Am J Blood Res 2022;12(3):82-87
www.AJBlood.us /ISSN:2160-1992/AJBR0142124

Review Article

Consensus recommendations on appropriate coagulation tests during emicizumab administration in Saudi Arabia

Tarek Owaidah[1], Abdulakareem Almomen[2], Ahmed Tarawah[3], Ashraf Warsi[4], Fawaz Alkasim[5], Hazzaa Alzahrani[6], Mahassen Saleh[7], Ohoud Kashari[8], Wasil Jastaniah[9,10]

[1]Hematology and Transfusion Medicine, Department of Pathology and Laboratory Medicine (MBC 10), King Faisal Specialist Hospital and Research Center Alfaisal University, Riyadh, Saudi Arabia; [2]Medicine-Hematology, King Saud University Medical City and Blood and Cancer Center, Riyadh, Saudi Arabia; [3]Paediatric Haematology, Madina Maternity and Children Hospital, Medina, Saudi Arabia; [4]Adult Haematology, College of Medicine, Umm Al-Qura University, Mecca, Saudi Arabia; Princess Norah Oncology Centre, King Abdelaziz Medical City, Ministry of National Guard Health Affairs-WR, Jeddah, Saudi Arabia; [5]Paediatric Haematology, Maternity and Children Hospital, Riyadh, Saudi Arabia; [6]Adult Haematology, King Faisal Specialist Hospital and Research Centre, Riyadh, Saudi Arabia; [7]Paediatric Haematology, King Faisal Specialist Hospital and Research Centre, Riyadh, Saudi Arabia; [8]Paediatric Haematology, East Jeddah General Hospital, Jeddah, Saudi Arabia; [9]Pediatrics and Pediatric Hematology/Oncology/BMT, College of Medicine, Umm Al-Qura University, Mecca, Saudi Arabia; [10]Princess Norah Oncology Center, King Abdulaziz Medical City, Ministry of National Guard Health Affairs WR, Jeddah, Saudi Arabia

Received January 31, 2022; Accepted May 5, 2022; Epub June 20, 2022; Published June 30, 2022

Abstract: Introduction: Emicizumab is a bispecific monoclonal antibody with the ability to bridge FIXa and FX, mimic FVIII, and restore normal hemostasis in patients with hemophilia A. Moreover, substantial evidence has shown that emicizumab-treated patients do not require monitoring, except before surgery or invasive procedures. However, introducing this novel drug to the market poses some challenges to physicians and clinical laboratories due to its interaction with conventional coagulation tests. Methods: Given the challenges and laboratory interactions posed by this novel drug, there is an unmet clinical need to develop clear recommendations for emicizumab laboratory monitoring to highlight which laboratory tests should be used, which tests should be avoided, and when these tests should be performed. These expert recommendations are essential to prevent inappropriate testing or misleading interpretations and reduce the extra costs of unnecessary monitoring. Results: A consensus meeting was conducted in December 2019, including top experts on hemophilia from Saudi Arabia, to discuss this issue. Conclusion: The experts agreed that, aPTT (activated Partial Thromboplastin Time)-based tests are not suitable for laboratory monitoring patients treated with emicizumab. Only FVIII chromogenic assays based on bovine FIX and FX proteins can be used to measure FVIII levels. They reviewed and recommended the type and time of testing for anti-factor VIII antibodies. Drug levels should be measured using the recommended test only when the anti-drug antibody (ADA) is clinically suspected and after excluding other causes (such as patient non-compliance).

Keywords: Consensus, coagulation test, emicizumab, haemophilia, Kingdom of Saudi Arabia

Introduction

The deficiency of coagulation factor VIII (FVIII) causes hemophilia A (HA). Patients with HA require lifelong treatment with FVIII replacement therapy starting at an early age [1, 2]. However, approximately 20-30% of the patients with severe HA develop antibodies (inhibitors) that neutralize FVIII and compromise treatment outcomes [3]. In Saudi Arabia, approximately 29% of patients with HA develop FVIII inhibitors, and those with FVIII inhibitors tend to have a severe form of the disease [4].

Emicizumab is a bispecific monoclonal antibody, functionally similar to FVIII, enabling it to bridge activated FIX and FX together to restore hemostasis. Routine prophylaxis is recommended to prevent or reduce bleeding episodes in adult and pediatric patients, including new-

borns, with HA (congenital factor VIII deficiency), with or without factor VIII inhibitors. In 2019, the Saudi Food and Drug Authority (SFDA) approved emicizumab at a loading dose of 3 mg/kg body weight through subcutaneous injection once every week for the first four weeks, followed by a maintenance dose of 1.5 mg/kg once every week, 3 mg/kg once every two weeks, or 6 mg/kg once every four weeks [5-7].

Current coagulation assays

Current laboratory coagulation tests evaluate the coagulation potential of the patients. Activated partial thromboplastin time (aPTT) is a global coagulation assay used to assess the coagulation potential in individuals with coagulation disorders. Current laboratory tests for FVIII activity include (1) the one-stage FVIII clotting assay, (2) the two-stage FVIII clotting assay, and (3) the chromogenic substrate assay.

The one-stage FVIII clotting assay is the most widely used coagulation assay to measure plasma FVIII activity. The assay evaluates the ability of the patient's plasma to shorten the aPTT after mixing it with FVIII-deficient plasma [8-11]. The two-stage FVIII clotting assay, developed as an alternative to the one-stage FVIII clotting assay, is based on the same idea of considering FVIII concentration as the rate-limiting step of the reaction [12]. The FVIII chromogenic substrate assay measures the FVIII-dependent activation of FX using purified human or bovine coagulation factors [13]. This test consists of two steps. In the first step, patient plasma is added to a reaction mixture containing FIXa, FX, calcium ions, phospholipids, and trace amounts of thrombin. Thrombin triggers the activation of FVIII and the subsequent FIXa-mediated activation of FX. FXa production is proportional to the concentration of FVIII in the plasma samples. In the second step, the amount of FXa produced is quantified using a chromogenic peptide substrate that binds selectively to FXa [14].

Assays to detect FVIII inhibitors in patients with HA were used to monitor hemostasis. The test is based on comparing the residual FVIII activity in a mixture of patient plasma samples and normal pooled plasma with the residual FVIII activity in a mixture of diluent and normal pooled plasma. This comparison allows the quantification of the reduction in FVIII activity due to FVIII inhibitors. The standardized assay to measure FVIII inhibitors is the Bethesda assay, later modified as the Nijmegen-Bethesda assay [15]. In the presence of emicizumab, the clot-based Bethesda assay is not specific, hence, a modified chromogenic Bethesda assay was developed. This assay uses the same methodology as the one-stage-based assay, with the difference in the detection step; the bovine chromogenic substrate that offers a more specific and accurate endpoint is used to detect residual FVIII [16, 17].

Effect of emicizumab on the current coagulation tests

Emicizumab acts by bridging the activated factor IX and factor X, without the need for FVIIIa, making it different from other drugs that aim to replace deficient FVIII [18]. Since emicizumab has no structural homology with FVIIIa, routine laboratory tests that depend on factor VIII levels give misleading results and are unreliable in patients with HA being treated with emicizumab [19].

In patients receiving emicizumab, these clotting-based tests provide false readings and should not be used to make clinical-treatment decisions.

1. Activated partial thromboplastin time (aPTT) is overcorrected in the presence of emicizumab.

2. One-stage, aPTT-based, single-factor assays (i.e., FVIII activity) appear to give results >150%.

3. Bethesda assays (clotting-based) for FVIII inhibitor titers will yield false-negative results.

4. Chromogenic FVIII activity tests can be performed using either human or bovine coagulation proteins. Assays containing human coagulation factors are responsive to emicizumab but may overestimate the clinical hemostatic potential of emicizumab. In contrast, assays containing bovine coagulation factors are insensitive to emicizumab (no activity measured) and can be used to monitor endogenous or infused FVIII activity or measure FVIII inhibitors [18].

A summary of the effects of emicizumab on the current coagulation assays is shown in **Table 1**.

Table 1. Shows a summary of the effect of emicizumab on the different coagulation assays

	Assay name	Sensitivity to emicizumab	Use in emicizumab-treated hemophilia A patients
Coagulation Assays	aPTT	Oversensitive	Should not be used in emicizumab-treated patients
FVIII activity	FVIII one-stage assay	Oversensitive	Should not be used in emicizumab-treated patients
	FVIII two-stage assay	Oversensitive	Should not be used in emicizumab-treated patients
	FVIII chromogenic substrate assay (human components)	Sensitive	For measurement of emicizumab effect
	FVIII chromogenic substrate assay (bovine components)	Insensitive	Can be used in emicizumab-treated patients to measure FVIII activity but not emicizumab activity
Hemostasis monitoring	Clotting-based Bethesda assays	False-negative	Should not be used in emicizumab-treated patients
	Chromogenic Bethesda assay (bovine components)	Insensitive	Can be used in emicizumab-treated patients to measure FVIII inhibitors

Table 2. Areas of uncertainty identified by the steering committee

Situation	Area of uncertainty
Congenital hemophilia A patients with inhibitors	• *Testing during immune tolerance induction (ITI) with FVIII while using emicizumab*
	• *Testing during breakthrough bleeds while using Emicizumab*
	• *Testing before, during, and after the surgical intervention while using Emicizumab*
Congenital hemophilia A patients without inhibitors	• *Testing during breakthrough bleeds while using Emicizumab*
	• *Testing during surgical intervention while using Emicizumab*
General	• *Measurement of emicizumab level during treatment*
	1. do we need to test
	2. when do we need to request emicizumab test
	• *Measurement of emicizumab anti-drug antibodies (ADA)*
	1. Do we need to test?
	2. when do we need to request ADA testing

Methods

A consensus meeting was conducted in Dec 2019 by a steering committee of top experts in hemophilia from Saudi Arabia to discuss the topic. The committee included ten members whose expertise was supported by reputation, attendance at national and international scientific meetings, and participation in clinical trials and expert panels. A literature review was performed to gain insight into current recommendations concerning coagulation tests and to identify controversial issues in patients with HA taking emicizumab. Based on the selected literature and their clinical experience, the steering committee identified the critical areas of uncertainty and reliable laboratory tests for patients with HA taking emicizumab under different conditions (**Table 2**).

Recommendations

After revising the identified literature reports and clinical expert experience, the steering committee discussed and produced the following statements about the identified situations of uncertainty regarding laboratory testing in patients with emicizumab-treated congenital HA (**Table 3**).

Patients with congenital HA with inhibitors

Testing during using emicizumab during Immune Tolerance Induction (ITI) with FVIII: Chromogenic Bethesda assay with bovine components could be used to measure FVIII inhibitors during ITI with frequency as per the United Kingdom (UK) guidelines for the treatment of congenital HA with inhibitors [20].

Testing during using emicizumab during breakthrough bleeds: This panel recommends the use of a chromogenic FVIII assay (bovine components) to monitor FVIII levels. Both indigenous and infused FVIII can be used to monitor the response.

Testing during using emicizumab during surgical intervention: Experts believe that FVIII inhibitor titer can be measured using the chromogenic Bethesda assay (bovine components) before, during, and after surgery in emicizumab-treated patients. However, to monitor FVIII levels before and after surgery, the FVIII chro-

Table 3. Summary of the consensus statements and the expert agreement rates on each statement

	Consensus statement	Agreement rate
For emicizumab-treated congenital hemophilia A patients with inhibitors		
During ITI	We recommend that in order to measure FVIII inhibitors during ITI, chromogenic Bethesda assay with bovine components can be used with frequency as per standard guidelines of treatment of congenital hemophilia A with inhibitors.	100%
During breakthrough bleeds	If required in patients with low titer inhibitors, we recommend FVIII chromogenic assay (bovine components) to monitor the infused or indigenous FVIII level.	100%
During surgical intervention	We recommend that FVIII inhibitors can be measured before, during, or after surgery in emicizumab-treated patients.	100%
	If required in patients with low titer inhibitors, we recommend FVIII chromogenic assay (bovine components) to monitor the FVIII level before and after surgery.	100%
	We recommend that only when needed, the chromogenic Bethesda assay (bovine components) can be used to measure FVIII inhibitors.	100%
For emicizumab-treated congenital hemophilia A patients without inhibitors		
During breakthrough bleeds	We recommend the use of the chromogenic FVIII assay (bovine components) with FVIII replacement therapy during breakthrough bleeds to monitor FVIII level and replacement.	100%
During surgical intervention	We recommend the use of the chromogenic FVIII assay (bovine components) to monitor FVIII levels before, during, and after surgical intervention.	100%
When do we need to measure the emicizumab level during treatment?		
We do not recommend to test the Emicizumb level regularly. However, hematologists may need to measure emicizumab level in specific situations (e.g., to confirm correct dosing and patient adherence to therapy, or in case of lack of efficacy).		100%
When do we need to measure the emicizumab ADA level during treatment?		
Testing the emicizumab level can be used to detect low concentrations due to ADA unless there is another cause (inappropriate dose or patient non-compliance). If ADAs are suspected based on clinical evaluation, the investigational test is available. However, the aPTT and the one-stage FVIII activity assay are complementary laboratory tests that may be used. If emicizumab exposure is lost, aPTT would be prolonged, and FVIII activity would be low; however, even at very low plasma emicizumab concentrations, aPTT would be normal.		100%

mogenic assay (bovine components) can be used if required in patients with low titer inhibitors.

Patients with congenital hemophilia without inhibitors

Testing during using emicizumab during breakthrough bleeds: The panel recommends the use of a chromogenic FVIII assay (bovine components) with FVIII replacement therapy during breakthrough bleeds to monitor FVIII levels and replacement.

Testing during using emicizumab during surgical intervention: The panel recommends the use of a chromogenic FVIII assay (bovine components) to monitor FVIII levels before, during, and after surgical intervention.

General testing for emicizumab level

Testing of emicizumab level during treatment: The panel of experts did not recommend regular testing of the emicizumab level. However, hematologists may need to measure emicizumab levels in specific situations (e.g., to allow physicians to confirm correct dosing and patient adherence to therapy or in case of lack of efficacy when the presence of Antidrug antibodies (ADA) is suspected.

Testing emicizumab ADA: Although the incidence of ADA to emicizumab is low [21], the panel recommends to test ADAS when tested, emicizumab concentrations levels are low after exclusion of inappropriate doses or patient non-compliance when the test is available. If emicizumab exposure is lost, aPTT would be prolonged and FVIII activity would be low; however, even at very low plasma emicizumab concentrations, aPTT would be normal.

Discussion

Emicizumab is a bispecific monoclonal antibody with functional similarities to FVIII, enabling it to bridge activated FIX and FX together and restore hemostasis. However, structural differences emicizumab interfere with coagulation assays commonly used to monitor patients with HA.

Recently, emicizumab has been approved and marketed in several countries, including Saudi Arabia. Introducing this novel drug in the mar-

ket imposes unprecedented challenges for physicians and clinical laboratories because monitoring this drug using conventional essays produces inaccurate results. Therefore, physicians and laboratory staff should be aware of emicizumab interference with standard coagulation assays. Appropriate monitoring tests need to be selected and interpreted in different clinical situations. Since there are no clear guidelines for testing protocols for patients on emicizumab, a panel of experts developed guidelines to provide expert recommendations for physicians and laboratories across the Kingdom of Saudi Arabia for emicizumab monitoring.

The recommendations are in line with those set by hematology groups in other parts of the world. The French BIMHO group suggested biological monitoring of patients treated with emicizumab in different clinical scenarios [22], recommending monitoring only before an invasive operation or in cases of bleeding. APT-based tests are not accurate, and normalization does not necessarily mean that patient hemostasis is normalized. Furthermore, they suggested that a chromogenic method using non-human reagents should be used to determine FVIII. These recommendations by the French BIMHO group were concordant with our expert consensus. Similar proposals were also made by the UK Hemophilia Center, proposing guidelines for laboratory testing of emicizumab-treated patients [23].

Conclusion

The experts agree that aPTT-based tests are not suitable for laboratory monitoring of emicizumab-treated patients. Only FVIII chromogenic assays based on bovine FIX and FX proteins can be used to measure FVIII levels. In addition, drug levels should be measured using the investigational test only when ADA is clinically suspected and after excluding other causes (such as patient non-compliance).

Acknowledgements

Medical writing support for the development of this manuscript was done by Editage's editorial support.

The consensus recommendations and algorithms presented in this manuscript were discussed and formulated through an advisory board that was funded by Roche Saudi Arabia. Medical writing support in the development of this manuscript was provided by Clinart MENA and funded by Roche Saudi Arabia.

Disclosure of conflict of interest

None.

Address correspondence to: Dr. Tarek Owaidah, Hematology and Transfusion Medicine, Department of Pathology and Laboratory Medicine (MBC 10), King Faisal Specialist Hospital and Research Center, Alfaisal University, Riyadh 11211, Saudi Arabia. Tel: +966-11-4424204; E-mail: towaidah@kfshrc.edu.sa

References

[1] Franchini M and Mannucci P. Past, present and future of hemophilia: a narrative review. Orphanet J Rare Dis 2012; 7: 24.
[2] Zimmerman B and Valentino LA. Hemophilia: in review. Pediatr Rev 2013; 34: 289-294; quiz 295.
[3] Pratt KP. Inhibitory antibodies in hemophilia A. Curr Opin Hematol 2012; 19: 399-405.
[4] Owaidah T, Momen AA, Alzahrani H, Almusa A, Alkasim F, Tarawah A, Nouno RA, Batniji FA, Alothman F, Alomari A, Abu-Herbish S, Abu-Riash M, Siddiqui K, Ahmed M, Mohamed SY and Saleh M. The prevalence of factor VIII and IX inhibitors among Saudi patients with hemophilia. Medicine (Baltimore) 2017; 96: e5456.
[5] Uchida N, Sambe T, Yoneyama K, Fukazawa N, Kawanishi T, Kobayashi S and Shima M. A first-in-human phase 1 study of ACE910, a novel factor VIII-mimetic bispecific antibody, in healthy subjects. Blood 2016; 127: 1633-1641.
[6] FDA Approves Emicizumab-Kxwh for Prevention and Reduction of Bleeding in Patients with hemophilia A with Factor VIII Inhibitors. 2017.
[7] FDA Approves Emicizumab-Kxwh for hemophilia A with or without Factor VIII Inhibitors. 2018.
[8] Teichman J, Chaudhry HR and Sholzberg M. Novel assays in the coagulation laboratory: a clinical and laboratory perspective. Transfus Apher Sci 2018; 57: 480-484.
[9] Moser KA and Adcock Funk DM. Chromogenic factor VIII activity assay. Am J Hematol 2014; 89: 781-784.
[10] Kitchen S, Kershaw G and Tiefenbacher S. Recombinant to modified factor VIII and factor IX - chromogenic and one-stage assays issues. Haemophilia 2016; 22: 72-77.
[11] Peyvandi F, Oldenburg J and Friedman KD. A critical appraisal of one-stage and chromogenic assays of factor VIII activity. J Thromb Haemost 2016; 14: 248-261.

[12] Al-Samkari H and Croteau SE. Shifting landscape of hemophilia therapy: Implications for current clinical laboratory coagulation assays. Am J Hematol 2018; 93: 1082-1090.
[13] Amiral J and Seghatchian J. Usefulness of chromogenic assays for potency assignment and recovery of plasma-derived FVIII and FIX concentrates or their recombinant long acting therapeutic equivalents with potential application in treated pediatric hemophiliac patients. Transfus Apher Sci 2018; 57: 363-369.
[14] Rodgers S and Duncan E. Chromogenic factor VIII assays for improved diagnosis of hemophilia A. Methods Mol Biol 2017; 1646: 265-276.
[15] Duncan E, Collecutt M and Street A. Nijmegen-Bethesda assay to measure factor VIII inhibitors. Methods Mol Biol 2013; 992: 321-333.
[16] Miller CH. Laboratory testing for factor VIII and IX inhibitors in haemophilia: a review. Haemophilia 2018; 24: 186-197.
[17] Allain JP and Frommel D. Antibodies to factor VIII. I. Variations in stability of antigen-antibody complexes in hemophilia A. Blood 1973; 42: 437-444.
[18] Knight T and Callaghan MU. The role of emicizumab, a bispecific factor IXa- and factor X-directed antibody, for the prevention of bleeding episodes in patients with hemophilia A. Ther Adv Hematol 2018; 9: 319-334.
[19] Adamkewicz JI, Chen DC and Paz-Priel I. Effects and interferences of emicizumab, a humanised bispecific antibody mimicking activated factor VIII cofactor function, on coagulation assays. Thromb Haemost 2019; 119: 1084-1093.
[20] Collins PW, Liesner R, Makris M, Talks K, Chowdary P, Chalmers E, Hall G, Riddell A, Percy CL, Hay CR and Hart DP. Treatment of bleeding episodes in haemophilia A complicated by a factor VIII inhibitor in patients receiving Emicizumab. Interim guidance from UKHCDO Inhibitor Working Party and Executive Committee. Haemophilia 2018; 24: 344-347.
[21] Paz-Priel I, Chang T, Asikanius E, Chebon S, Emrich T, Fernandez E, Kuebler P and Schmitt C. Immunogenicity of emicizumab in people with hemophilia A (PwHA): results from the HAVEN 1-4 studies. Blood 2018; 132 Suppl 1: 633-633.
[22] Nougier C, Jeanpierre E, Ternisien C, Proulle V, Hezard N, Pouplard C and Lasne D. Emicizumab treatment: impact on coagulation tests and biological monitoring of haemostasis according to clinical situations (BIMHO group proposals). Eur J Haematol 2020; 105: 675-681.
[23] Jenkins PV, Bowyer A, Burgess C, Gray E, Kitchen S, Murphy P, Platton S, Riddell A, Chowdary P and Lester W. Laboratory coagulation tests and emicizumab treatment A United Kingdom Haemophilia Centre Doctors' Organisation guideline. Haemophilia 2020; 26: 151-155.