Laboratory Diagnosis of Acquired Hemophilia A: Limitations, Consequences, and Challenges

Andreas Tiede, MD, PhD[1] Sonja Werwitzke, MD, PhD[1] Rüdiger E. Scharf, MD, PhD[2]

[1] Department of Hematology, Hemostasis, Oncology, and Stem Cell Transplantation, Hannover Medical School, Hannover, Germany
[2] Department of Clinical and Experimental Hemostasis, Hemotherapy and Transfusion Medicine, Heinrich Heine University Medical Center, Düsseldorf, Germany

Address for correspondence Andreas Tiede, MD, PhD, Department of Hematology, Hemostasis, Oncology and Stem Cell Transplantation, Hannover Medical School, Carl Neuberg Str. 1, 30625 Hannover, Germany (e-mail: tiede.andreas@mh-hannover.de).

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Abstract

Acquired hemophilia A (AHA) should be suspected in patients with a new onset of bleeding and an isolated prolongation of activated partial thromboplastin time. About 10% of patients do not bleed at the time of diagnosis, but are at risk of future bleeding, particularly during interventions or surgery. Diagnosis of AHA is confirmed by demonstrating markedly reduced factor VIII activity (FVIII:C) and neutralizing anti-FVIII antibodies, so-called inhibitors. Several limitations and pitfalls exist with the assays used to diagnose AHA. Interference can result from anticoagulants or lupus anticoagulant. The Bethesda assay used to measure inhibitor potency assumes a log–linear relationship between inhibitor concentration and effect on residual FVIII:C activity to allow exact quantification. However, this relationship is not present for the type 2 inhibitors typically seen in AHA. Therefore, this assay only provides a rough estimate of inhibitor potency. These limitations can explain, in part, why laboratory data, such as inhibitor potency, failed to predict bleeding or response to treatment in AHA. This article reviews the diagnostic approach to AHA, discusses assay-specific limitations and addresses some of the challenges for future research.

Keywords
- acquired hemophilia
- factor VIII
- inhibitor
- autoantibody

Acquired hemophilia A (AHA) is caused by autoantibody inhibitors against coagulation factor VIII (FVIII:C). AHA is a serious condition with increased risk of bleeding, high morbidity, and high mortality and can occur in previously healthy men and women of every age.[1,2] Making a rapid and precise diagnosis of AHA are vital to patients to recognize their risk of bleeding from trauma or surgery and to provide adequate hemostatic support, whenever needed.

With an estimated annual incidence of 1.4 per million, AHA is a rare disorder. It is often associated with other medical conditions (►**Table 1**). Most patients with AHA are primarily seen by physicians, who may not have had previous personal experience with AHA. It is therefore important to increase the awareness of physicians for clinical symptoms and laboratory signs of AHA.[3] Once suspected, the diagnostic work-up of AHA is usually straightforward. The assays used, however, show considerable variability among laboratories and may also have some pitfalls that require specific consideration.

This review examines the diagnostic work-up of AHA. Specifically, limitations and possible pitfalls of laboratory assays are discussed. Another objective is to provide a source of education to physicians involved in the diagnosis of AHA, but also to highlight gaps that warrant further evaluation and research.

Methods

Literature Search

Literature searches were performed using the indexed online database Medline/PubMed with the terms "acquired h(a)emophilia," "acquired factor VIII inhibitor(s)," "acquired

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Table 1 Conditions frequently associated with acquired hemophilia

Condition	Estimated frequency	
	EACH2 study (%)[7]	UK surveillance study (%)[44]
Malignancy	12	15
Autoimmune disorders	13	17
Postpartum period	8	2
Dermatological conditions	1	3
Infections	4	Not recorded
Drugs	3	Not recorded
Others	12	Not recorded
None (idiopathic AHA)	52	63

Abbreviation: AHA, acquired hemophilia A.

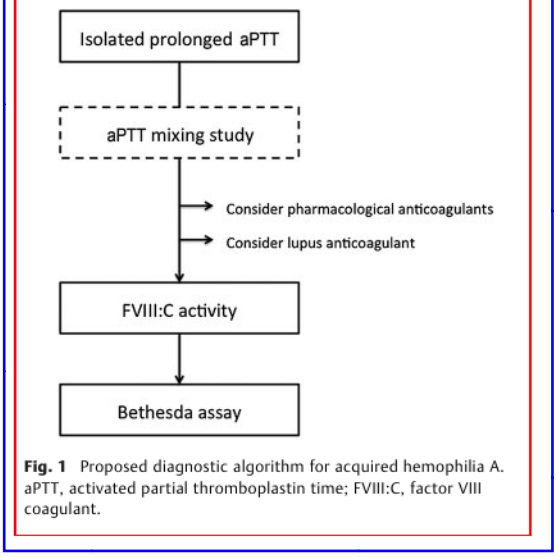

Fig. 1 Proposed diagnostic algorithm for acquired hemophilia A. aPTT, activated partial thromboplastin time; FVIII:C, factor VIII coagulant.

inhibitor(s)," and "Bethesda assay" in May 2014. The full articles of the relevant abstracts were retrieved. Additional relevant publications were identified in the references of those articles and from the authors' own libraries.

Patient Examples

The patients reported herein were treated in the Department of Hematology, Hemostasis, Oncology, and Stem Cell Transplantation at Hannover Medical School. Clinical information was extracted by A.T. from medical records. Laboratory data were retrieved by A.T. and S.W. from the laboratory information system of the departmental routine hemostasis laboratory.

Diagnostic Algorithm

Various diagnostic algorithms have been suggested by an international consensus group[4] and by others.[2,5,6] We propose a simplified algorithm, as depicted in ►**Fig. 1**. Isolated prolongation of activated partial thromboplastin time (aPTT) is usually the first clue of AHA, particularly in patients with a recent onset of bleeding. About 10% of AHA patients have not yet had a bleed at time of diagnosis.[7] We therefore recommend appropriate work-up of any unexplained aPTT prolongation until a diagnosis is made.

aPTT mixing studies are often suggested as a first step to screen for the presence of an inhibitor.[1,2] However, these mixing assays are poorly validated and may not be specific and sensitive enough to confirm or to rule out AHA (see later). Their value may depend on local conditions and the time needed to obtain specific testing.[8,9]

Where factor VIII coagulant (FVIII:C) testing is rapidly available, it appears straightforward to order this assay irrespective of the aPTT mixing study results. In cohorts of AHA, FVIII:C activity was usually reduced to < 30 IU/dL, in 75% of the patients to < 5 IU/dL.[7] Depending on the population, deficiency in factors IX, XI and XII have to be considered alternative (or even more frequent) causes of isolated aPTT prolongation. Thus, ordering these tests together with FVIII:C may provide more clarity.

If FVIII:C activity is markedly reduced, demonstration and quantification of an inhibitor can be obtained by the Bethesda assay. Lupus anticoagulant (LA) and pharmacological inhibitors of coagulation should also be considered during the work-up. Both settings may sometimes result in an artificially low FVIII:C activity and a false-positive Bethesda assay. However, it should be noted that neither the presence of an LA nor therapy with an anticoagulant agent can formally exclude AHA. Consequently, it may be required to perform additional tests or to obtain new samples from the patient (see later).

Differential Diagnosis

Prolongation of aPTT can result from many causes (►**Table 2**). Of these differential diagnoses, only deficiency in factor VIII (hemophilia A and von Willebrand disease [VWD]), factor IX (hemophilia B), and factor XI are associated with increased bleeding. Medical history and bleeding pattern in these conditions are very different from AHA. Heparins and many other anticoagulants including vitamin K antagonists (VKA) and direct oral anticoagulants may also cause aPTT prolongation.

Differential diagnoses of a reduced FVIII:C activity include congenital hemophilia A (with or without inhibitor), VWD, and the acquired von Willebrand syndrome (AVWS). The differentiation is usually straightforward by history taking and laboratory diagnosis (►**Table 3**).[10,11] Inhibitory alloantibodies against FVIII:C in patients with mild hemophilia A are rare, but they can sometimes decrease the residual procoagulant activity and may resemble AHA clinically.[12,13] In cases of doubt, particularly when the patient's history and exposure to FVIII products is unclear, molecular analysis may be

1. Anchor Name: Adapted Figure Illustration [OEEL (Erin Ooi Ee Ling)]

Table 2 Causes of aPTT prolongation

Cause	Note
Factor VIII deficiency	Congenital or acquired hemophilia A, some forms of von Willebrand disease or acquired von Willebrand syndrome
Factor IX deficiency	Hemophilia B
Factor XI deficiency	Less severe bleeding disorder
Factor XII, prekallikrein, and HWMK deficiency	Do not cause bleeding
Other coagulation factor deficiencies • Factor X • Factor V • Prothrombin • Fibrinogen (incl. dysfibrinogenemia)	Also cause prolongation of prothrombin time
Lupus anticoagulant	Increased risk of thromboembolism
Pharmacological anticoagulants	
• Unfractionated heparin	
• Indirect factor Xa inhibitors (low–molecular-weight heparin and fondaparinux)	• Only with higher (therapeutic) doses
• Direct factor Xa inhibitors (rivaroxaban and apixaban)	• Effect on prothrombin time often stronger than on aPTT
• Direct thrombin inhibitors (dabigatran, argatroban, and lepirudin)	• Effect on aPTT often stronger than on prothrombin time

Abbreviations: aPTT, activated partial thromboplastin time; HMWK, high-molecular-weight kininogen.

Table 3 Differential laboratory diagnosis of AHA

Differential diagnosis	Interference with AHA diagnosis	Appropriate action
Von Willebrand disease or acquired von Willebrand syndrome	Patients may have low FVIII:C (and thus prolonged aPTT) due to VWF deficiency	VWF testing (VWF:Ag, VWF:Ac), FVIII binding capacity of VWF; multimer analysis.
Lupus anticoagulant	• Prolongs aPTT • Acts as (fast reacting) inhibitor in aPTT mixing study • May sometimes inhibit FVIII:C assays • May cause false-positive Bethesda assay	• Negative dRVVT-based assay may exclude LA, but up to 20% of patients with FVIII inhibitors are positive in dRVVT-based mixing and confirmation tests • Use more than one dRVVT assay to test for LA • Clinical consideration
Vitamin K antagonists	Prolong aPTT	• Normal prothrombin time/INR excludes agent • aPTT mixing study, FVIII:C, and Bethesda assay unaffected
Unfractionated heparin	• Prolongs aPTT • Acts as (fast reacting) inhibitor in mixing test	• Normal thrombin time excludes heparin • FVIII:C and Bethesda assay affected only by very high doses
Low–molecular-weight heparin and heparinoids	Prolong aPTT with some reagents	• Anti-Xa assay to confirm or exclude anticoagulant drug action • Withdraw drug and repeat testing • FVIII:C and Bethesda assay affected only with very high doses
Direct Xa inhibitors (rivaroxaban and apixaban)	Prolong aPTT and interfere with FVIII:C assays with some reagents	• Anti-Xa assay to confirm or exclude anticoagulant drug action • Withdraw drug and repeat testing
Direct thrombin inhibitors (lepirudin, argatroban, and dabigatran)	Prolong aPTT and interfere with FVIII:C assay with some reagents	• Thrombin time • Withdraw drug and repeat testing

Abbreviations: aPTT, activated partial thromboplastin time; dRVVT, diluted Russel viper venom time; INR, international normalized ratio; LA, lupus anticoagulant; VWF:Ag, von Willebrand factor antigen; VWF:Ac, von Willebrand factor activity (or ristocetin cofactor).

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helpful to differentiate between AHA and congenital hemophilia with inhibitors.

LA causes a thrombophilic state, but can artificially result in low FVIII:C activity. This is true for one-stage clotting tests but also chromogenic substrate assays. LA may interfere with the Bethesda assay. It should be noted, however, that patients with AHA can have concomitant LA.[14–16] In bleeding patients, AHA should be considered a likely diagnosis even if LA is suggested by appropriate methods (see later). A summary of the differential laboratory diagnosis between AHA and LA is provided in ►**Table 3**.

Activated Partial Thromboplastin Time Mixing Test

A positive mixing test may help to differentiate between an inhibitor and simple coagulation factor deficiency. Patient plasma is mixed 1 + 1 with normal pooled plasma (NPP). Failure of normal plasma to correct the aPTT by > 50% is indicative of an inhibitor,[17–19] but there is no consensus about this definition. Inhibitors detected by this assay may include anti-FVIII inhibitors, LA, and pharmacological anticoagulants such as heparin.[9]

FVIII inhibitors often require incubation at 37°C for 2 hours to cause a prolongation of aPTT in the mixing test.[20] In contrast, LA and anticoagulant agents result in a prompt prolongation. An immediate prolongation, however, does not rule out AHA, as high-titer FVIII inhibitors may act very fast, or be at such high level as to neutralize FVIII in a seemingly fast fashion. In summary, the aPTT mixing study is of limited value, as it cannot confirm or rule out the diagnosis of AHA.[21]

Excluding Lupus Anticoagulant

LA may sometimes inhibit FVIII:C activity assays. Importantly, LA can even cause false-positive results in the Bethesda assay. Therefore, LA should be considered as a priority differential diagnosis, particularly in patients who do not bleed. Different behaviors of LA and FVIII inhibitors in the aPTT mixing study have been described,[20] but these differences may not be sufficient to rule out either condition.

LA can be excluded in most patients by diluted Russel viper venom time (dRVVT)-based assays that use snake venom to activate factor X and induce clotting in the presence of phospholipids and calcium. As coagulation is activated downstream of FVIII, dRVTT-based assays are less sensitive to FVIII deficiency than aPTT-based assays and are used in integrated systems comprising mixing tests (with normal plasma) and confirmation tests (with higher phospholipid concentrations).[22] Despite the lower sensitivity to FVIII inhibitors, Tripodi et al found borderline or positive results in 22% of their congenital hemophilia patients with inhibitors using a commercial dRVVT reagent.[23]

In patients with LA not bleeding but showing borderline or positive Bethesda assay, mixing tests and titrations based on chromogenic substrate assays of FVIII:C may be useful. These tests appear to be less sensitive to LA than the classical Bethesda assay.[24]

In summary, LA and inhibitory autoantibodies against FVIII:C may coexist in some patients or may interfere with one another in laboratory tests. As a clear discrimination is not always possible, clinical history and symptoms need to be considered along with laboratory tests to diagnose difficult cases.

Excluding Pharmacological Anticoagulants

Patients may develop AHA while on VKA, heparins or other anticoagulants.[25] The withdrawal of these drugs or using antidotes should be considered in bleeding patients. If prolongation of the aPTT persists, determination of FVIII:C activity is required immediately. The interference of anticoagulants on assays used to diagnose AHA is detailed in ►**Table 3**.

Bethesda Assay

The Bethesda assay is used to demonstrate and quantify inhibitory anti-FVIII antibodies based on their ability to block FVIII:C activity. Non-neutralizing antibodies directed against FVIII will not be detected by this test.

Assay Principle

Patient plasma serially diluted in buffer is mixed with an equal amount of NPP (►**Fig. 2A**). For control, NPP is mixed

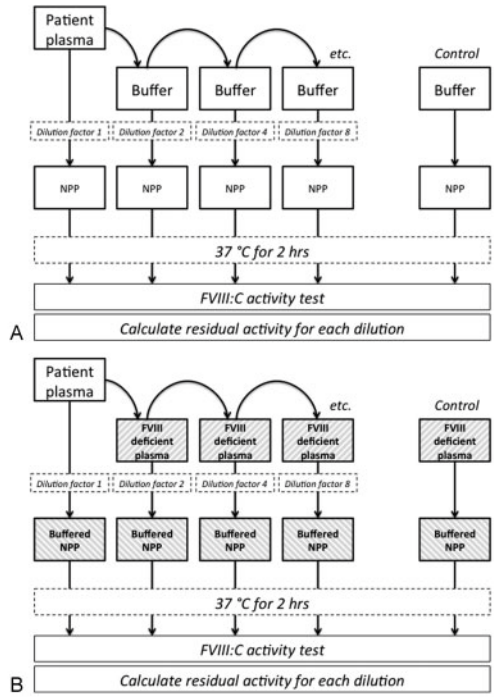

Fig. 2 Schematic description of (A) classical Bethesda assay and (B) Nijmegen modification of Bethesda assay. NPP, normal pooled plasma.

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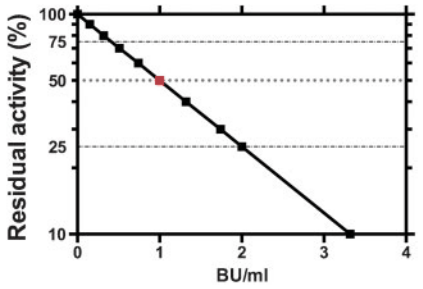

Fig. 3 Bethesda assay nomogram. 1 BU/mL is defined as the amount of inhibitor that inactivates 50% of FVIII:C activity. The nomogram should be used for residual activities between 25 and 75%.

with buffer only. The mixture is incubated at 37°C for 2 hours, and FVIII:C activity is subsequently determined, usually by a one-stage coagulation assay. The residual activity (RA) is calculated for each dilution as:

$$RA = FVIII{:}C_{dilution}/FVIII{:}C_{control} \times 100\%$$

For dilutions with RA between 25 and 75%, the Bethesda units per mL can be read from a nomogram (►**Fig. 3**) and multiplied by the dilution factor to obtain the final inhibitor concentration C_{inh}. Alternatively, it can be calculated as:

$$C_{inh}[BU/mL] = (2 - \log_{10} RA)/\log_{10} 2 \times dilution$$

The calculation is best performed in standard working sheets (e.g., ►**Table 4**). If more than one RA is between 25 and 75%, the dilution closest to 50% should be preferred to determine C_{inh}.

Type 1 versus Type 2 Inhibitors

The mathematical model behind the Bethesda assay assumes a log–linear relationship between dilution and RA. This is the hallmark of type 1 inhibitors that occur in approximately 30% of patients with congenital hemophilia A. Plotting RA (linear axis) against dilution (log axis) will result in a more or less straight line for RA between 25 to 75% (►**Fig. 4A**). Neighboring dilutions in this window will provide very similar C_{inh} (►**Table 4** and ►**Fig. 4B**). At high inhibitor concentration (low dilution), RA is very low (< 1%).

Type 2 inhibitors are typically seen in patients with AHA and have different characteristics. The RA at the lowest dilution is often greater than 1%, and the slope of the curve may be smaller than expected for an inhibitor that follows the earlier mentioned log–linear relationship between RA and dilution (►**Fig. 4C**).[26,27] The determined C_{inh} then increases with progressive dilution (►**Table 4** and ►**Fig. 4D**). The RA closest to 50% should be used to determine C_{inh}, but if two or more RA are equally distant to 50%, the result will be unreliable (►**Fig. 5**). Therefore, the Bethesda assay provides a rough estimate of the inhibitor potency in AHA only.

The reason for the different behavior of inhibitors in AHA is unknown. Differences in binding epitopes, immunoglobulin (sub)class, or affinity properties, do not provide an explanation for the striking difference in the Bethesda assay.[28–30]

Nijmegen Modification of the Bethesda Assay

Not surprisingly, data from external quality assessments demonstrate high interlaboratory coefficients of variation of approximately 50% for the classical Bethesda assay.[31] The Nijmegen modification improved sensitivity and specificity by buffering the normal plasma and replacing the diluent buffer by inhibitor-free FVIII-deficient plasma (►**Fig. 2B**).[32,33] However, interlaboratory variability of the Nijmegen-modified Bethesda test is only slightly better than that of the original assay.[34,35]

The source of FVIII-deficient plasma may affect results in the Nijmegen-modified Bethesda assay: immuno-depleted, VWF-free plasma gave rise to decreased inhibitor titers, whereas chemically depleted plasma appeared to give falsely

Table 4 Typical examples of type 1 and type 2 inhibitors in Bethesda assay

	Patient sample dilutions										Control
Dilution factor	1	2	4	8	16	32	64	128	256	512	
Type 1											
FVIII:C (IU/dL)	< 1	< 1	< 1	0.5	3.3	8.2	19.7	29.1	40.8	41.0	42.5
RA (%)	0	0	0	1.2	7.8	19.2	46.3	68.5	96.0	96.5	–
C_{inh} (BU/mL)	–	–	–	51.3	59.0	76.2	71.1	69.9	15.1	26.5	–
Final result							71.1				
Type 2											
FVIII:C (IU/dL)	4.1	6.2	7.2	10.4	12.4	16.6	19.7	25.9	33.1	42.4	49.2
RA (%)	8.4	12.6	14.7	21.1	25.3	33.7	40.0	52.6	67.4	86.3	–
C_{inh} (BU/mL)	3.6	6.0	11.1	18.0	31.7	50.2	84.6	118.6	145.7	108.8	–
Final result								118.6			

Abbreviations: C_{inh}, inhibitor concentration; RA, residual (FVIII) activity.
Note: Examples correspond to ►**Fig. 4A** (type 1) and B (type 2).

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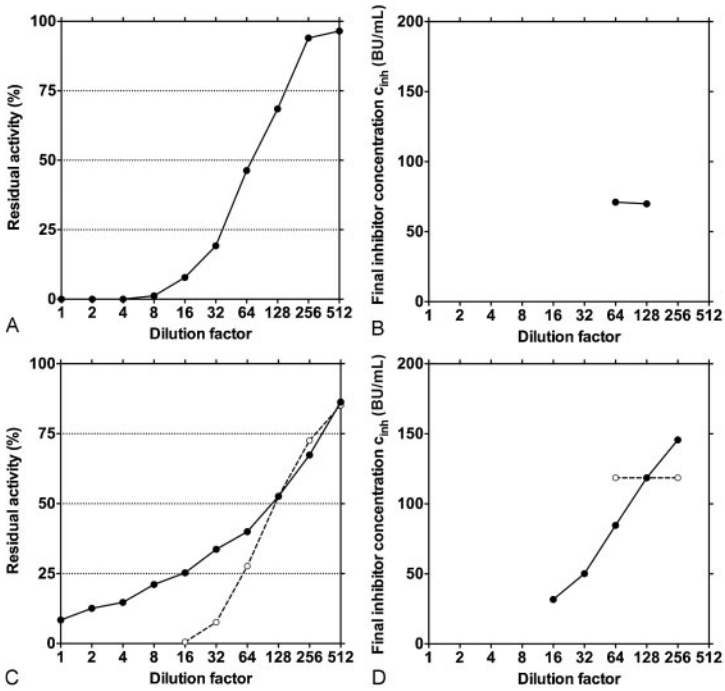

Fig. 4 Typical examples of different inhibitor types. (A) Type 1 inhibitor of 71.1 BU/mL (compare ►**Table 4** for raw data). Note log–linear part of the curve for residual FVIII:C activities of 25 to 75%. (B) Final inhibitor concentration of the inhibitor in panel A, determined for all residual activities between 25 and 75%. Note that the final titer is very similar for different dilutions. The inhibitor can be "diluted out." (C) Type 2 inhibitor of 118.6 BU/mL (closed symbols, solid line, compare ►**Table 4** for raw data). For comparison, a theoretical type 1 inhibitor of the same potency is included (open symbols, dashed line). Note the residual activity $>$ 1 IU/dL for the highest inhibitor concentration and the smaller slope of the curve. (D) Final inhibitor concentration for the inhibitor in panel C, determined for all residual activities between 25 and 75% (closed symbols, solid line). Note that the inhibitor concentration appears to increase with increasing dilution. This is not the case for the theoretical type 1 inhibitor of the same potency (open symbols, dashed line).

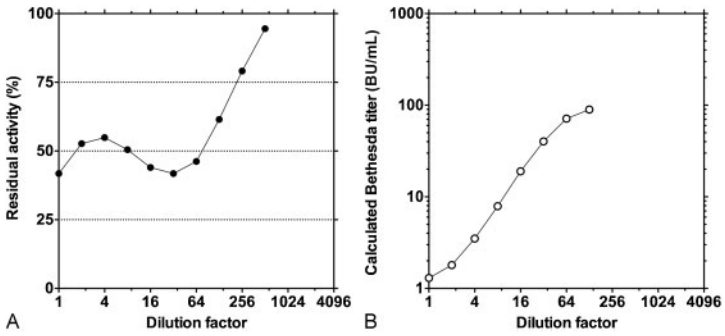

Fig. 5 Atypical inhibitor. Note that the residual activity over dilution curve (panel A) crosses 50% several times. Depending on the choice of the cutoff, the inhibitor concentration (panel B) can be very different, yielding an unreliable result.

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elevated titers in comparison with other types of FVIII-deficient plasma.[36] These sources of variation may explain some of the variability seen in external quality assessments.

Alternative Assays for Anti-FVIII Antibodies

A source of inaccuracy in the classical or Nijmegen-modified Bethesda assay may be the residual FVIII:C activity and the (probably high amounts of) FVIII antigen in patients with AHA. Activity and possibly some of the antigen can be destroyed by thermal treatment, although denaturation may not necessarily remove all binding sites of the autoantibody. In fact, treatment at 58°C for 90 minutes has been shown to improve the sensitivity of inhibitor detection in serial samples of patients with AHA, who were about to achieve remission.[37] Whether the sensitivity at first diagnosis or the interlaboratory precision of the assay would also be improved by this method remains unclear. The same study also demonstrated that sensitivity of an anti-FVIII enzyme-linked immunosorbent assay (ELISA), particularly after thermal treatment of the samples, was higher than that of the Nijmegen-modified Bethesda assy.[37]

Laboratory Results and Clinical Outcome

In congenital hemophilia, the residual FVIII:C activity (RA) is used to define severity of the disease because RA closely correlates with the bleeding risk, particularly with the risk of hemarthrosis.[38] Moreover, the Bethesda assay has been traditionally used to guide treatment in hemophilia patients with inhibitory alloantibodies against FVIII:C: bleeds in patients with inhibitors of up to 5 BU/mL can be successfully treated with high-dose FVIII replacement therapy, whereas inhibitors $>$ 5 BU/mL require bypassing agents to control acute bleeds.[39,40] By contrast, in AHA, residual FVIII:C activity and inhibitor concentration have not been shown so far to correlate with the risk of bleeding or the response to hemostatic treatment.[7,41]

Bleeding Risk

The lack of association between residual FVIII:C activity or inhibitor concentration and the risk of bleeding may be due in part to the design of studies that explored the course and outcome of AHA. For example, laboratory data were collected at time of initial diagnosis, and these may not necessarily correlate with the risk of bleeding before diagnosis or during the course of disease. However, the limitations of both FVIII:C and inhibitor assessment may also contribute to the lack of association between laboratory results and bleeding risk. Treatment recommendations regarding the choice of hemostatic agent have been given based on clinical severity of bleeding rather than on residual FVIII:C activity or inhibitor concentration.[21] This is justified as the efficacy of bypassing agents (recombinant factor VIIa [NovoSeven; Novo Nordisk, Bagsvaerd, Denmark] and activated prothrombin complex concentrate [FEIBA; Baxter, Vienna, Austria]) was significantly higher (93%) than that of FVIII concentrates (68%)[41] and the Bethesda titer does not appear to predict response of FVIII concentrates.

Success of Immunosuppressive Treatment

Immunosuppressive treatment (IST) is used to suppress (and eradicate) autoantibody formation and to induce remission in AHA. The probability of achieving remission with IST ranges between 60 and 80%, whereby, the time needed to achieve remission varies between a few days and several months.[42–46] Some of the available registries and case series reported that remission is obtained more likely and faster with combination therapy of steroid and cyclophosphamide as compared with steroid only,[42,45] while others did not find this association.[44] Rituximab is increasingly used in a wide variety of regimens, which makes it difficult to assess its effects.[45,47]

Laboratory results, particularly the baseline inhibitor titer or residual FVIII:C activity, are of potential value to guide IST intensity. The largest registry of AHA reported so far, European Acquired Hemophilia Registry (EACH2), found that patients with higher baseline FVIII:C and lower inhibitor concentrations achieved remission faster, but this analysis was not adjusted for treatment choice.[45]

The German, Austrian, and Swiss Society on Thrombosis and Hemostasis performed a registry of patients treated according to a uniform IST protocol. The registry included 102 patients over a period of 3 years. Interim results recently reported indicate that baseline FVIII:C activity is indeed a predictor of the time needed to achieve remission, and also of overall survival.[48] Of note, the inhibitor titer (Bethesda assay), which was assessed by the local studies sites according to their routine procedures, did not have an impact on prognosis.

Conclusion

The diagnosis of AHA is usually straightforward. However, there are some pitfalls associated with the assays employed, in particular with the Bethesda assay. Possible interference with lupus anticoagulant or pharmacological anticoagulants needs to be considered. Moreover, the high interlaboratory variation that is not substantially improved by the Nijmegen modification is a concern with this assay. Most importantly, because of the nature of type 2 inhibitors in AHA, the Bethesda method is of limited use to assess inhibitor potency. Several methods, including thermal denaturation of samples and ELISA-based inhibitor assessment, have been suggested and await further validation for their diagnostic application in AHA.

References

1 Collins PW. Therapeutic challenges in acquired factor VIII deficiency. Hematology (Am Soc Hematol Educ Program) 2012; 2012:369–374
2 Franchini M, Mannucci PM. Acquired haemophilia A: a 2013 update. Thromb Haemost 2013;110(6):1114–1120
3 Coppola A, Favaloro EJ, Tufano A, Di Minno MN, Cerbone AM, Franchini M. Acquired inhibitors of coagulation factors: part I-

acquired hemophilia A. Semin Thromb Hemost 2012;38(5): 433–446
4 Collins P, Baudo F, Huth-Kühne A, et al. Consensus recommendations for the diagnosis and treatment of acquired hemophilia A. BMC Res Notes 2010;3:161
5 Mulliez SM, Vantilborgh A, Devreese KM. Acquired hemophilia: a case report and review of the literature. Int J Lab Hematol 2014; 36(3):398–407
6 Sborov DW, Rodgers GM. How I manage patients with acquired haemophilia A. Br J Haematol 2013;161(2):157–165
7 Knoebl P, Marco P, Baudo F, et al; EACH2 Registry Contributors. Demographic and clinical data in acquired hemophilia A: results from the European Acquired Haemophilia Registry (EACH2). J Thromb Haemost 2012;10(4):622–631
8 Kershaw G, Jayakodi D, Dunkley S. Laboratory identification of factor inhibitors: the perspective of a large tertiary hemophilia center. Semin Thromb Hemost 2009;35(8):760–768
9 Kershaw G, Orellana D. Mixing tests: diagnostic aides in the investigation of prolonged prothrombin times and activated partial thromboplastin times. Semin Thromb Hemost 2013;39(3): 283–290
10 Tiede A, Priesack J, Werwitzke S, et al. Diagnostic workup of patients with acquired von Willebrand syndrome: a retrospective single-centre cohort study. J Thromb Haemost 2008;6(4):569–576
11 Tiede A, Rand JH, Budde U, Ganser A, Federici AB. How I treat the acquired von Willebrand syndrome. Blood 2011;117(25): 6777–6785
12 Holstein K, Marx G, Lentz B, Bokemeyer C, Langer F. Successful eradication of a FVIII inhibitor in a 60-year-old patient with mild haemophilia A using single-agent prednisolone. Hamostaseologie 2012;32(Suppl 1):S48–S51
13 Peerlinck K, Jacquemin M. Mild haemophilia: a disease with many faces and many unexpected pitfalls. Haemophilia 2010;16 (Suppl 5):100–106
14 Blanco AN, Cardozo MA, Candela M, Santarelli MT, Pérez Bianco R, Lazzari MA. Anti-factor VIII inhibitors and lupus anticoagulants in haemophilia A patients. Thromb Haemost 1997;77(4):656–659
15 Seethala S, Collins NP Jr, Comerci G Jr. An unusual etiology for elevation of activated partial thromboplastin time (aPTT) in SLE: acquired hemophilia and lupus anticoagulant. Case Rep Hematol 2013;2013:521785
16 Taher A, Abiad R, Uthman I. Coexistence of lupus anticoagulant and acquired haemophilia in a patient with monoclonal gammopathy of unknown significance. Lupus 2003;12(11):854–856
17 Lossing TS, Kasper CK, Feinstein DI. Detection of factor VIII inhibitors with the partial thromboplastin time. Blood 1977; 49(5):793–797
18 Ewing NP, Kasper CK. In vitro detection of mild inhibitors to factor VIII in hemophilia. Am J Clin Pathol 1982;77(6):749–752
19 Kasper CK. Measurement of factor VIII inhibitors. Prog Clin Biol Res 1984;150:87–98
20 Cohen AJ, Kessler CM. Acquired inhibitors. Baillieres Clin Haematol 1996;9(2):331–354
21 Huth-Kühne A, Baudo F, Collins P, et al. International recommendations on the diagnosis and treatment of patients with acquired hemophilia A. Haematologica 2009;94(4):566–575
22 Pengo V, Tripodi A, Reber G, et al; Subcommittee on Lupus Anticoagulant/Antiphospholipid Antibody of the Scientific and Standardisation Committee of the International Society on Thrombosis and Haemostasis. Update of the guidelines for lupus anticoagulant detection. J Thromb Haemost 2009;7(10):1737–1740
23 Tripodi A, Mancuso ME, Chantarangkul V, et al. Lupus anticoagulants and their relationship with the inhibitors against coagulation factor VIII: considerations on the differentiation between the 2 circulating anticoagulants. Clin Chem 2005;51(10):1883–1885
24 Blanco AN, Alcira Peirano A, Grosso SH, Gennari LC, Pérez Bianco R, Lazzari MA. A chromogenic substrate method for detecting and titrating anti-factor VIII antibodies in the presence of lupus anticoagulant. Haematologica 2002;87(3):271–278
25 Uggla B, Linder O, Schulman S. Acquired hemophilia masked by warfarin therapy: report on two cases. Blood Coagul Fibrinolysis 2003;14(8):769–772
26 Gawryl MS, Hoyer LW. Inactivation of factor VIII coagulant activity by two different types of human antibodies. Blood 1982;60(5): 1103–1109
27 Ling M, Duncan EM, Rodgers SE, et al. Classification of the kinetics of factor VIII inhibitors in haemophilia A: plasma dilution studies are more discriminatory than time-course studies. Br J Haematol 2001;114(4):861–867
28 Gharagozlou S, Sharifian RA, Khoshnoodi J, et al. Epitope specificity of anti-factor VIII antibodies from inhibitor positive acquired and congenital haemophilia A patients using synthetic peptides spanning A and C domains. Thromb Haemost 2009;101(5): 834–839
29 Lewis KB, Hughes RJ, Epstein MS, et al; PATH (Personalized Alternative Therapies for Haemophilia) Study Investigators. Phenotypes of allo- and autoimmune antibody responses to FVIII characterized by surface plasmon resonance. PLoS ONE 2013; 8(5):e61120
30 Whelan SF, Hofbauer CJ, Horling FM, et al. Distinct characteristics of antibody responses against factor VIII in healthy individuals and in different cohorts of hemophilia A patients. Blood 2013;121(6): 1039–1048
31 Favaloro EJ, Verbruggen B, Miller CH. Laboratory testing for factor inhibitors. Haemophilia 2014;20(Suppl 4):94–98
32 Verbruggen B, Novakova I, Wessels H, Boezeman J, van den Berg M, Mauser-Bunschoten E. The Nijmegen modification of the Bethesda assay for factor VIII:C inhibitors: improved specificity and reliability. Thromb Haemost 1995;73(2):247–251
33 Verbruggen B. Diagnosis and quantification of factor VIII inhibitors. Haemophilia 2010;16(102):20–24
34 Meijer P, Verbruggen B. The between-laboratory variation of factor VIII inhibitor testing: the experience of the external quality assessment program of the ECAT foundation. Semin Thromb Hemost 2009;35(8):786–793
35 Favaloro EJ, Bonar R, Kershaw G, Mohammed S, Duncan E, Marsden K; RCPA Haematology QAP Haemostasis Committee. Laboratory identification of factor VIII inhibitors in the real world: the experience from Australasia. Haemophilia 2010;16(4):662–670
36 Verbruggen B, Giles A, Samis J, Verbeek K, Mensink E, Nováková I. The type of factor VIII deficient plasma used influences the performance of the Nijmegen modification of the Bethesda assay for factor VIII inhibitors. Thromb Haemost 2001;86(6):1435–1439
37 Batty P, Platton S, Bowles L, Pasi KJ, Hart DP. Pre-analytical heat treatment and a FVIII ELISA improve factor VIII antibody detection in acquired haemophilia A. Br J Haematol 2014;166(6): 953–956
38 White GC II, Rosendaal F, Aledort LM, Lusher JM, Rothschild C, Ingerslev J; Factor VIII and Factor IX Subcommittee. 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
39 Sørensen B, Dargaud Y, Kenet G, et al. On-demand treatment of bleeds in haemophilia patients with inhibitors: strategies for securing and maintaining predictable efficacy with recombinant activated factor VII. Haemophilia 2012;18(2):255–262
40 Haya S, Moret A, Cid AR, et al. Inhibitors in haemophilia A: current management and open issues. Haemophilia 2007;13 (Suppl 5):52–60
41 Baudo F, Collins P, Huth-Kühne A, et al; EACH2 registry contributors. Management of bleeding in acquired hemophilia A: results from the European Acquired Haemophilia (EACH2) Registry. Blood 2012;120(1):39–46

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42 Delgado J, Jimenez-Yuste V, Hernandez-Navarro F, Villar A. Acquired haemophilia: review and meta-analysis focused on therapy and prognostic factors. Br J Haematol 2003;121(1):21–35
43 Collins P, Macartney N, Davies R, Lees S, Giddings J, Majer R. A population based, unselected, consecutive cohort of patients with acquired haemophilia A. Br J Haematol 2004;124(1):86–90
44 Collins PW, Hirsch S, Baglin TP, et al; UK Haemophilia Centre Doctors' Organisation. Acquired hemophilia A in the United Kingdom: a 2-year national surveillance study by the United Kingdom Haemophilia Centre Doctors' Organisation. Blood 2007;109(5):1870–1877
45 Collins P, Baudo F, Knoebl P, et al; EACH2 registry collaborators. Immunosuppression for acquired hemophilia A: results from the European Acquired Haemophilia Registry (EACH2). Blood 2012;120(1):47–55
46 Borg JY, Guillet B, Le Cam-Duchez V, Goudemand J, Lévesque H; SACHA Study Group. Outcome of acquired haemophilia in France: the prospective SACHA (Surveillance des Auto antiCorps au cours de l'Hémophilie Acquise) registry. Haemophilia 2013;19(4):564–570
47 Tiede A, Huth-Kühne A, Oldenburg J, et al. Immunosuppressive treatment for acquired haemophilia: current practice and future directions in Germany, Austria and Switzerland. Ann Hematol 2009;88(4):365–370
48 Tiede A, Blumtritt JM, Klamroth R, et al. Prognostic parameters for survival and remission from acquired hemophilia: results from the GTH-AH 01/2010 Study. Blood 2013;122(21):205

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