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Laboratory coagulation tests and emicizumab treatment A United Kingdom Haemophilia Centre Doctors' Organisation guideline

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Abstract

Introduction: The factor VIII mimetic emicizumab (Hemlibra, Hoffman-la Roche, Basel, Switzerland) has a novel mode of action that affects the laboratory monitoring of patients receiving this treatment.

Aim: This guideline from the United Kingdom Haemophilia Centre Doctors Organisation (UKHCDO) aims to provide advice for clinical and laboratory staff on appropriate use of laboratory assays in patients with Haemophilia A treated with emicizumab.

Methodology: The guideline was prepared by a review of the available literature and discussion and revision by the authors.

Results: The guideline describes the effect of emicizumab on commonly used coagulations tests and provides recommendations on the use of assays for measurement of factor VIII and factor VIII inhibitor in the presence of emicizumab. The guideline also provides recommendations on measurement of emicizumab.

Conclusion: Knowledge of the effect of emicizumab on coagulation tests and factor assays is required to ensure appropriate testing and monitoring of therapy in patients receiving this drug.

KEYWORDS

bispecific antibody, chromogenic factor VIII assay, emicizumab, factor VIII assay, haemophilia, Hemlibra

1 | INTRODUCTION

The recent development of emicizumab (Hemlibra, also previously referred to as ACE910; Hoffman-la Roche) extends treatment options for haemophilia A patients, with and without anti-Factor (F) VIII inhibitors, and provides an alternative to FVIII replacement therapy for patients with severe haemophilia A.[1,2] The novel nature and mode of action of the molecule have implications for the laboratory testing of coagulation parameters in patients receiving this treatment.

Methodology: Relevant publications were identified using the search terms ACE910, emicizumab and Hemlibra in addition to a review of laboratory guidance documents produced by the manufacturers (Hoffman-la Roche) and roundtable discussions by the writing group. Due to the paucity of publications regarding laboratory testing in patients treated with emicizumab a 'GRADE' system for the recommendations has not been used.

2 | WHAT IS EMICIZUMAB AND HOW DOES IT WORK?

Emicizumab is an engineered IgG4 bispecific antibody that binds both factor IXa (FIXa) and its substrate factor X (FX). This interaction co-localises the components of the intrinsic tenase complex and improves

the ability of FIXa to activate FX in the absence of FVIIIa. It therefore is a FVIII mimetic, in that it acts as a cofactor for FIXa activation of FX.[3]

3 | WHAT ARE THE DIFFERENCES BETWEEN FVIII AND EMICIZUMAB?

The mode of action and regulation of emicizumab is very different to FVIII. In addition to being able to bind FIXa and FX, emicizumab is capable of binding zymogen FIX and FXa. Emicizumab does not bind to the phospholipid surface and also, as a bispecific antibody, emicizumab is not regulated by the activation and inactivation mechanisms that regulate FVIII activity.[4]

The recommended treatment regimens result in stable, prolonged levels of drug with a described half-life of approximately 30 days; emicizumab therefore persists in the circulation many months after the last dose.[5] These differences between emicizumab and FVIII, together with its long half-life, have significant implications for laboratory testing of persons with haemophilia receiving this treatment, summarized in Table 1. The recommendations are summarized in Table 2.

4 | LABORATORY TESTS AND EMICIZUMAB

A recently published study,[6] together with information provided by the manufacturer, has provided some information on the influence of emicizumab on certain coagulation tests, measurement of FVIII in the presence of emicizumab and measurement of emicizumab levels.[7] The summary of product characteristics also provide some detailed recommendations.[8] However, published data are very limited.

5 | EFFECTS OF EMICIZUMAB ON COAGULATION TESTS

A summary of the described effects of emicizumab on coagulation tests is presented in Table 1.

5.1 | Activated partial thromboplastin time (APTT) and related single factor assays

Emicizumab, being a bispecific antibody designed to interact with human FIXa and FX, affects all tests that involve human FIX(a)and FX(a). The APTT is considerably shortened by the presence of the drug to within, or shorter than, the normal reference range, even at subtherapeutic levels, for example during initiation of therapy.[5,9] Similarly, the presence of emicizumab also affects all related APTT-based assays including, FVIII, FIX, FXI, FXII assays and also APTT-based activated protein C resistance (APC-R) and APTT-based Protein S assays.[10] The effect on patient APTT and APTT-based coagulation assays may be evident for up to 6 months after treatment discontinuation due to the prolonged half-life of emicizumab.[9] The activated clotting time (ACT) is proposed as being affected but no experimental data demonstrating this have been published.

5.2 | Prothrombin time (PT) and related single factor assays

Emicizumab is described as having a modest and clinically irrelevant effect on the PT and an extremely small effect on the INR.[10] The extent of the effect varied according to the manufacturer and brand of PT reagent. The presence of emicizumab did not affect PT-based single factor assays.

5.3 | Thrombin time and clauss fibrinogen

The thrombin time and Clauss fibrinogen assay are unaffected by the presence of emicizumab.

5.4 | Chromogenic assays and immunogenic-based assays

Chromogenic assays for antithrombin, protein C and anti–Xa based chromogenic assays are described as unaffected by the presence of emicizumab. All immunogenic-based assays are unaffected by the presence of emicizumab.

5.5 | Recommendation

APTT-based tests are unsuitable for the measurement of coagulation factors or inhibitors in patients being treated with emicizumab. Alternative non–APTT based assays should be used.

Local verification of PT reagent response should be considered for measurement of the PT in patients treated with emicizumab.

6 | MEASUREMENT OF FVIII IN THE PRESENCE OF EMICIZUMAB

Although emicizumab therapy reduces the risk of bleeding, additional treatment, such as use of FVIII concentrate products or bypassing agents, may be required for patients with breakthrough bleeds or those undergoing surgery. It follows from the known mechanism of action of emicizumab and its effect on the APTT, that emicizumab will affect the FVIII one-stage clotting assay (OSCA). Apparent FVIII levels are artificially raised and FVIII activity therefore should not be measured by OSCA in patients being treated with emicizumab, as the result is uninterpretable and invalid.

Similarly, emicizumab will show significant interference in FVIII chromogenic assays (CSA) that use human FIXa and FX. In contrast,

TABLE 1 Coagulation tests and emicizumab effects (adapted from[7])

Test results affected by emicizumab	Test results unaffected by emicizumab
APTT reduced	
PT reduced [described as a clinically insignificant effect]	PT-based assays
APTT-based single factor assays (increased)	Thrombin time
APTT-based inhibitor screens (false negative)	Clauss fibrinogen
APTT-based factor inhibitor titres (false negative)	Immuno-based assays
Chromogenic FVIII assays using human-derived FIXa and FX (increased)	Chromogenic FVIII assays using bovine-derived FIXa and FX
Activated clotting time (ACT) (possibly reduced)	Chromogenic assays for coagulation factors or treatments other than FVIII.

TABLE 2 Summary of recommendations

Laboratory testing in patients treated with emicizumab. Summary of recommendations	
Coagulation tests	APTT-based tests are unsuitable for the measurement of coagulation factors or inhibitors. Local verification of PT reagent response should be considered for measurement of the PT.
FVIII level	FVIII should be measured by a FVIII chromogenic assay that utilises bovine FIXa and FX components. Local verification of alternative chromogenic kits with bovine FX and human FIXa is recommended prior to use.
FVIII inhibitor	FVIII inhibitor levels in patients receiving emicizumab should be measured using chromogenic reagents containing bovine FIXa and FX components, including prior to treatment initiation. As above, local verification of alternative chromogenic kits with bovine FX and human FIXa is recommended.
Antidrug antibody and emicizumab level	We recommend measurement of the drug level in a patient with a suspected inhibitor to emicizumab. Emicizumab levels should be measured using a modified OSCA calibrated with emicizumab-specific calibrators.

emicizumab does not interfere with chromogenic assays where both FIXa and FX are of bovine origin. CSA kits that use human FIXa and bovine FX components have been described as capable of accurately measuring FVIII in the presence of emicizumab.[11] There is a theoretical risk that the FVIII level obtained may be artificially raised using such kits by interaction of emicizumab with the human FIXa of the kit and endogenous patient FX.

There are currently no alternative methods to chromogenic assays containing bovine FX for the accurate determination of FVIII levels in patients treated with emicizumab. There is insufficient evidence to recommend the use of viscoelastic or thrombin generation tests as alternative assays. If the chromogenic assay is not available, monitoring of FVIII treatment in patients is limited to the assessment of clinical outcomes.

6.1 | Recommendation

FVIII levels, if required in patients being treated with emicizumab, should be measured by a FVIII chromogenic assay that utilises bovine FIXa and FX components. Examples include Coamatic FVIII assay (Chromogenix), Coatest SP (Chromogenix), FVIII Chromogenic Assay (Siemens).

Verification of kits containing bovine FX and human FIXa, for example Technochrom FVIII:C (Technoclone) and Rossix FVIII (Rossix), as suitable for measuring FVIII in the presence of emicizumab is recommended, prior to use.

7 | MEASUREMENT OF FVIII INHIBITORS IN THE PRESENCE OF EMICIZUMAB

Screening for and measurement of FVIII inhibitors may be needed during treatment with emicizumab. Pre-analytical heat inactivation methods, routinely used to inactivate residual FVIII prior to performing the Bethesda assay, will not completely remove emicizumab, an IgG4 bispecific antibody.[12,13] FVIII OSCA or human component CSA, therefore, are not suitable for measuring FVIII inhibitors using the Bethesda assay or the modified Nijmegen Bethesda assay[10] as the continuing presence of emicizumab will result in false negative results. FVIII inhibitor levels should be measured by CSA with bovine components.

To ensure appropriate sequential monitoring and test consistency, the patient FVIII inhibitor titre should be measured in a pre-treatment sample using the same CSA method.

7.1 | Recommendation

FVIII inhibitor levels in patients receiving emicizumab should be measured using chromogenic reagents containing bovine FIXa and FX components, including prior to treatment initiation.

Verification of kits with bovine FX and human FIXa, for example Technochrom FVIII:C (Technoclone) and Rossix FVIII (Rossix), as suitable for measuring FVIII inhibitors in the presence of emicizumab is recommended, prior to use.

8 | ANTIDRUG ANTIBODIES AND MEASUREMENT OF EMICIZUMAB DRUG LEVEL

Routine measurement of the drug for the purposes of dose adjustment is not considered necessary for a variety of reasons: the treatment regimen is designed to achieve a steady-state level of emicizumab; and bioequivalence between drug concentrations and apparent cofactor activity has not been established. However, the presence of antidrug antibodies (ADA) has been detected in clinical trials in patients treated with emicizumab at a prevalence of approximately 3%; though drug neutralising antibodies are thought to occur in less than 1% of cases.[14] Further data may become available with increased use and availability of the drug.

There is currently no commercially available assay available for detection of ADA. Although the presence of a neutralising antibody would be hypothesised to prolong the APTT, the test is sensitive to even low levels of emicizumab. A shortened APTT would suggest the presence of drug but is not a measure of drug level and should not be considered as an exclusion of ADA. Measurement of emicizumab levels would be indicative of whether ADA are present in a compliant patient.

If knowledge of the emicizumab level is required, unmodified FVIII OSCA or human component CSA are not suitable as surrogate markers of emicizumab levels. Emicizumab-specific calibrators for measurement of the drug are available (R^2 diagnostics; Enzyme Research Laboratories). These are CE marked for use as calibrators in a modified human OSCA. To date, there is limited experience in the use of the assay. A recent study showed that use of the product-specific calibrators in a modified OSCA delivered close agreement between in results using seven different APTT reagents (Actin, Actin FS, Actin FSL, Pathromtin SL, Synthasil, APTT-SP and Synthafax).[15] No data are available regarding the use of the calibrator for measurement of emicizumab using chromogenic assays with human components, though in principle this should be possible.

8.1 | Recommendation

We recommend measurement of the drug level in a patient with a suspected inhibitor to emicizumab.

Emicizumab can be measured using a modified OSCA with emicizumab-specific calibrators and performed in accordance with best practice guidelines.[16] Verification of the assay method with reagents in local use must be performed by the testing laboratory.

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