ECAT Information: Emicizumab pilot study

In Autumn 2020 a pilot study was performed on the laboratory testing of emicizumab, a bispecific factor IXa- and factor X-directed antibody. This antibody was developed to bring together factor IXa and factor X without the need for factor VIII. It is therefore able to restore blood coagulation in patients with haemophilia A. This pilot study consisted of two parts. Part I investigated the inter-laboratory variability in the quantitative measurement of emicizumab, while part II investigated the interference of emicizumab in a range of different coagulation tests. In the previous newsletter we gave a summary of the results of part I of this pilot study. Now we report on the results of part II.

In part II the participants had the possibility of investigating the interference of emicizumab in all kinds of coagulation tests. Two different samples were provided. One sample was a negative control sample (Factor VIII deficient plasma), the other sample was a positive sample (Factor VIII deficient plasma spiked with approx. 50 µg/mL emicizumab). In total 45 participants participated in the interference study.

Eight participants also measured the emicizumab concentration in these two samples. The table below shows the results.

Sample	Description	N	Mean (µg/mL)	Range (µg/mL)
Negative	Factor VIII deficient plasma	8	0.4	0.0 – 1.6
Positive	Factor VIII deficient plasma spiked with approx. 50 µg/mL emicizumab	8	51.1	42.6 – 60.4

Effect of emicizumab on basic coagulation tests

The table below shows the effect of emicizumab on different global coagulation tests, such as aPTT, PT, fibrinogen and thrombin time.

Parameter	Unit	N	Negative sample	Positive sample
APTT	Sec.	44	81 – 120	17 - 26
PT	Sec.	39	8.8 – 14.7	8.8 – 14.8
Thrombin time	Sec.	24	10.0 – 25.4	12.0 – 24.5
Fibrinogen	g/L	31	2.16 – 2.90	2.18 – 2.80

Emicizumab has no effect on the measurement of the PT, fibrinogen and thrombin time. This is in line with what was expected on the basis of the mode of action of emicizumab. However, a significant effect of emicizumab could be observed on the measurement of the aPTT. With the concentration of approximately 50 µg/mL a normalisation of the aPTT could be observed. This is in line by what has been published previously [1]. A similar effect was observed for all 10 different aPTT reagents used by the participants (IL HemosIL APTT-SP, IL HemosIL SynthASil, Siemens Actin FS, Siemens Actin FSL, Siemens Pathromtin SL, Stago Cephalin / Kaolin / CKPrest, Stago Cephascreen, Tcoag TriniCLOT APTT S, Tcoag TriniCLOT APTT HS).

Effect of emicizumab on aPTT-based coagulation factor tests

Several participants investigated the effect of emicizumab on the laboratory testing of the clotting factors VIII, IX, XI and XII by either a APTT-based one-stage clotting assay or a chromogenic assay. The table below shows the summary of the results.

Parameter	Unit	N	Negative sample	Positive sample
Factor VIII (one-stage)	IU/mL	32	0.01 – 0.03	3.1 – 7.0
Factor VIII (chromogenic)	IU/mL	11	0.00 – 0.03	0.0 – 0.5
Factor IX (one-stage)	IU/mL	38	0.75 – 1.19	1.00 – 2.81
Factor IX (chromogenic)	IU/mL	7	0.83 – 1.12	0.83 – 1.11
Factor XI (one-stage)	IU/mL	29	0.77 – 1.29	1.53 – 3.42
Factor XII (one-stage)	IU/mL	24	0.80 – 1.22	1.25 – 3.23

The measurement of the clotting factors FVIII, FIX, FXI and FXII by an APTT-based one-stage clotting assay is significantly affected by the presence of emicizumab.

The measurement of FVIII with a chromogenic method is not affected by emicizumab, except when the Hyphen Biophen FVIII method is used, which employs human-type of reagents (data not shown). The other methods employed use bovine-type of reagents. The measurement of FIX using a chromogenic method is not affected by emicizumab.

Effect of emicizumab on the measurement of FVIII inhibitor

Eleven participants performed a Factor VIII inhibitor assay. Four participants used a one-stage clotting assay and seven participants a chromogenic assay. All participants reported a negative result, i.e. a result below the lower limit of detection or a very low titre (negative sample: 0.1 BU/mL ; positive sample: 0.2 BU/mL). Because not all participants provided detailed information about the method or reagents used, no conclusion could be drawn about which method or which reagent demonstrates interference by emicizumab. Several studies have demonstrated an interference by emicizumab in the FVIII inhibitor assay [1-3]. Therefore, each laboratory should validate their FVIII inhibitor method taking into account the interference by emicizumab.

Effect of emicizumab on PT-based coagulation factor tests

Several participants also investigated the effect of emicizumab on the laboratory testing of the clotting factors II, V, VII and X by PT-based clotting assays. The table below shows the summary of the results.

Parameter	Unit	N	Negative sample	Positive sample
Factor II	IU/mL	25	0.77 – 1.04	0.75 – 1.09
Factor V	IU/mL	26	0.59 – 0.80	0.58 – 0.79
Factor VII	IU/mL	27	0.99 – 1.39	0.99 – 1.47
Factor X	IU/mL	26	0.79 – 1.07	0.79 – 1.08

The measurement of the clotting factors FII, FV, FVII and FX using a PT-based one-stage clotting assay is not affected by the presence of emicizumab.

Effect of emicizumab on thrombophilia markers

No effect of emicizumab was observed on the measurement of antithrombin activity, protein C activity (chromogenic) and free protein S antigen. However, protein C activity measured with a clotting assay was significantly affected by emicizumab (see table below).

Parameter	Unit	N	Negative sample	Positive sample
Protein C (clot assay)	IU/mL	8	0.91 – 1.16	0.26 – 0.46
Protein C (chromogenic)	IU/mL	15	0.92 – 1.01	0.90 – 1.00

There is also a trend for somewhat lower APC resistance ratios in the sample with emicizumab when using the Siemens ProC global and IL HemosIL FV Leiden methods (data not shown).

The results of this interference study demonstrates that some of the laboratory tests regularly used in the laboratory are significantly affected by emicizumab. We thank all participants in this pilot study for their contribution. We hope that the results of this study may help you to be aware of the potential effects of emicizumab in laboratory tests.

In 2021 ECAT will begin a regular survey programme for the quantitative measurement of emicizumab (2 surveys / year). If you are interested in participating, please contact the ECAT office (info@ecat.nl).

References

1. Adamkewicz, J.I., D.C. Chen and I. Paz-Priel, Effects and Interferences of Emicizumab, a Humanised Bispecific Antibody Mimicking Activated Factor VIII Cofactor Function, on Coagulation Assays. Thromb Haemost, 2019; 119: 1084-1093.
2. Lowe, A., S. Kitchen, I. Jennings, D.P. Kitchen, T.A.L. Woods and I.D. Walker, Effects of Emicizumab on APTT, FVIII assays and FVIII Inhibitor assays using different reagents: Results of a UK NEQAS proficiency testing exercise. Haemophilia, 2020; 26: 1087-1091.
3. Nogami, K., T. Soeda, T. Matsumoto, Y. Kawabe, T. Kitazawa and M. Shima, Routine measurements of factor VIII activity and inhibitor titer in the presence of emicizumab utilizing anti-idiotype monoclonal antibodies. J Thromb Haemost, 2018; 16: 1383-1390.