The Diagnosis and Evaluation of Women and Girls with Hemophilia and Hemophilia Carriers: Guidance from the SSC of the ISTH

Abstract:

The impact of Hemophilia in affected women and girls (WG) is insufficiently recognized, resulting in sub-optimal evaluation of bleeding symptoms, delay in diagnosis and missed management opportunities. The aim of this collaborative International Society on Thrombosis and Haemostasis (ISTH) Scientific Subcommittee (SSC) project amongst Pediatric and Neonatal Thrombosis and Haemostasis SSC, Factor VIII, IX and Rare Coagulation Disorders SSC, and Women's Health Issues in Thrombosis and Haemostasis SSC is to provide recommendations regarding screening and diagnosis of Hemophilia carriers (HC)/WG with hemophilia (WGwH) in addition to the previously published revised nomenclature by the ISTH SSC. We provide several guidance recommendations regarding the utilization of the ISTH-Bleeding Assessment Tool (ISTH-BAT) as a tool to quantify bleeding phenotype, factor VIII/IX assays for testing, assay discrepancy, genetic testing and testing during pregnancy and postpartum, to help guide hemophilia providers to streamline the screening and diagnosis of HC/WGwH in a uniform manner. We hope, once the screening and diagnosis of HC/WGwH becomes a uniform standard practice globally, this will then pave the way for improved management, bringing gender equity in hemophilia care a step forward.

Introduction:

The recognition of the impact of hemophilia in women and girls (WG) as a bleeding disorder is improving thanks to several recent studies and the prior publication of the International Society on Thrombosis and Haemostasis (ISTH) Scientific Subcommittee (SSC) for nomenclature clarification[1]. Despite this, a recent ISTH survey[2] documented several inconsistent practices in the evaluation and diagnosis of women and girls with Hemophilia A (WGwHA) and Hemophilia A carriers (HAC) amongst global providers. Less than 50% of the providers surveyed felt that WGwHA/HAC are being effectively diagnosed. Comparing provider approaches in diagnosing Hemophilia A (HA) in utilizing factor assays, chromogenic assay (CSA) was shown to be ordered by fewer providers in WG compared to men and boys (MB) and one stage assay (OSA) utilized more often than CSA to diagnose WGwHA and to measure baseline factor (F) VIII level in HAC. Diagnostic tools including the ISTH Bleeding Assessment Tool (BAT)[3] score and genetic testing were not shown to be routinely used to evaluate and diagnose WGwHA/HAC by one-third of the global providers surveyed. Lastly, global providers reported that diagnosis of WGwHA/HAC can be enhanced by consistent screening and improved utilization of the tools and FVIII assays available. Recent publications[4, 5] have also documented the prevalence of assay discrepancies in WGwHA/HAC. These studies and surveys underline the need to have guidelines and recommendations regarding evaluation, diagnosis and management of women and girls with Hemophilia (WGwH) and Hemophilia carriers (HC0, which are currently lacking due to the dearth of clinical trials. Experts' consensus and a guidance document will enable clinicians to streamline the process of evaluation, diagnosis and management of WGwH/HC and to address the practical challenges encountered by global providers.

The aim of this collaborative ISTH SSC project amongst Pediatric and Neonatal Thrombosis and Haemostasis SSC, Factor (F) VIII, IX and Rare Coagulation Disorders SSC, and Women's Health Issues in Thrombosis and Haemostasis SSC is to revisit the previously recommended nomenclature by the ISTH SSC,[1] and provide recommendations regarding screening and diagnosis of WGwH/HC including the utilization of the BAT score, FVIII/FIX assays for testing, assay discrepancy, genetic testing and testing during pregnancy and postpartum.

Methods

This collaborative SSC project started in 2025. Experts in WGwH/HC were convened based on their clinical and academic expertise, who conducted a review of available literature in PubMed on the topics of WGwH/HC nomenclature, screening, diagnosis, FVIII/IX assays, prevalence of assay discrepancies, genetic testing and testing during pregnancy and postpartum. Reference lists of publications identified were reviewed and articles retrieved where relevant. Randomized controlled trials or observational studies that addressed the diagnosis and evaluation of WGwH/HC were included. Case reports were excluded. The term "advise" is used when consensus between authors was achieved in combination with evidence from multiple higher quality clinical studies with greater certainty of evidence, indicating a strong guidance statement, where clinicians should generally implement the guidance recommendation. The term "suggest" is used for author consensus but with a lack of higher quality clinical studies with lower certainty of evidence, indicating a weak guidance statement, whereby strong evidence to support the statement is lacking; and the clinician may implement the guidance recommendation, but an alternative practice may also be appropriate. Consensus was achieved by serial revision of proposed statements in response to co-author feedback. Conflicts amongst the co-authors were resolved by discussion and modifications as required. The initial draft of the recommendations was presented at the meeting of the SSCs on FVIII/IX & Rare Coagulation Disorder during the ISTH congress in June 2025 in Washington DC, USA. The final recommendations were presented to the Factor VIII/FIX and Rare Coagulation Disorders SSC, Pediatric and Neonatal Thrombosis and Haemostasis SSC, and Women's Health Issues in Thrombosis and Haemostasis SSC and their feedback was incorporated into the final Guidance statement.

Throughout this paper, the terms women and girls will be used to discuss medical concerns affecting persons born with a uterus. The authors wish to emphasize the importance of addressing reproductive tract bleeding health in any person with a uterus, regardless of gender identity.

Definition of WGwH and HC

Guidance recommendations:

1. The guidance panel advises that the overarching diagnosis of HC be used to denote all WG with pathogenic *F8/F9* gene variant for Hemophilia A/B, with/without low FVIII/IX levels, respectively. This includes WGwH with FVIII/IX <0.40 IU/mL (WG with mild, moderate and severe H A/B) as well as symptomatic and asymptomatic HC A/B.

2. The guidance panel advises that WG with FVIII/IX levels <0.40 IU/mL be diagnosed with Hemophilia (WGwH) and be managed accordingly and counselled about carrier status. Their Hemophilia, in concordance with the ISTH diagnosis criteria[6], may be mild (FVIII/IX levels >0.05-<0.40 IU/mL), moderate (FVIII/IX levels 0.01-0.05 IU/mL), or severe (FVIII/IX levels <0.01 IU/mL).
3. The guidance panel advises that WG with FVIII/IX levels ≥0.40 IU/mL and a pathogenic *F8/F9* gene variant with bleeding symptoms as defined in the ISTH-BAT be evaluated thoroughly for other bleeding disorders; those who test negative for other bleeding disorders are considered symptomatic HC and be managed similar to WG with mild Hemophilia and be counselled about carrier status.
4. The guidance panel advises that WG with FVIII/IX levels ≥0.40 IU/mL and a pathogenic *F8/F9* gene variant without bleeding symptoms as defined in the ISTH-BAT score are considered asymptomatic HC and be counselled about carrier status and potential bleeding complications with periodic monitoring for bleeding symptoms/signs and prior to procedures.

Several studies demonstrate that WGwH/HC can have low coagulation factor levels[7, 8, 9]. In HC, the random X chromosome inactivation, namely lyonization, can lead to a wide range of factor levels. Plug et al[7] demonstrated a median factor level of 0.60 IU/ml (range 0.05–2.19) in WGwH, with 28% having factor levels <0.40 IU/ml. Labarque and colleagues[8] found that the median FVIII level in HAC (0.68 IU/mL; range 0.19-2.00 IU/mL) was significantly lower than in non-carriers (1.28 IU/mL; range 0.49-2.30 IU/ml) ($p < .001$), with 77% of tested carriers having FVIII levels ≥0.5 IU/mL. WGwH/HC can have bleeding manifestations, including mucocutaneous bleeding, heavy menstrual bleeding (HMB), post-partum hemorrhage, post-trauma/surgery bleeding, and joint bleeding, which affects their quality of life[7, 8, 9]. This increased bleeding is reported not only in those with FVIII/IX level <0.40 IU/mL, but also in those with levels ≥0.40 IU/mL without an established scientific rationale[7, 8, 9]. This led to the ISTH SSC recommendation for a new nomenclature[1], with an overarching diagnosis of HC, and further classifying them as WG with mild, moderate or severe hemophilia if FVIII/IX level is >0.05-<0.40 IU/mL, 0.01-0.05 IU/mL, or <0.01 IU/mL respectively and, if FVIII/IX level is >0.40 IU/mL, as symptomatic or asymptomatic HC based on the presence or absence of bleeding symptoms/signs respectively. WGwH is a preferred primary nomenclature for females with FVIII/IX levels <0.40 IU/dL rather than HC, as it highlights that firstly, these WG have a bleeding disorder, as well as secondly, they are a carrier of Hemophilia; also genotypically these females may be heterozygotes for *F8*/F9 mutation and have a bleeding phenotype like MB with Hemophilia (MBwH) or they may also be homozygotes, compound heterozygotes or hemizygotes (in WG with Turner's syndrome) for *F8/F9* mutation[10]. Where resources are tied to a

diagnostic code, this classification enables hemophilia providers to manage WGwH/symptomatic HC with appropriate hemostatic therapy based on their factor level and type of bleeding, similar to MBwH versus asymptomatic HC, who can be managed with counseling and periodic monitoring for bleeding symptoms/signs. This nomenclature was a first step in equitable care for this group, with remaining issues to be considered such as overuse of the term "carrier" which could convey a non-pathologic state and lack of clarity on WGwH with levels ≤0.40 IU/ml, which has been designated as Hemophilia by other governing bodies.

Screening and Diagnosis of HA:

A. ISTH Bleeding Assessment Tool (BAT) Score:

Guidance recommendations:

5. The guidance panel advises that WGs with genetic variant for Hemophilia and WGs suspected of being WGwH/HC with bleeding symptoms/signs including HMB have a bleeding assessment completed utilizing the ISTH-BAT and score documented.
6. The guidance panel advises that WGs including girls with a proven pathogenic *F8/F9* variant as well as 'potential' HC whose genetic testing is being delayed until they can consent themselves or for other reasons, be assessed for bleeding at the initial visit with ISTH-BAT score.
7. The guidance panel advises that those diagnosed as WGwH or symptomatic/asymptomatic HC be periodically reassessed every 6 to 12 months and their ISTH-BAT score updated, as they transition into the adolescence, menarchal age and into adulthood, prior to experiencing menstruation, pregnancy, postpartum status, menopause and other surgical/procedural hemostatic challenges.

According to the ISTH SSC recommendation on new nomenclature for WGwH[1], Hemophilia diagnosis should be assigned based on the patient's bleeding phenotype and factor levels. Bleeding scores are used to quantitatively assess and report the bleeding phenotype in bleeding disorders. Several bleeding scores have been assessed in WGwH/HC. The Condensed Molecular and Clinical Markers for the Diagnosis and Management of type 1 von Willebrand Disease (MCMDM-1VWD) Bleeding Questionnaire to evaluate bleeding phenotype[9, 11] and the Pictorial Bleeding Assessment Chart (PBAC) score[11] to evaluate HMB, have been studied in this population; HC had higher bleeding score using both tools when compared to controls.

The ISTH-BAT score was developed as a standardized measure to quantify bleeding phenotype in all bleeding disorders, with a summative score of ≥6 in adult women (≥18 years) and a score of ≥3 in pediatric and adolescent females (<18 years) considered abnormal[12]. In adolescents after menarche, it is reasonable to adopt a score of ≥5 since the menstruation domain is then added to the ISTH-BAT[13].

A more recent study[14] assessed variability in ISTH-BAT bleeding scores among healthy adult females across a range of ages and showed the normal range to be 0 to 4 in those aged 18 – 30 years, and 0 to 6 in those aged 52 – 88 years. A large multinational study aimed to validate the ISTH-BAT score in this population demonstrating significantly higher scores in HC/WGwH when compared to age matched controls[15]. Confirming this data, a single center study showed abnormal (higher) ISTH-BAT scores in 57% adult women and 35% pediatric females and 66% post-menarchal and 27% pre-menarchal females respectively with HA/HAC[3]. Other studies have confirmed these findings demonstrating abnormal (higher) bleeding scores in HC and WGwH A and B[9, 16,17]. More recently, a large study utilizing the American Thrombosis and Hemostasis Network (ATHN) dataset documented that nearly half of pediatric HC A and B had factor activity levels ≤0.40 IU/mL[18]. Interestingly, only 13.5% had an abnormal bleeding score for age, noted in more patients with factor levels less than ≤0.40 IU/mL (23%) than those >0.40 IU/mL (9.7%). It is likely that there may be a bias as patients with no bleeding are less likely to be registered in the ATHN dataset. As girls might not have lived long enough to face hemostatic challenges, it is important to periodically reassess their symptoms as they transition into the adolescence, menarche and eventually into adulthood. This is applicable to women as well, especially those who have not previously faced hemostatic challenges. Importantly, the ISTH-BAT score is not routinely used as our ISTH international survey showed that one-third of the global hemophilia providers do not use the ISTH-BAT score routinely in evaluating WGwHA/HAC[2].

Documenting the ISTH-BAT score in WG with a genetic variant for Hemophilia as well as WG presenting with bleeding including HMB can help to evaluate and quantify their bleeding phenotype. This can in turn prompt FVIII/IX testing, especially in WG presenting with bleeding, and enable ruling in or out Hemophilia diagnosis.

B. F VIII/IX assays:

1. Screening WG relatives of Persons with Hemophilia

Guidance recommendation:

8. The guidance panel advises that WG relatives of persons with Hemophilia A and B (PwH) with pathogenic *F8/F9* genetic variants and obligate carriers have FVIII/IX levels evaluated at the earliest possible time, with factor assay/s in order to categorize them as WGwH versus symptomatic/asymptomatic HC.

Given that WG relatives of PwH who have a pathogenic *F8/F9* gene variant may have F VIII/IX activity <0.40 IU/mL or activity ≥0.40 IU/mL and have a bleeding phenotype[7, 8, 9], it is important to measure and document their baseline FVIII/IX levels. As recommended in mild MBwHA[19], both one-stage assay (OSA) and/or chromogenic assay (CSA) should be considered to document baseline FVIII levels in WG. The advantages of OSA are the following: as a one-stage procedure, the assay is simple, rapid, inexpensive and easy to automate and hence widely available and used for clinical diagnosis[21]. The disadvantages are that this assay has a wide variety of assay kits, reagents, assay conditions and analyzers, leading to potentially high interlaboratory variability in the FVIII:C levels. In comparison, CSA has less interlaboratory variability; the disadvantages are that CSA is more expensive, less readily available, and perceived to be technically complex and difficult to automate[20].

In the recent international survey assessing global providers practices and perception of evaluating WGwHA, 62% reported using OSA, 6% using CSA, and 30% using both assays[2]. The use of different assays was based on various factors such as availability at their center/hospital, availability after hours and during weekends, reliability of results, expense of the assay, sensitivity to diagnose FVIII deficiency, less complexity and insurance coverage. Interestingly, statistically significant difference ($p<0.001$) was noted when comparing provider approaches in diagnosing HA, with CSA with/without OSA ordered by less providers in WGwHA (36%) compared to MBwHA (59%)[2]. Efforts need to be undertaken to improve global providers' awareness and utilization of OSA and/or CSA to test WGwHA, and to improve availability, cost and coverage by different health systems, enabling an appropriate diagnosis.

Hemophilia B diagnosis has been made historically with OSA, and the FIX CSA has been a recent addition[21]. The principle of FIX CSA is also generation of FXa, with subsequent measurement by cleavage of a specific chromogenic substrate. There are few FIX CSA kits available when compared to FVIII CSA, however these also vary in their constituents and assay conditions similar to FVIII CSA.

2. Screening WGs with Bleeding

Guidance recommendations:

9. The guidance panel advises that WG with bleeding symptoms/signs as defined in the ISTH-BAT, as part of their evaluation for underlying bleeding disorders, have FVIII/IX levels evaluated with factor assay/s.

Historically, WGwH are identified as HC based on family history of Hemophilia in their male relatives. Nevertheless, WG may also present to medical attention with bleeding symptoms first and subsequently be diagnosed with Hemophilia. *F8/F9* genetic mutation may only become evident first in an affected female, with no family history. Several studies have reported a wide range of bleeding symptoms and signs in WGwH including mucocutaneous, post-trauma and post-surgical bleeding, HMB and pregnancy and post-partum bleeding[22-25]. It is essential to evaluate WG with bleeding symptoms and signs thoroughly for FVIII/IX deficiency (inherited or acquired), in addition to other bleeding disorders (combined FV/FVIII deficiency, other factor deficiencies, von Willebrand disease and platelet disorders), as detection of Hemophilia in WG will not only enable appropriate care for the index patient, but can also lead to extended testing of affected family members and genetic counseling and required for off-springs testing[26].

C. F VIII Assay Discrepancy:

Guidance recommendations:

10. The guidance panel advises that all WG relatives of PwHA, WGs with *F8* genetic variant and WGs with bleeding symptoms/signs have their FVIII levels measured with both the OSA and CSA at least once, if both assays are readily available, to assess for discrepancy (ISTH SSC recommended assay discrepancy definition: OSA/CSA ratios of >2.0 and <0.5[19]).
11. The guidance panel advises that if discrepancy is present in symptomatic HAC and WGwHA, the assay with lower value that correlates with their bleeding symptoms/signs be considered the diagnostic assay, and be the assay utilized for follow-up monitoring.
12. The guidance panel advises that if discrepancy is present, asymptomatic HAC be periodically reassessed for bleeding as they transition into the adolescence, menarchal age and into adulthood experiencing pregnancy and postpartum status; if and when they develop bleeding symptoms and signs, their diagnosis be changed to symptomatic HAC and assay discrepancy addressed accordingly.

13. When one assay (e.g., CSA) is not routinely available outside normal working hours, it is possible to manage discrepant patients with the available assay provided the relationship between the different assays is known in the individual patient.
14. If discrepancy is not present, either OSA or CSA can be used for follow-up monitoring taking into consideration availability (during weekends, after hours), cost, insurance coverage and other assay-related factors.
15. The guidance panel suggests that in limited resource settings, if only one assay is available (OSA or CSA), FVIII level may be assessed by the available assay initially. A normal FVIII level with a single assay does not completely exclude HA. When feasible, the alternate assay can be completed and documented through a send out test to an outside lab or center, to rule out HA and verify if there is an assay discrepancy.
16. The guidance panel advises that when reporting a discrepancy, the results be reported as the ratio of OSA over CSA.
17. The guidance panel advises that all abnormal tests be confirmed on a repeat sample; initial tests and repeat tests be done at a time without any underlying inflammation, pregnancy or estrogen use.

Several studies in MBwHA[27-32] have documented assay discrepancies in up to one-third of the patients with non-severe HA, with the diagnosis accuracy and severity assignment affected in up to 10% due to the discrepancies. However, a large study of an international cohort of patients with non-severe HA with central measurements of FVIII levels, showed low prevalence of assay discrepancy in the study population, supporting their hypothesis that assay discrepancy is largely driven by laboratory variations[33]. Most studies in WGwHA have reported OSA results primarily. Chiffre-Rokatoarivony et al[18] utilized both OSA and CSA, nevertheless did not address the prevalence of assay discrepancies in this population. The recently published study[3], the first to evaluate assay discrepancies in an exclusive WGwHA population, documented both direct discrepancies (in 20 to 40% of patients studied), as well as inverse discrepancies (in 7 to 11% of patients studied), and reported this as both >1.5 and 2-fold differences as well as absolute differences of 0.10 and 0.20 IU/mL. This is also the first study[3] to document the comparison between absolute differences and fold differences in assay discrepancies, showing that direct discrepancy of >1.5-fold and inverse discrepancy of >0.6-fold differences align closer to absolute difference of 0.20 IU/mL. Up to 17% of patients in this study would have been misdiagnosed as HAC and not as having Hemophilia and 1.5% would have been assigned incorrect Hemophilia severity categorization based on one assay results only. ISTH SSC recommended assay discrepancy definition as

OSA/CSA ratios of >2.0 (discrepancy) and <0.5 (inverse discrepancy) have been recommended by for MBwHA.[19] Alternately, discrepancy can be reported as an absolute difference of 0.10 or 0.20 IU/mL between the 2 assays, which has been shown in some studies on MBwHA[19] and WGwHA[3].

Assay discrepancy between OSA and CSA have been reported in few studies in MBwHB, ranging between little discrepancy (1/51 patients tested; 2%)[33] to higher rates of discrepancy (15/44 samples tested; 34%; 12/17 patients tested; 70%)[34, 35] with ≥2-fold higher FIX results with OSA when compared to CSA. This has however not been reported in WGwHB/HBC.

D. Genetic testing:

Guidance recommendations:

18. The guidance panel advises that all WG identified as potential HC undergo genetic testing if available, regardless of FVIII/IX levels, targeting the specific *F8/F9* gene variant found in their relative PwH.
19. The guidance panel advises that genetic testing be recommended for obligate WG HC for the specific genetic variant identified in their family.

Genetic testing encompasses all the genotyping methods aimed at identifying gene variants, such as molecular intron analysis, next-generation sequencing, Sanger sequencing and multiplex ligation-dependent probe amplification, among others. WGwH who inherit the affected X chromosome may not have a bleeding phenotype, as their unaffected X chromosome enables them to produce factor levels in the normal range. However, lyonization can lead to random inactivation of the unaffected X chromosome, resulting in unpredictable low factor levels in the range of mild, moderate, or rarely severe Hemophilia. Obligate HC are defined as a) the biological daughter of a father with Hemophilia, b) the biological mother of a child with hemophilia and at least one more relative with Hemophilia or known HC and c) the biological mother of two or more children with Hemophilia[36]. Genetic testing should be recommended for obligate HC, targeting the same genetic variant as their relatives (MBwH and/or WG who are identified as HC), also taking into consideration non-paternity in a small subset of patients, and is mandatory for those wishing to pursue prenatal diagnosis, preimplantation genetic testing and/or assisted reproductive technology. Potential HC, on the other hand, will need genetic testing to rule in/out their carrier status, particularly if their FVIII/IX level is within the normal range. These include a biological WG relative of a MBwH and no known family history of Hemophilia/HC and biological WG relative of a WG HC. The genetic testing will be for the specific *F8/F9* gene variant found

in their relative PwH. Additionally, X chromosome inactivation studies to identify random or non-random inactivation can be undertaken in WG potential HC and WGwH with bleeding phenotype, although these assays may not be routinely available in all centers and countries[37]. However, there have been no reports of differential patient's clinical behavior or healthcare conduct in response to this finding, and there is no guidance on how to interpret these in the setting of a normal FVIII/FIX level.

Since the publication of the ISTH guidance on the nomenclature of HC, there has been considerable discussion regarding the proper terminology, with patient advocacy groups suggesting limiting the use of the term HC, describing the inheritance as X-linked (leaving out recessive or dominant qualifier) and explaining that bleeding can occur with normal levels with interindividual differences likely explained by skewed lyonization. The authors acknowledge this and recommend the use of the term HC when having discussions about inheritance and for documentation. Whereas when having discussion about clinical management, focus should be on the severity of the factor deficiency and the bleeding symptoms for clinical classification.

WG with a bleeding phenotype and identified low factor levels should have a detailed family history emphasized on the presence or absence of biological relatives with Hemophilia/HC. Those with negative family history should also undergo genetic testing to clarify their HC status, as new mutations for Hemophilia can also occur in WGs. This can be done in a stepwise fashion as in the genetic testing of MBwH. WGwH genotypically may be heterozygotes for a *F8/F9* variant similar to MBwH or they may be homozygotes, compound heterozygotes or hemizygotes for *F8/F9* mutation[10]. Karyotype with high-resolution of X to identify X chromosome abnormality may also be required in select cases where X chromosomal abnormality larger than *F8/9* mutation is suspected.

Authors of the European principles of care for WG with inherited bleeding disorders[38] advise to perform genetic testing for Hemophilia in adolescence or early adulthood and in certain cases considering it in children; however, this practice varies by cultural values and available resources. In the United States, current practice involves conducting genetic testing in all potential WG HC[3, 18]. Knowing the HC status enables optimal, continued clinical care for WGwH as well as symptomatic HC, allows for consideration of informed reproductive choices, preimplantation and prenatal diagnostics, and if the patient chooses to conceive, enables proactive planning about the timing of pregnancy, mode of delivery, close medical monitoring and care during pregnancy, delivery and post-partum, and consideration of testing of the fetus/neonate and optimal care of the mother and the neonate to prevent/manage bleeding

complications. In addition, if genetic testing does not reveal a pathogenic *F8/F9* variant, this can relieve anxiety and avoid unnecessary use of medical resources.

In addition, genetic testing in MBwHA can identify gene defects responsible for factor OSA/CSA discrepancies; however, the available information in WGwHA and HAC is scarce and likely varies from genetic variants results in MBwHA[4]. Our recent ISTH survey[2] documented that genetic testing is rarely used to evaluate/diagnose WGwHA by 33% of respondents. These results bring to attention the need for efforts to educate global providers on appropriate steps for thorough evaluation and diagnosis of WGwH including genetic testing when indicated and to provide ready access for genetic testing for global hemophilia providers, including low-resource countries that might require adaptations of these strategies based on the population's characteristics, molecular facilities, and quality assurance to provide the largest benefits for WGwH and HC[39-42].

E. Testing during pregnancy and postpartum:

Guidance recommendations:

20. The guidance panel advises that FVIII/IX levels be measured during pregnancy, and at minimum during the 3rd trimester in order to guide plans for delivery and the post-partum period. FVIII/IX levels should also be measured during the 2nd trimester to guide occasional pre-term births.
21. The guidance panel advises that if a woman presents for the first-time during pregnancy, for Hemophilia A, both OSA and CSA FVIII be undertaken at presentation (where the assays are available); and both assays be repeated at least 6 weeks post-partum in order to establish baseline levels.
22. The guidance panel advises that genetic testing be recommended if not performed earlier, especially if prenatal diagnosis (PND) is desired; prenatal genetic testing be available as an option in case of a potentially affected male fetus; early PND if pregnancy termination in case of an affected male fetus is considered, or late PND to guide perinatal management plans.

During pregnancy there is an increase in FVIII and von Willebrand factor and the levels subsequently reduce rapidly in the first 7-10 days postpartum[42, 43]. For WwHA/HAC, FVIII:C levels can increase significantly, and into the 'normal' range in the later stages of pregnancy. However, the FVIII:C levels remain lower than for someone without Hemophilia, and the risk of postpartum haemorrhage remains increased, in particular for carriers of severe Hemophilia[44]. In case of HBC, FIX levels rise considerably less during pregnancy compared to FVIII:C although some increase is observed[45].

Counselling of the HC, and working with a multidisciplinary team on Hemophilia, with specialists in both adult and paediatric Hemophilia and obstetric medicine, is extremely important to support both the pregnancy and safe delivery of the child. Within this, identifying the underlying genetic variant is needed. For those with a known family history of Hemophilia, the affected male offspring will have the same phenotype as the other affected male family members. If the genetic variant is known prior to pregnancy, then carriers of severe Hemophilia may have the option of undergoing in vitro fertilization for preimplantation genetic testing of embryos if available as an accepted practice. HC should have determination of fetal sex by serum free DNA if available (at about 9 weeks gestation) if the couple considers terminating the pregnancy in case of an affected male fetus, although this is a rare occurrence with multidisciplinary counseling. In other cases, the testing can wait until sex determination is possible by ultrasound, usually at week 20, unless the unknown status causes severe distress in the pregnant women affecting a heathy pregnancy. If the genetic variant is known, then additional options include prenatal diagnosis of Hemophilia by chorionic villous sampling (placental biopsy performed during the first trimester of pregnancy) if early termination of pregnancy of an affected male fetus would be considered, or late amniocentesis (amniotic fluid sampling performed during the third trimester of pregnancy, usually at 34-36 weeks) to adjust the delivery plan with regards to the neonate. In case of an affected male fetus atraumatic delivery, without cranial blood sampling, forceps or vacuum extraction, is advised.

F. Testing in Low-income countries:

Guidance recommendations:

23. The guidance panel suggests that for low-income countries where resources (factor assays, genetic testing) are limited, a partnership with the World Federation of Hemophilia using programs like “A Cornerstone Initiative Country Project” that helps build infrastructure and training to certain regions and countries in the world be developed[47].
24. The guidance panel advises that when testing is not feasible, every potential WG Hemophilia carrier at the minimum be educated about the possibility of her carrier status versus mild, moderate or severe Hemophilia, risk for bleeding symptoms, and giving birth to a child with haemophilia.

G. Systematic testing in specialized centers:

Guidance recommendations:

25. The guidance panel advises that well-defined systematic approaches and testing pathways for proactive identification and screening of all potential WG hemophilia carriers among families of all PwHs be developed and routinely practiced in all Hemophilia Treatment Centers (HTC), to facilitate uniform testing, in order to minimize the potential for "missed" diagnoses[46]. This initiative would be a crucial step toward ensuring equitable access to hemophilia diagnosis and care for all potentially affected individuals, regardless of sex and gender.

Conclusion:

Creating a proactive, systematic approach to screen WGwH/HC and WG with bleeding phenotype including family history, bleeding evaluation employing ISTH-BAT as a diagnostic tool, factor level assessment, genetic testing, testing during pregnancy and postpartum in countries with resources and screening with partnership programs and/or educating potential HC in low income countries, are important for accurate diagnosis and to avoid missed diagnoses (Figure 1). Aligning with the 2020 WFH guideline recommendations to establish family pedigrees for genetic counseling and testing[47], implementing well defined and uniform testing pathways for carrier screening in all HTCs can avoid missed diagnoses and allow thorough testing of all WG relatives of PwH. As and when this effort of establishing family pedigrees in PWH and screening all WG relatives becomes a standard practice globally, this will then pave the way for gender equity in hemophilia care and will be a major stride towards ending gender disparities in hemophilia.

References:

1. van Galen KPM, d'Oiron R, James P, Abdul-Kadir R, Kouides PA, Kulkarni R, Mahlangu JN, Othman M, Peyvandi F, Rotellini D, Winikoff R, Sidonio RF. A new hemophilia carrier nomenclature to define hemophilia in women and girls: Communication from the SSC of the ISTH. J Thromb Haemost 2021; 19(8):1883-1887.
2. Srivaths L, Larson J, Fijnvandraat K, Ardila J, Escobar M. Chromogenic Versus One Stage Assay to Diagnose Women and Girls with Hemophilia A - Mapping Global Approaches and Assessing Challenges: Communication from the SSCs of the ISTH. J Thromb Haemost 2025; 23(8):2656-2662.
3. Elbatarny M, Mollah S, Grabell J, et al; Zimmerman Program Investigators. Normal range of bleeding scores for the ISTH-BAT: adult and pediatric data from the merging project. Haemophilia. 2014; 20(6):831-5.
4. Srivaths L, Larson J, Saroukhani S, Said M, Brown D, Rodriguez N, Menon N, Escobar M. Comparing one stage, chromogenic assay results and discrepancies with bleeding phenotype and genetic variants in females with hemophilia A. J Thromb Haemost 2025; 23(2):504-512.
5. Desage S, Lienhart A, Janbain M, Rezigue H, Leuci A, Dargaud Y. Discrepancy between one-stage clotting and chromogenic factor VIII activity in women with hemophilia A and hemophilia A carriers: a retrospective clinical study. J Thromb Haemost. 2025; 23(6):1817-1823.
6. White GC II, Rosendaal F, Aledort LM, et al. Definitions in hemophilia: recommendation of the Scientific Subcommittee on Factor VIII and Factor IX of the Scientific and Standardization Committee of the International Society on Thrombosis and Haemostasis. Thromb Haemost. 2001;85(3):560.
7. Plug I, Mauser-Bunschoten EP, Bröcker- Vriends AHJT, et al. Bleeding in carriers of hemophilia. Blood. 2006; 108:52-56.
8. Labarque V, Perinparajah V, Bouskill V, et al. Utility of factor VIII and factor VIII to von Willebrand factor ratio in identifying 277 unselected carriers of hemophilia A. Am J Hematol. 2017; 92: E94-E96.
9. Olsson A, Hellgren M, Berntorp E, Ljung R, Baghaei F. Clotting factor level is not a good predictor of bleeding in carriers of haemophilia A and B. Blood Coagul Fibrinolysis. 2014; 25:471-475.
10. Miller CH, Bean CJ. Genetic causes of haemophilia in women and girls. Haemophilia. 2021; 27(2): e164-e179.

11. Paroskie A, Gailani D, DeBaun MR et al. A cross-sectional study of bleeding phenotype in haemophilia A carriers. Br J Haematol 2015; 170: 223–8.
12. Elbatarny M, Mollah S, Grabell J et al. Normal range of bleeding scores for the ISTH-BAT: adult and pediatric data from the merging project. Haemophilia 2014;20: 831–5.
13. Jain S, Zhang S, Acosta M, Malone K, Kouides P, Zia A. Prospective evaluation of ISTH-BAT as a predictor of bleeding disorder in adolescents presenting with heavy menstrual bleeding in a multidisciplinary hematology clinic**.** J Thromb Haemost. 2020; 18(10):2542-2550.
14. Doherty D, Grabell J, Christopherson PA, Montgomery RR, Coller BS, Lavin M, et al.; Zimmerman Program Investigators. Variability in International Society on Thrombosis and Haemostasis-Scientific and Standardization Committee endorsed Bleeding Assessment Tool (ISTH-BAT) score with normal aging in healthy females: contributory factors and clinical significance. J Thromb Haemost. 2023 Apr;21(4):880-886.
15. James PD, Mahlangu J, Bidlingmaier C. Evaluation of the utility of the ISTH-BAT in haemophilia carriers: a multinational study. Haemophilia 2016;22: 912-918.
16. Young JE, Grabell J, Tuttle A, Bowman M, Hopman WM, Good D, et al. Evaluation of the self-administered bleeding assessment tool (Self-BAT) in haemophilia carriers and correlations with quality of life. Haemophilia 2017; 23: e536–e538.
17. Chiffré-Rakotoarivony D, Diaz-Cau I, Ranc A, Champiat M-A, Rousseau F, Gournay-Garcia C, et al. Bleeding risk in hemophilia A and B carriers: comparison of factor levels determined using chronometric and chromogenic assays. Blood Coagul Fibrinolysis. 2024; 35(5):232-237.
18. Puetz J, Hu B. Factor activity levels and bleeding scores in pediatric hemophilia carriers enrolled in the ATHN dataset. Pediatr Blood Cancer. 2023; 70(11):e30644.
19. Makris M, Oldenburg J, Mauser-Bunschoten EP, Peerlinck K, Castaman G, Fijnvandraat K; subcommittee on Factor VIII, Factor IX and Rare Bleeding Disorders. The definition, diagnosis and management of mild hemophilia A: communication from the SSC of the ISTH. J Thromb Haemost. 2018 Dec;16(12):2530-2533.
20. Peyvandi F, Oldenburg J, Friedman KD. A critical appraisal of one-stage and chromogenic assays of factor VIII activity. J Thromb Haemost. 2016;14(2):248-61.
21. Bowyer AE, Gosselin RC. Factor VIII and Factor IX Activity Measurements for Hemophilia Diagnosis and Related Treatments. Semin Thromb Hemost. 2023;49(6):609-620.
22. Mauser-Bunschoten EP, van Houwelingen JC, Sjamsoedin Visser EJ, van Dijken PJ, Kok AJ, Sixma JJ. Thromb Haemost. Bleeding symptoms in carriers of hemophilia A and B. 1988; 59(3):349-52.

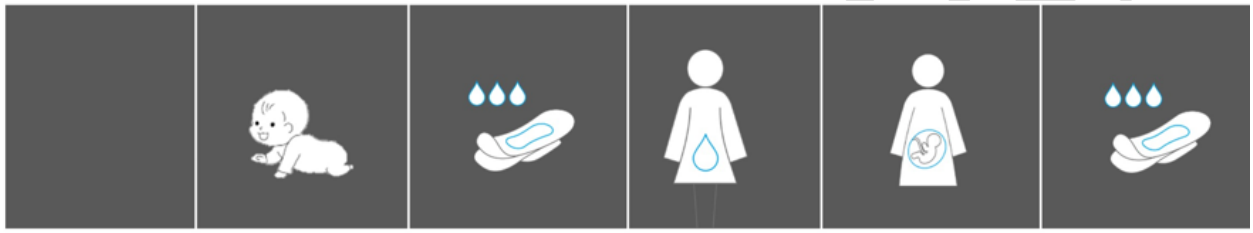
23. Staber J, Croteau SE, Davis J, Grabowski EF, Kouides P, Sidonio RF Jr. The spectrum of bleeding in women and girls with haemophilia B. Haemophilia. 2018; 24(2):180-185.
24. Punt MC, Waning ML, Mauser-Bunschoten EP, Kruip MJHA, Eikenboom J, Nieuwenhuizen L, et al. Maternal and neonatal bleeding complications in relation to peripartum management in women with Von Willebrand disease: a systematic review. Blood review 2020;39;100633
25. Paroskie A, Oso O, Almassi B, DeBaun MR, Sidonio RF Jr. Both hemophilia health care providers and hemophilia A carriers report that carriers have excessive bleeding. J Pediatr Hematol Oncol. 2014 May;36(4):e224-30.
26. Dardik R, Janczar S, Lalezari S, et al. Four Decades of Carrier Detection and Prenatal Diagnosis in Hemophilia A: Historical Overview, State of the Art and Future Directions. Int J Mol Sci. 2023 Jul 24;24(14):11846.
27. Poulsen AL, Pedersen LH, Hvas AM, Poulsen LH, Thykjaer H, Ingerslev J. Assay discrepancy in mild haemophilia A: entire population study in a National Haemophilia Centre. Haemophilia 2009; 15: 285–9.
28. Trossaert M, Lienhart A, Nougier C, Fretigny M, Sigaud M, Meunier S, Fouassier M, Ternisien C, Negrier C, Dargaud Y. Diagnosis and management challenges in patients with mild haemophilia A and discrepant FVIII measurements. Haemophilia 2014; 20: 550–8.
29. Pavlova A, Delev D, Pezeshkpoor B, Muller J, Oldenburg J. Haemophilia A mutations in patients with non-severe phenotype associated with a discrepancy between one-stage and chromogenic FVIII assays. Thromb Haemost 2014; 111: 851–61.
30. Cid AR, Calabuig M, Cortina V, Casana P, Haya S, Moret A, Cabrera N, Aznar JA. One-stage and chromogenic FVIII:C assay discrepancy in mild haemophilia A and the relationship with the mutation and bleeding phenotype. Haemophilia 2008; 14: 1049–54.
31. Pipe SW, Eickhorst AN, Mckinley SH, Saenko EL, Kaufman RJ. Mild hemophilia A caused by increased rate of factor VIII A2 subunit dissociation: evidence for nonproteolytic inactivation of factor VIIIa in vivo. Blood 1999; 93: 176–83.
32. Trossaert M, Boisseau P, Quemener A, Sigaud M, Fouassier M, Ternisien C, Lefrancois-Bettembourg A, Tesson C, Thomas C, Bezieau S. Prevalence, biological phenotype and genotype in moderate/mild hemophilia with discrepancy between one-stage and chromogenic factor VIII activity. J Thromb Haemost 2011; 9: 524–30.
33. Zwagemaker AF, Kloosterman FR, Gouw SC, Boyce S, Brons P, Cnossen MH, et al; DYNAMO study group. Little discrepancy between one-stage and chromogenic factor VIII (FVIII)/IX assays in a

large international cohort of persons with nonsevere hemophilia A and B. .J Thromb Haemost. 2023; 21(4):850-861.

34. Kihlberg K, Strandberg K, Rosén S, Ljung R, Astermark J. Discrepancies between the one-stage clotting assay and the chromogenic assay in haemophilia B. Haemophilia 2017;23(04): 620–627.
35. Schmidt DE, Truedsson Å, Strålfors A, Hojbjerg JA, Soutari N, Holmström M, Ranta S, Letelier A, Bowyer A, Ljung R, Antovic J, Bruzelius M. Clinical Implications of Discrepancy between One-Stage Clotting and Chromogenic Factor IX Activity in Hemophilia B. Thromb Haemost. 2024;124(1):32-39.
36. Srivastava A, Santagostino E, Dougall A. WFH Guidelines for the Management of Hemophilia, 3rd edition. Haemophilia. 2020; 26 Suppl 6:1-158.
37. Di Michele DM, Gibb C, Lefkowitz JM, Ni Q, Gerber LM, Ganguly A. Severe and moderate haemophilia A and B in US females. Haemophilia. 2014 Mar;20(2): e136-43.
38. Van Galen K, Lavin M, Skouw-Rasmussen N, Fischer Katelijn, et al. European principles of care for women and girls with inherited bleeding disorders. Haemophilia 2021;1–11
39. González-Ramos I-A, Mantilla-Capacho J-M, Luna-Záizar H, et al. Genetic analysis for carrier diagnosis in hemophilia A and B in the Mexican population: 25 years of experience**.** Am J Med Genet C Semin Med Genet. 2020 Dec;184(4):939-954.
40. Luz Karime Yunis LK, Linares A, Cabrera E, Yunis JJ. Systematic molecular analysis of hemophilia A patients from Colombia. Genet Mol Biol. 2018 Oct-Dec;41(4):750-757.
41. Peyvandi F. Carrier detection and prenatal diagnosis of hemophilia in developing countries. Semin Thromb Hemost. 2005 Nov;31(5):544-54.
42. James AH, Konkle BA, Kouides P, Ragni MV, Thames B, Gupta S, et al. Postpartum von Willebrand factor levels in women with and without von Willebrand disease and implications for prophylaxis. Haemophilia. 2015; 21(1):81-7.
43. Collins AEIC, Curry N, Raza-Burton S, Bignell P, Davies C, Michael Joffe M, et al. Factor VIII levels and bleeding according to factor 8 (F8) mutation in pregnant carriers of haemophilia A: a multicentre retrospective cohort study**.** Br J Haematol. 2021; 193(2):397-400.
44. Punt MC, Waning ML, Mauser-Bunschoten EP, Kruip MJHA, Eikenboom J, Nieuwenhuizen L, et al. Maternal and neonatal bleeding complications in relation to peripartum management in hemophilia carriers: A systematic review. Blood Rev. 2021; 49:100826.

45. Szesci PB, Jørgensen M, Klajnbard A, Andersen MR, Colov NP, Stender S. Haemostatic reference intervals in pregnancy. Thromb Haemost. 2010; 103: 718-727.
46. Krumb E, Lambert C, Van Damme A, Hermans C. Proactive systematic hemophilia carrier screening: a step toward gender equity in hemophilia care. Blood Adv. 2024 Oct 22;8(20):5268-5278.
47. Identification and diagnosis. https://wfh.org/identification-and-diagnosis/

Figure 1: Screening and Diagnosis of (Potential) Hemophilia Carriers



Life stage	Infancy/ childhood	Adolescence	Young adulthood	Pregnancy	Mid/late adulthood
Screening & Diagnosis	Bleeding assessment* Testing for FVIII/IX levels$ Consider genetic testing	Bleeding assessment Testing for FVIII/IX levels$ Genetic testing#	Bleeding assessment Testing for FVIII/IX levels$	Bleeding assessment Testing for FVIII/IX levels$ Genetic testing#	Bleeding assessment^

*While using the ISTH Bleeding assessment tool the following cut-off values are used:

Asymptomatic when the ISTH BAT score is ≥3 in pediatric females

Asymptomatic when the ISTH BAT score is ≥5 in adolescent females

^Asymptomatic when the ISTH BAT score is ≥6 in adult females – further follow up only on demand in case of questions or new abnormal bleeding symptoms occur

#If not yet performed

$preferably both with one-stage and chromogenic assay

Abbreviations:

FVIII/IX: Factor VIII/IX