CLINICAL STUDY REPORT

Apixaban and rivaroxaban in breast milk

An Open Label, Non-Randomised, Phase IV Clinical Trial to Determine the Transfer of Apixaban and Rivaroxaban in Breast Milk Following Oral Administration

Sponsor Protocol Code:	V6.0
EudraCT Number:	2018-003852-19
ClinicalTrials.gov Identifier:	NA
REC Number:	19/LO/0082
Investigational Drugs (IMPs):	Apixaban and rivaroxaban
Indication:	Venous thromboembolism
Development Phase:	IV
Study Begin (FPFV):	17 Jan 2020
Study End (LPLV):	07 Mar 2020
Report Version & Issue Date:	V1.0 07 Feb 2021
Co-sponsor Name and Address:	King's College London - King's College Hospital NHS Foundation Trust King's Health Partner's Clinical Trial Office, F16 Tower Wing, Guy's Hospital, Great Maze Pond, London SE1 9RT
Co-sponsor contact details:	Helen Critchley Telephone: 020 7188 5732, Fax: 0207 188 8330 Email: helen.critchley@kcl.ac.uk
Chief Investigator:	Professor Roopen Arya

SIGNATURE PAGE

By signing below I approve the contents of this Clinical Study Report, and confirm that to the best of my knowledge it accurately describes the conduct and results of the study. The clinical trial reported herein was conducted in accordance with the principles contained in the Declaration of Helsinki, Good Clinical Practice (GCP) and all applicable laws and regulations.

Chief Investigator: Professor Roopen Arya

Printed name	**Signature**	**Date**
ROOPEN ARYA		26/02/2021

CONTENTS

1. Ethics

Independent Ethics Committee or Institutional Review Board
The study protocol and amendments were reviewed and approved by a National Research Ethics Service (London - Fulham Research Ethics Committee – 19/LO/0082).

Ethical conduct of the study
The trial was conducted according to the protocol and in compliance with the principles of the Declaration of Helsinki (1996) as amended, the principles of Good Clinical Practice (GCP) and in accordance with Medicines for Human Use (Clinical Trials) Regulations 2004, as amended, the Research Governance Framework for Health and Social Care, the Data Protection Act 1998 and other regulatory requirements as appropriate. The trial protocol and substantial amendments were reviewed by the United Kingdom (UK) Medicines and Healthcare products Regulatory Agency (MHRA)

Subject information and consent
Participants were recruited from the advertisement on www.mums.net, local forums, and the outpatient clinic letters at the Haematology department of King's College Hospital NHS Foundation Trust. Those mothers who were interested in participating in the study actively contacted the researcher, and the researcher explained the study in details and booked the screening visit time slot. Informed written consent form was obtained when the participant attended the screening visit.

2. Data Monitoring

Monitoring of this trial was to ensure compliance with Good Clinical Practice and scientific integrity was managed and oversight retained, by the KHP-CTO Quality Team.

3. Sponsors, Investigators and Trial Sites

Co-Sponsors Name: King's College London - King's College Hospital NHS Foundation Trust co-sponsor	
Chief Investigator	***Professor Roopen Arya***

4. Co-Investigator(s), Statistician, Laboratories, Database Management

Co-Investigator	***Dr Jignesh Patel***
Co-Investigator	***Miss Yating Zhao***
Statistician/Laboratories/Database Management	***Miss Yating Zhao***

5. Study Synopsis

Title of clinical trial	An Open Label, Non-Randomised, Phase IV Clinical Trial to Determine the Transfer of Apixaban and Rivaroxaban in Breast Milk Following Oral Administration
Protocol Short Title/Acronym	Apixaban and rivaroxaban in breast milk
Study Phase	IV
Sponsor name	King's College London - King's College Hospital NHS Foundation Trust co-sponsor
Chief Investigator	Professor Roopen Arya
Eudract number	2018-003852-19
REC number	19/LO/0082
IRAS project ID:	243946
Medical condition or disease under investigation	Healthy volunteer
Purpose of clinical trial	To investigate whether apixaban and rivaroxaban transfer into breast milk
Primary objective	To determine if apixaban and rivaroxaban are excreted in breast milk following oral administration to breastfeeding mothers.
Secondary objective (s)	To determine apixaban and rivaroxaban concentration-time profiles in maternal plasma and breastmilk (if apixaban and/or rivaroxaban are detectable in breast milk).
Trial Design	Open label, non-randomised, single centre study
Endpoints	Primary endpoint: Rivaroxaban in plasma and breastmilk will be measured at 0, 2-3, 6, 10, 12, 24 hours after swallowing one 20mg tablet of rivaroxaban. Apixaban in plasma and breastmilk will be measured at 0, 3-4, 7, 12, 14, 16, 24 hours after swallowing one 5mg tablet of apixaban (1 tablet twelve hours apart). Sampling of milk will be from both breasts. Secondary endpoints: If apixaban and/or rivaroxaban are detectable in breast milk, the following PK parameters in breast milk and maternal plasma will be the endpoints: AUC from zero to the time of the last quantifiable concentration (AUC(0-t)); AUC from zero to the time of 24 hours (AUC(0-24)).
Planned number of subjects	Apixaban Group: 2; Rivaroxaban Group: 2
Summary of eligibility criteria	Breastfeeding mothers aged ≥18, and are at least 6 weeks postpartum with negative pregnancy test when enter the study and about to stop breastfeeding their infants during the trial period, and are able to provide written informed consent; Mothers < 6 weeks postpartum will be eligible if they had already weaned or plan to wean their infants after the study.
IMP, dosage and route of administration	Apixaban (APX) group: Subjects will receive two oral doses of apixaban (1 x 5 mg tablet twice daily). Rivaroxaban (RVX) group: Subjects will receive a single oral dose of rivaroxaban (1 x 20 mg tablet once daily).

Active comparator product(s)	NA
Maximum duration of treatment of a subject	24 hours
Version and date of protocol amendments	V6.0 05 Feb 2019

6. Glossary of terms

APTR	activated partial thromboplastin time ratio
APTT	activated partial thromboplastin time
APX	apixaban
AUC	area under concentration-time curve
Cavg	average concentration
Cmilk	drug concentration in milk
Cavg, m	average maximum concentration
Cavg, p	average drug concentrations in plasma
Dinf	daily infant dosage
DOACs	direct oral anticoagulants
DVT	deep vein thrombosis
GFR	glomerular filtration rate
INR	international normalised ratio
kg	kilogram
LMWH	low molecular weight heparin
M/P	milk to plasma ratio
mg	milligram
mL	millilitre
ng	nanogram
PE	pulmonary embolism
PT	prothrombin time
RID	relative infant dose
RIV	rivaroxaban
TT	thrombin time

UHPLC-MS/MS	ultra-high-performance liquid chromatography - tandem mass spectrometry
VKA	vitamin K antagonist
VTE	veous thromboembolism
µL	microlitre

7. Publication (reference)

Zhao, Y., Couchman, L., Kipper, K., Arya, R., Patel, J.P., 2020. A UHPLC-MS/MS method to simultaneously quantify apixaban, edoxaban and rivaroxaban in human plasma and breast milk: for emerging lactation studies. Journal of Chromatography B, 1144:122095. doi: 10.1016/j.jchromb.2020.122095.

Zhao, Y., Ding, A., Arya, R., Patel, J.P., 2018. Factors influencing the recruitment of lactating women in a clinical trial involving direct oral anticoagulants: a qualitative study. International journal of clinical pharmacy, 40(6), pp.1511-1518.

8. Study period (years)

FPFV was on 17 Jan 2020, and LPLV was on 07 Mar 2020. Volunteer recruitment was completed on 07 Mar 2020. The study period was terminated before the recruitment of the 4th volunteer, due to the pandemic of COVID-19.

9. Phase of development

Phase IV (post-marketing)

10. Objectives

The purpose of this trial is to investigate whether apixaban and rivaroxaban are excreted in breastmilk.

Primary objective

To determine if apixaban and rivaroxaban are excreted in breastmilk following single dose oral administration to breastfeeding mothers.

Secondary objectives

To evaluate the concentration-time profiles of apixaban and rivaroxaban in the plasma and breastmilk of breastfeeding mothers, and therefore to establish the potential exposure of breastfed infants to apixaban and rivaroxaban.

11. Background and Context

Venous Thromboembolism

Venous Thromboembolism (VTE) is a disease that encompasses both deep vein thrombosis (DVT) and pulmonary embolism (PE). DVT is a condition where a blood clot develops in the deep veins, most often in the legs. PE occurs when a part of the clot breaks off and travels from the deep veins to the arteries of the lungs, which can be lethal. VTE is a major disease burden with incidences ranging from 0.75 to 2.69 per 1000 persons in the general population globally every year.

The management of VTE involves prophylaxis and acute treatment. VTE prophylaxis is frequently

prescribed to patients assessed to be at increased risk of VTE. Low molecular weight heparin (LMWH) is most commonly prescribed for prophylaxis, started as soon as possible after risk assessment has been completed and continued until the patient is no longer at increased risk of VTE.

Anticoagulant therapy is the mainstay for the treatment of VTE and is classically divided into three phases: the acute phase of the first 5–10 days following VTE diagnosis, a maintenance phase of 3–6 months, and an extended phase beyond this period. The options of anticoagulant therapy include subcutaneous LMWH or fondaparinux, intravenous UFH, or the direct oral anticoagulants (DOACs). Anticoagulant therapy should be continued for at least 3 months to prevent VTE recurrence. The optimum duration is determined by the estimations of the anticipated risks of recurrent VTE balanced with the risk of bleeding with continued anticoagulation use.

VTE in the postpartum period

It is well established that there is an elevated risk of VTE during pregnancy, which continues during post-partum period. The risk of post-partum VTE is as much as 5 times higher than during pregnancy and 2.5 to 84 times greater than in non-pregnant women. PE remains a leading direct cause of maternal death in the UK for over 20 years. The duration of the increased VTE risk after childbirth varies based on the type of risk factors and the risk can extend up to the first 3 to 6 weeks post-partum.

LMWH is the commonest prescribed anticoagulant agent for thromboprophylaxis during both pregnancy and the post-partum period because it does not cross placenta and is reported to be safe during breastfeeding. Although warfarin use in pregnancy is restricted, it is reported to be safe to use during breastfeeding.

In the UK, the timing of initiation and duration of post-partum thromboprophylaxis depends on the type of and the number of existing risk factors of postnatal VTE. Anticoagulation treatment for the management of VTE should be initiated immediately once DVT or PE is clinically suspected, until the diagnosis is excluded by objective testing. The maintenance of therapeutic doses of subcutaneous LMWH should be continued until at least 6 weeks postnatally, and the continuing risk of thrombosis should be assessed before discontinuing treatment minimum of 3 months in total. In place of LMWH, warfarin can be prescribed in this setting after the fifth day following birth in breastfeeding women. DOACs have not been tested in breastfeeding women, thus have not been recommended in breastfeeding women who need treatment for post-partum VTE.

Direct oral anticoagulants (DOACs)

Historically, vitamin K antagonists (VKAs), such as warfarin, have been the standard of care and the only oral anticoagulant option. Many limitations are associated with warfarin: narrow therapeutic window and requires frequent monitoring due to its considerable variability in dose response among patients. Its variability is attributed to genetic polymorphism and other factors including interactions with drugs and diet. The DOACs, have recently been introduced because of the practical deficits of VKAs.

DOACs, including direct anti-Xa inhibitors (rivaroxaban, apixaban and edoxaban) and thrombin inhibitors (dabigatran), can directly inhibit the pathways in the coagulation cascade. Apixaban, rivaroxaban and edoxaban selectively and reversibly bind to factor Xa, competitively inhibiting both free and clot-bound factor Xa and prothrombinase activity, inhibiting both thrombin formation and development of thrombin, and eventually interrupting the intrinsic and extrinsic pathway of the blood coagulation cascade. Dabigatran blocks the procoagulant activity by competitively and reversibly binding to the active site of free and fibrin-bound thrombin, preventing the conversion of fibrinogen into fibrin during the coagulation cascade. These four DOACs are currently available in the UK, for the treatment and prevention of VTE.

A variety of clinical trials and post-marketing studies have demonstrated that fixed doses of apixaban and rivaroxaban have improved or similar efficacy and noninferior safety compared to warfarin or enoxaparin for prevention and treatment of arterial and venous thrombotic diseases, because their pharmacokinetics are dose proportional and predictable. The most severe side effect of apixaban and rivaroxaban is major bleeding. Apixaban and rivaroxaban exhibit a dual mode of elimination via the kidney and via feces, thereby attenuating the risk of drug accumulation in patients with renal impairment, in comparison with dabigatran.

Previous non-clinical and clinical studies/cases on DOACs in breastmilk
Previous preclinical animal studies indicate that DOACs are secreted into rat milk. For example, the excretion of dabigatran into milk varies from 0.08 to 0.13% of the dose administered to the lactating rats. A single oral dose of apixaban administered to lactating rats was extensively secreted into rat milk with a high milk to maternal plasma ratio (Cmax M/P about 8, AUC M/P about 30). Similarly, rivaroxaban and edoxaban were excreted into the milk of rats, although the maternal plasma ratio has not been published (Xarelto 10 mg film-coated tablets - Summary of Product Characteristics (SPC) - (Emc), 2015; Lixiana 15mg Film-Coated Tablets - Summary of Product Characteristics (SPC) - (Emc), 2015).

Few published clinical cases revealed some information referring to the use of DOACs in postpartum women. A recent case reported that a patient was prescribed rivaroxaban 15 mg twice daily on the fifth day after delivery and demonstrated that a small amount of rivaroxaban passes into human breastmilk (M/P about 0.4), while the safety of rivaroxaban in nursing mothers and their breastfed infants remains to be verified. A recent completed clinical study indicated that small amount of dabigatran was excreted in breast milk, yet the Cmax M/P and AUC M/P were varied between the two breastfeeding women involved in the study (0.04 vs. 0.12 for Cmax M/P; 0.02 vs. 0.1 for AUC M/P).

12. Methodology

12.1. Potential participants identification

Potential study subjects were invited to participate in this open-label, single-center, phase IV trial via multiple routes, such as advertisements post on websites, and invitation letters enclosed with PIS sent to those women who had delivered their babies at King's College Hospital NHS Foundation Trust in the preceding year, and had been administrated LMWH during pregnancy or the postpartum period. Subjects interested in the trial actively contacted investigators, following which a pharmacist trained in haematology would discuss the PIS with them via phone. If a subject remained interested, she was then invited to the screening visit at the Haematology Outpatient Clinic to assess her eligibility, with the Chief Investigator involved.

12.2. Screening

The screening visit was designed to assess participants' demographics and eligibility to the study and was completed by the chief investigator and a clinical pharmacist. The assessment included participant's age, duration of gestation, date of delivery, feeding regimen (exclusive breastfeeding or mixed feeding), smoking history, alcohol history, medical history, ongoing medication, blood pressure, and pulse. Additionally, a variety of laboratory tests were performed, including haematology tests, biochemistry tests, pregnancy test, and serology test. Written informed consent was obtained.

The assessment results of participants were discussed, and it was determined whether the participant was eligible to participate in the study by research investigators. Eligible participants were then contacted to confirm the home visits date and time for DOAC administration and sample collection.

12.3. Interventions and Samples Collection

Participants were allocated to rivaroxaban and apixaban sequentially in the order of their recruitment; apixaban followed by rivaroxaban. Those allocated to rivaroxaban were administered a single oral dose of 20 mg, whilst those allocated to apixaban were given two doses of apixaban 5mg, 12 hours apart. Home visits were provided to collect blood and breastmilk from volunteers according to the pre-specified sampling timepoints (Fig. 1).

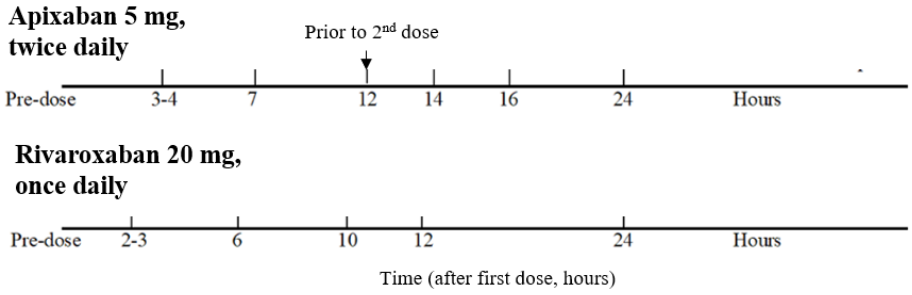

Figure: Sampling timepoints for plasma and breastmilk sample collection

About 5 mL of breastmilk was collected by an electric pump or hand expression from one breast at each sampling time, depending on each volunteer's preference.

13. Number of patients (planned and analysed)

13.1 Planned

Four volunteers

13.2 Analysed

Three completed the study before the outbreak of COVID-19

Table: The reasons for patient withdrawal from the study

Participant	Comments
APX001	The mother felt that she was not ready to interrupt breastfeeding her baby before the outbreak of COVID-19, then the study was terminated due to the COVID-19 pandemic.

14. Diagnosis and main criteria for inclusion

The inclusion criteria are as follows:

Participants must have provided written informed consent for participation in the study, capacity of adhering to applicable protocol requirements, such as visit schedule and interrupting breastfeeding during the sampling period, negative pregnancy test, normal renal function (serum creatinine less than 90 µmol/L), normal liver function (serum alanine aminotransferase no more than 40 IU/L), not taking any medication interacting with apixaban or rivaroxaban, and no contraindications for taking apixaban or rivaroxaban. Participants must have been a minimum age of 18 years old, and at least 6 weeks postpartum when they participate in the study.

15. Test product, dose and mode of administration

Baseline therapy

NA

IMP

Apixaban (Eliquis®) capsule and rivaroxaban (Xarelto®) tablet

Table: Dose of IMP administered to each study particpant

Subject ID	IMP	Dose
APX002	Eliquis®	2 tablets x 5 mg (1 tablet 12 hours apart)
RIV001	Xarelto®	1 tablet x 20 mg
RIV002	Xarelto®	1 tablet x 20 mg

16. Duration of treatment

Table: Duration of treatment

Study Drug	**Study Drug Dose**	**Subjects**	**Duration for each subject*** **(Day -28 to 3)**		
Apixaban	5 mg (twice daily)	1	Screening (day -28 to -3)	Treatment (day 1 to 2, 24 hours): Two doses administration, post-dose PK.	Telephone follow-up (day 3): safety review & study closure
Rivaroxaban	20 mg (once daily)	2		Treatment (day 1 to 2, 24 hours): Single dose administration, post-dose PK.	

17. Reference therapy, dose and mode of administration

NA

18. Criteria for evaluation: Endpoints

18.1 Efficacy

Primary end-point

Apixaban concentrations in plasma and breast milk were measured at 0, 3-4, 7, 12,14, 16, 24 hours after swallowing two 5mg tablets of apixaban (1 capsule 12 hours apart).
Rivaroxaban concentrations in plasma and breast milk were measured at 0, 2-3, 6, 10, 12, 24 hours after swallowing one 20mg tablet of rivaroxaban.
The validated UHPLC - MS/MS assay was used to evaluate the concentration levels.
Milk to plasma ratio (M/P), the calculation of which was based on the average concentration of drug over 24 hours (Cavg,0-24h). The value of M/P was used to calculate the estimated Dinf and the percentage of estimated daily infant dosage to the approved dose (known as relative infant dose (RID)), with the equation below:

$$Estimated\ Dinf = M/P \times C_{avg,p} \times 150mL/kg/day \text{ or } C_{avg,\,m} \times 150mL/kg/day$$

$$RID\ (\%) = \frac{Dinf \text{ or } Estimated\ Dinf\ (mg/kg/day)}{Maternal\ Dosage\ (mg/kg/day)}$$

Secondary Efficacy Parameters

The following parameters for apixaban and rivaroxaban in milk and plasma were the endpoints: area under the concentration-time curve (AUC) from zero to the time of the last quantifiable concentration (AUC(0-t)); AUC from zero to the time of 24 hours (AUC(0-24)), which was used to calculate Cavg, 0-24h.
These parameters were estimated by means of noncompartmental pharmacokinetic analysis using the lin up/log down trapezoidal rule in R (version 3.6.2).

18.2 Safety

Safety Parameters

The safety parameters include adverse events that were documented by questioning of the participants by the investigators and by spontaneous reporting by the participants. Additionally, liver function and renal function tests were performed for the blood sample at the last sampling time for safety purposes.

Specific Safety Endpoints

Pharmacodynamic endpoints including INR, activated partial thromboplastin time ratio (APTR), prothrombin time (PT), and thrombin time (TT) were assessed at each sampling time, which helped the investigators judge if apixaban and rivaroxaban had been eliminated to ensure mothers could re-start breastfeeding after 48 hours of the first dose, if they wished.

19. Statistical Methods

Due to the nature of the study, descriptive statistics were used to analyse the data and describe the study population.

20. Summary – Conclusions

20.1 Demographic data

The following tables summarise the demographics of the study population:

Table: Demographic data and baseline characteristics for all participants

Characteristics	APX002	RIV001	RIV002
Age (yrs)	44	27	42
Ethnicity	White	Mixed	White
Weight (kg)	47.0	122.4	87.3
Gestational age at delivery (weeks+days)	39+0	37+1	40+4
Stage of lactation	Weaning	Late	Late
Length of time postpartum (months)	23	8	8
Breastfeeding regimen	Breastfeed and supplement with solids	Breastfeed and supplement with formula and solids	Breastfeed and supplement with formula and solids
eGFR (mL/min/1.73 m²)	>90	65	90
Concurrent medicines	No	No	No

20.2 Primary outcome

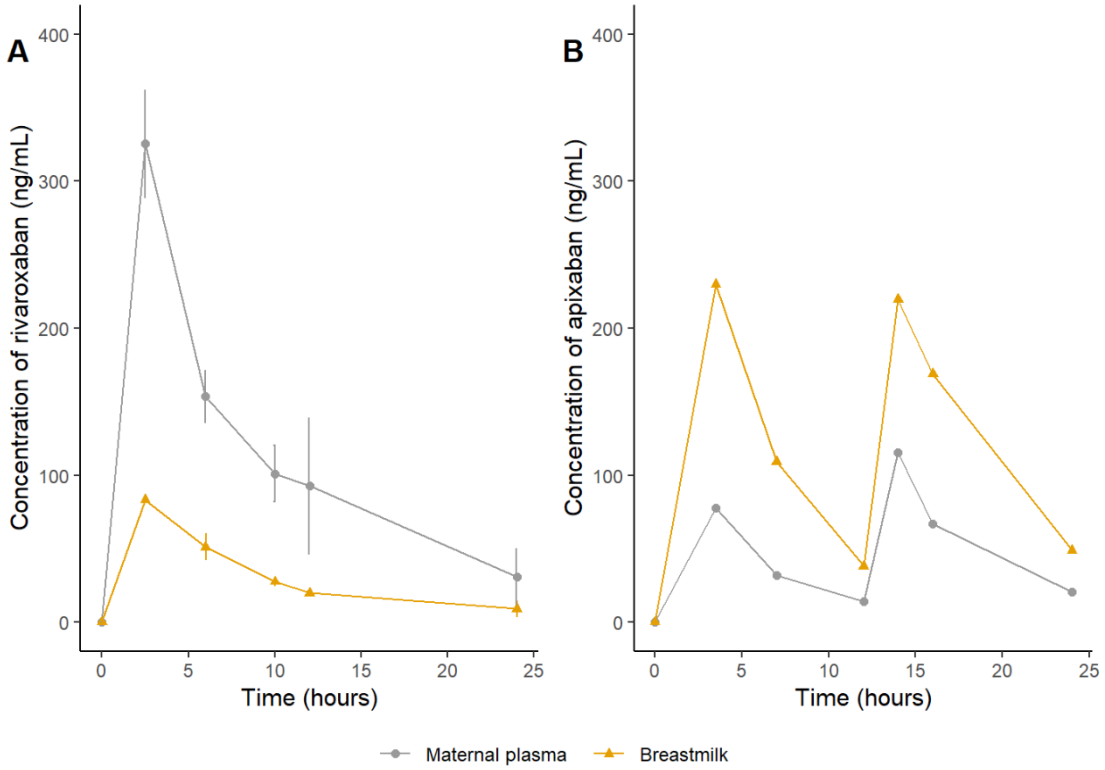

Figure: Concentration - time profile for rivaroxaban (n=2) (A), and apixaban (B) in maternal plasma and breastmilk. For rivaroxaban, data are mean ± standard deviation (SD) for two subjects.

Table: Apixaban and rivaroxaban pharmacokinetic parameters in plasma and breastmilk

Parameter	**Rivaroxaban 20mg (once daily) (N = 2)**	**Apixaban 5 mg (twice daily) (N = 1)**
$AUC_{0\text{-}24\ h}$, milk, ng*h/mL (±SD)	693.77 (23.84)	2725.33
$Cavg_{0\text{-}24}$, milk, ng/mL (±SD)	28.91 (0.99)	113.56
$AUC_{0\text{-}24}$, maternal plasma, ng*h/mL (±SD)	2569.55 (312.73)	1045.82
$Cavg_{0\text{-}24}$, maternal plasma, ng/mL (±SD)	107.06 (13.03)	43.58

Table Calculated apixaban and rivaroxaban exposure parameters

Parameter	**Rivaroxaban 20mg (once daily)** **(N = 2)**	**Apixaban 5 mg** **(twice daily)** **(N = 1)**
M/P ratio (±SD)	0.27 (±0.02)	2.61
Daily infant dosage, mg/kg/day	0.0043	0.017
RID (%) (±SD)	1.63 (±0.01)	12.78

20.3 Safety results

One woman reported gum bleeding when she brushed her teeth after the first dose of apixaban, but she had the same event before when she did not take apixaban, so it was not considered drug related adverse event. No other women suffered or reported any adverse effects. All women commenced breastfeeding at the recommended time, with no adverse outcomes reported on the follow-up.

20.4 Conclusion

This clinical trial found a significant distribution of apixaban into human breastmilk, while rivaroxaban distribution was significantly less. The results suggest that rivaroxaban may hold promise for breastfeeding women and further research from women in the early period after delivery is needed, to confirm this.

21. Date of Report

This is version 1.0 of the Clinical Study Report synopsis, dated 26/Feb/2021.