that a BLS of 5 or higher in adolescent girls was predictive of having a bleeding disorder. As previously described, we defined a BLS of 5 or higher as abnormal for adolescents and 3 or higher as abnormal for children.¹ The participant's age at the time of BLS determination was used to determine if the score was abnormal or not. For subjects with multiple reported ISTH-BAT BLS we selected the earliest record. The one-stage factor activity level is the predominant methodology used to determine factor activity levels in the United States, and factor activity levels reported in this study were presumed to be from a one-stage assay. For subjects submitting more than one factor activity level, the lowest reported level (baseline) was used. The proportion of subjects who had a genotype determined was collected to describe the population. A detailed analysis of genetic information was beyond the scope of this study. Because this updated query covered a different timeline than prior studies of hemophilia carriers using the ATHNdataset, sample size differences were expected.

Descriptive statistics were used to define the population. Data sets with a sample size less than 50 were considered to be too small for valid statistical comparisons. Pearson's Chi squared test, Fisher's exact test, Wilcoxon rank sum and Welch Two Sample t-tests were used for group comparisons. When able to be measured, a p value of less than 0.05 was considered statistically significant. The original, de-identified data can be obtained by contacting ATHN at support@athn.org.

Results

As of June 2022, 743 females under 18 years of age who were identified as having hemophilia A or B or were carriers of hemophilia A or B were enrolled in the ATHNdataset. Demographic information regarding these subjects is found in table 1. P values for all comparisons were >0.05 . Three-hundred-forty-three were children under 11 years of age and 400 were adolescents between 11 and 17.99 years of age. Roughly three-

fourths were hemophilia A carriers or had hemophilia A, and one-fourth were hemophilia B carriers or had hemophilia B. Of the 626 with known factor activity levels, 305 (49%) had activity levels of 40 IU/dL or less, thereby meeting criteria for having hemophilia. Of these, 18 had activity levels in the severe range, and 9 were moderate. Adolescents with hemophilia B were more likely to report factor activity levels less than 40 IU/dL than adolescents with hemophilia A ($p=0.02$). There was no statistical difference in children reporting factor activity levels < 40 IU/dL between hemophilia A and B ($p=0.3$). The mean factor activity (standard deviation) for children was 45 (28) and for adolescents was 47 (28) ($p=0.5$). Figure 1 shows the distribution of factor activity levels for the entire pediatric population of subjects enrolled in the ATHNdataset.

Two-hundred-twenty-three subjects reported an ISTH-BAT BLS and demographics regarding this population is found in table 2. Ninety-eight percent of subjects reporting a BLS participated in the *MLOF* genotype initiative. The mean BLS for adolescents (1.35) compared to children (0.86), was statistically different ($p=0.03$), but not a clinically relevant difference. Only 30 (13.5%) subjects reported an abnormal bleeding score for age. Seventeen percent of adolescents reported an abnormal BLS for age compared to 7.7% of children ($p=0.045$). There was no statistically significant difference in those reporting an abnormal BLS between hemophilia A and B for children or adolescents ($p=0.3$ and $p=0.8$). Of the 223 subjects who submitted an ISTH-BAT BLS, 195 submitted both a BLS and factor activity level. Twenty-seven (13.8%) reported an abnormal BLS for age. Figure 2 shows the proportion with an abnormal bleeding score by factor activity level for children and adolescents. Fourteen (23%, 3 children) subjects with factor activity levels under 40 IU/dL reported an abnormal BLS for age while 13 (9.7%, 4 children) of those with factor activity levels over 40 IU/dL did. Because the sample size of subjects reporting an abnormal BLS was so small, additional evaluations to determine factors contributing to an abnormal BLS were not undertaken.

Discussion

Previous investigations of adult hemophilia carriers, including an investigation of the ATHNdataset, have shown that roughly one-fourth meet criteria for having hemophilia.^{1,4} Our investigation of pediatric carriers enrolled in the ATHNdataset showed nearly half of pediatric carriers meet current criteria for having hemophilia. There are several potential explanations for the high proportion of pediatric carriers in this study having hemophilia. One is that there could be ascertainment bias in the ATHNdataset. Pediatric carriers with hemophilia (and more likely to have abnormal bleeding) may be more likely to be seen at a HTC, thereby skewing the data. However, of those reporting a BLS, the majority reported a normal BLS for age, and the median BLS was 0. This suggests that recruitment of subjects with a higher bleeding risk may not be skewing the data to a significant extent. Also, similar ascertainment bias may exist in studies of adult carriers. Pre-analytical or analytical errors are another possible explanation. However, these specimens were handled by HTCs well versed in handling blood specimens for hemophilia patients. Also, many of the subjects in this study are seen at the same HTCs that also see adults. Factor VIII is an acute phase response protein and if anything, the stress of phlebotomy in a child might lead to falsely elevated factor VIII activity levels. Pre-analytical and analytical issues are unlikely to explain the high proportion of pediatric carriers with hemophilia enrolled in the ATHNdataset compared to adult carriers with hemophilia enrolled in the same database. It is known that factors VIII and IX levels rise with age, and the mean factor activity level in child and adolescent carriers was lower than that reported for adults.^{2,8,9} This could explain the difference in proportion of those reporting a factor activity below 40 IU/dL in adults compared to pediatric carriers.

Of those reporting a BLS, a minority (9.7%) of pediatric carriers with factor activity levels >40 IU/dL in the ATHNdataset report BLS that were abnormal for age. Abnormal bleeding with normal factor activity levels is a consistent finding in multiple studies of adult carriers as well and seems to be more prevalent than in children.¹⁻³ Again, it is possible that bias could explain this finding. A very small, single institution study showed a higher BLS for carriers that knew their carrier status prior to obtaining their BLS compared to carriers that did not.¹⁰ In addition, cultural difference in the reporting of BLS in carriers have been reported.^{1,11,12} It is possible that carriers with normal factor activity levels do not truly have abnormal bleeding, and the apparent abnormal BLS are due to methodologic/cultural issues used to determine the BLS. However, this should not be assumed to be the explanation. To date, studies demonstrating a strong

correlation between factor VIII and IX activity levels and bleeding risk have been done exclusively in males. It is not unreasonable to propose that factors VIII and/or IX have gender related hemostatic functions that are not measured by aPTT based assays. Factor VIII, at least, is known to have a non-hemostatic function that is not measured by an aPTT based assay.¹³ The factor VIII:Von Willebrand complex is important for bone homeostasis, and female carriers have recently been shown to have higher rates of osteoporosis and fractures compared to an unaffected population.^{13,14} In factor XI deficiency, there is a no correlation between factor activity levels and clinical bleeding.¹⁵ It has been proposed that this is due to hemostatic effects of factor XI that are not measured by aPTT based assays.^{16,17} As shown in table 3, moderate to weak correlation between factor activity and clinical bleeding is seen in other coagulation factor deficiencies.^{15,18} Additional investigation into possible hemostatic functions of factor VIII and IX outside those measured by aPTT based assays are needed.

Another finding of our study was that most pediatric females with hemophilia that reported a BLS had a normal BLS for age. This is also seen in adult carriers with hemophilia.¹ However, an overwhelming majority with hemophilia had factor activity levels between 16 IU/dL and 40 IU/dL, levels that are expected to have a lower risk of abnormal bleeding.¹⁹ Alternatively, this could be another example of the inadequacy of aPTT based factor assays in predicting bleeding symptoms in this population.

Several guidelines recommend against testing potential carriers for genetic diseases during childhood.^{20,21} A principal reason for this stems from ethical concerns regarding the loss of the child's future autonomy. However, these guidelines have not met with universal acceptance.²² A more recent policy statement from British Medical Health allows for carrier testing so long as no harm comes to the child.²² The American Academy of Pediatrics and American College of Medical Genetics most recent guideline also recommends against carrier testing unless a child is at risk for childhood onset conditions.²¹ Our study suggests that hemophilia carriers are at significant risk for having hemophilia and/or abnormal bleeding. Because factor activity levels may not be diagnostic of carrier status and may not be predictive of bleeding risk, genetic testing of potential/obligate carriers seems indicated.

This study has several strengths, even compared to other investigations of adult carriers, including data submitted from multiple institutions, genotyping of a significant proportion of subjects, standardized measure of bleeding symptoms, and a sample size that is larger than most studies involving adult hemophilia carriers.^{2,3,23} Even with this large sample size, only a small number of pediatric carriers reported an abnormal BLS for age. Thus, we could not make valid analysis of other factors that might contribute to an abnormal BLS. Further improvement of the study would have been inclusion of additional details regarding clinical bleeding such as joint or menstrual bleeding. However, this type of data is not consistently submitted by participating HTC and would lead to an underestimation of bleeding symptoms.

Our investigation suffers from a weakness common to many previous studies of hemophilia carriers, namely, they only investigate carriers seen at a HTC. It is unknown to what degree carriers seen at a HTC are representative of all hemophilia carriers, most of whom are not seen at a HTC. The majority of subjects in this study did not submit a BLS, and of those that did, nearly all were participants in the *MLOF* genotype initiative. Despite this limitation, the sample size of pediatric subjects that did submit a BLS was larger than most studies of adult carriers that report BLS, thereby making this data of value.

We relied on the ISTH-BAT BLS to describe bleeding symptoms. Bleeding scores remain the best objective measure of clinical bleeding and have been validated for carriers.²³ However, there are limitations, which may be particularly relevant for pediatric hemophilia carriers. For instance, a 2-year-old carrier with a single untreated joint bleed and no other abnormal bleeding would get an ISTH-BAT BLS score of 2. This would not flag as abnormal for age in this study, but most clinicians would consider this abnormal bleeding. Unlike Von Willebrand disease, investigations of carriers have noted a poor correlation between factor activity levels and bleeding symptoms.^{1, 24-26} It is unknown if this is due to the inadequacies of the BLS, factor activity levels, or both.

The benefits of a large sample size can be offset by errors associated with large databases, including en-

rollment, data collection and submission, and query errors. We attempted to limit this by restricting this investigation to continuous variables (factor activity and BLS) and categorical variables that are audited by ATHN. This study was limited to pediatric carriers residing in the United States who participated in the ATHNdataset and may not be reflective of pediatric carriers residing in other areas of the world.

Despite the limitations of this study, as one of the few investigations of hemophilia carriers focusing exclusively on the pediatric population, we believe it makes important contributions. Significant knowledge gaps remain regarding clinical bleeding in this population and additional investigation is needed. Also needed are investigations into the potential hemostatic functions not measured by aPTT based assays. This would have applicability beyond hemophilia carriers and include all patients with coagulation factor deficiencies.

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Conflicts of Interest: The authors report no conflicts of interest for this project.

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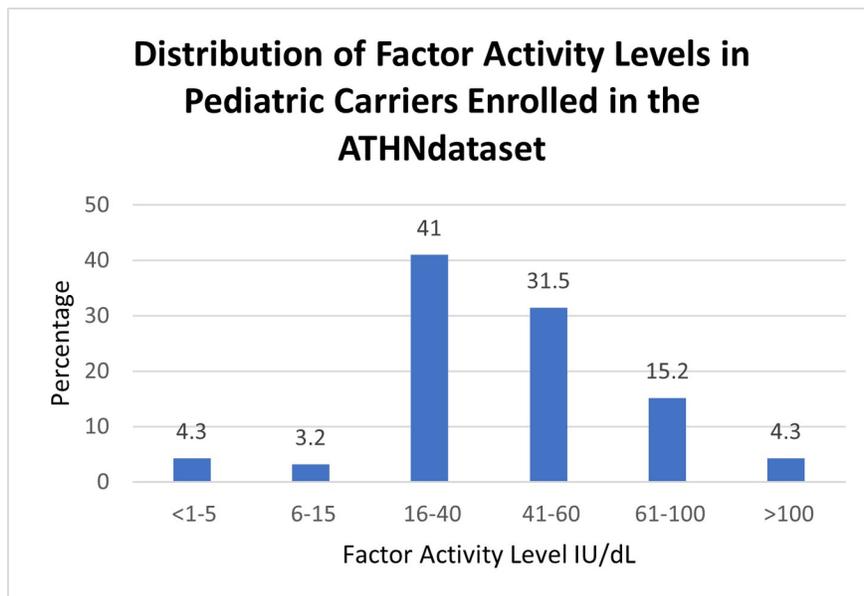
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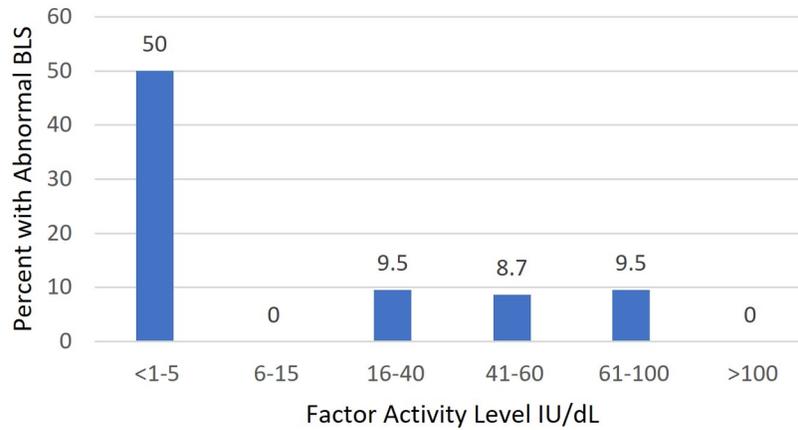
Legends:

Figure 1. Shows the distribution of factor activity levels for all pediatric carriers enrolled in the ATHNdataset.

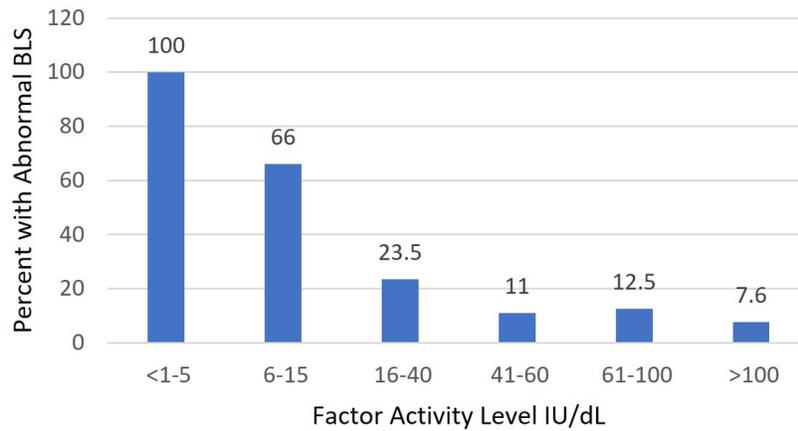
Figure 2. Shows the percentage with an abnormal BLS by factor activity for child and adolescent hemophilia carriers.



Percentage with an Abnormal BLS by Factor Activity for Children Ages 0-10



Percentage with an Abnormal BLS by Factor Activity for Adolescents Ages 11-17



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