Study protocol, Statistical analysis plan and Informed Consent Forms

Official study title: **Assessing drug exposure risk of infants breastfed by women with Inflammatory bowel disease**

NCT number: **03397108**

Contents

ASSESSING DRUG EXPOSURE RISK OF INFANTS BREASTFED BY WOMEN WITH INFLAMMATORY BOWEL DISEASE

Principal Investigator: Shinya Ito

SCOPE

The inflammatory bowel disease (IBD) shows the highest incidence among people of childbearing age. Indeed, it is not uncommon that pregnant or lactating women with IBD require drug therapy including monoclonal antibodies against Tumor Necrosis Factor-alpha (TNFα). However, these patients face challenges because information on pregnancy and breastfeeding safety of these new drugs is lacking due to their exclusion from drug development processes. Whereas data necessary for fetal safety assessment is accumulating gradually, significant gaps in the research efforts and our understanding on excretion of TNFα antagonists into milk remain. Although experts consider it acceptable to use the TNFα antagonists during breastfeeding due to the relatively low levels in milk, the existing data on milk levels of these drugs are highly inconsistent, probably because previous reports gave no consideration to potential interference from high levels of endogenous TNFα in milk. As a result, a comprehensive picture of TNFα antagonists in milk remains obscure. Moreover, TNFα-dependent milk chemokines have been recently shown to play a role in shaping the postnatal programming of brain development, implying that altered disposition of endogenous TNFα and other chemokines in milk during anti-TNFα therapy has an impact on brain development of the infants of those women with IBD. The present proposal describes the first step to address this issue by uncovering the TNFα dependent 'lactocrine' pathway and disposition of TNFα antagonists in milk.

BACKGROUND

Breastfeeding has tangible benefits for the mother and infant

The health benefits of breastfeeding are evident for both mother and infant.[1] For infants, epidemiological data indicate reduction of mortality[1-3] and morbidity from infection, [1,4] and decrease in some adult-onset conditions.[1, 5,6] A randomized study further showed positive impact of breastfeeding on infant cognitive function, [7] confirming previous results.[1] Exclusive breastfeeding is now recommended to continue at least for 6 months after birth.[1] Moreover, a history of nursing is associated with reduced incidence of cancer in mothers, particularly breast cancer. Although there are inconsistencies among reports on these maternal benefits of breastfeeding, [8, 13] studies showing positive associations continue to emerge.[1, 9-11]

TNFα antagonists are used in the treatment of IBD

IBD includes ulcerative colitis (UC) and Crohn's disease (CD). Incidence of IBD is increasing worldwide, and at present the reported prevalence rate in North America is more than 500 per 100,000 persons[12]. The incidence of IBD is highest during reproductive age, although female predominance of IBD is not a consistent finding.[12] For moderate to severe IBD, TNFα antagonists including infliximab and adalimumab are used with an immunosuppressant such as Azathioprine and Methotrexate (MTX). [14] Metabolism of these monoclonal antibodies against TNFα (TNFmAb) occurs by proteolysis in the reticuloendothelial system.[15, 16] TNFmAb bind to the receptor (the neonatal Fc receptor: FcRn) at acidic pH in the endosome. This binding protects them from degradation, thereby contributes to prolongation of their elimination half-life to approximately 2-7 weeks.[17] Presence of FcRn is reported in human mammary gland epithelial cells.[18]

Various information resources[19-21] consider TNFmAb to be compatible with breastfeeding based on several notions: 1) Reported milk levels are low; 2) No adverse effects on infant development has been

shown; and 3) the drug is likely to be destroyed in the infant's gastrointestinal tract; although a possibility of local immune suppression in the gastrointestinal tract is often acknowledged. Indeed, experts[22, 23] state a low risk to the nursing infant and recommend continuous breastfeeding during the TNFmAb therapy. Although this view is a logical consequence of the existing data, our understanding requires revision because the previous studies did not take into account the highly elevated endogenous TNFα levels in milk, which may interfere with the drug measurement, as described later. Azathioprine and Methotrexate (MTX) are also used with TNFmAb to prevent antibody formation against TNFmAb. Experts consider Azathioprine to be compatible with breastfeeding, as milk levels are invariably low or undetectable.[19, 20] On the other hand, use of MTX during breastfeeding is controversial and safety information is extremely limited with only two case reports, in both of which milk MTX levels were either below the lower range of the current standard MTX single oral dose (per kg) for pediatric patients with rheumatoid arthritis[35,36] or below the quantitation limit.[37] Despite the reported low milk levels, MTX is not usually used in pregnancy and lactation.

TNFmAb is excreted into milk, but our understanding is incomplete

The mechanism of TNFmAb transfer into milk may be similar to an FcRn binding/internalization pathway for endogenous IgG, as FcRn is expressed on the mammary epithelial cells.[18] Although conflicting views exist, most authorities consider infliximab as safe during breastfeeding because previously published studies were unable to detect the drug in breast milk.[19-23] However, recently reported infliximab concentrations in breast milk were in the range of approximately 100- 475 ng/mL around 1-6 days post-infusion.[26-28] Although the levels are below the therapeutic range in serum, the local effect on infant mucosa remains unknown and infant infliximab clearance is not established. In one report, [27] a breastfed infant had a serum concentration of 1700 ng/mL, contradicting the previous reports that demonstrated undetectable infant serum infliximab levels. This data suggests that infliximab (and potentially other TNFmAbs) may be absorbed through the infant gut and into systemic circulation, potentially via the FcRn, expressed in neonatal tissues.

Overall, there are major inconsistencies among the reports on infliximab excretion into breast milk. Moreover, previous reports used methods to detect free TNFmAb, [27, 29] which are not bound (or only partially bound) to TNFα. Importantly, milk is enriched with endogenous TNFα as described later. Therefore, no comprehensive picture is available for milk TNFmAb levels to which the infant is exposed through milk. Even if TNFmAb absorption through infant gut is small, basic understanding on its disposition in milk is important because TNFmAb inactivates milk TNFα, which apparently has physiological function for the offspring (see below).

Endogenous TNFα levels in milk are very high compared to serum.

In serum: An average TNFα concentration in serum of healthy individuals is 0.02 ng/L.[40] In contrast; patients with IBD show serum TNFα concentrations of about 10 ng/L, [40] that is 500-fold higher than the level in healthy control. This huge difference between TNFα concentrations between IBD patients and healthy subjects remained the same across the disease activity spectrum (i.e., inactive vs. active stages).[40]

In breast milk: Milk of healthy lactating women contains variable concentrations of TNFα and other chemokines produced by epithelial and immune cells in the mammary gland.[41] Current studies[42, 43] suggest that milk TNFα levels in healthy women have large individual variations, depending on infant age and stages of milk (i.e. the highest in colostrum, followed by transitional/mature milk), and that an "average" level may be around 1-150 ng/L. It is remarkable that the milk TNFα concentrations could be 7,500-fold higher than serum levels. Given the high serum TNFα levels in IBD patients as described above, [40] reflecting hyperactivity of TNFα producing cells, milk of IBD women may show even higher

TNFα levels, which may interfere with measurement of TNFmAb. However, no data is available for TNFα and other chemokines in breast milk of patients with IBD. Clearly, an obvious question is their physiological roles.

TNFα-dependent lactocrine system affects infant brain development

Milk chemokines modulate infant immune development.[44, 45] A recent mouse study[25] has made unexpected observations that milk chemokines (reduced in Tnf-knockout mice: IP10 [CXCL10], MIP-1 beta [CCL4], MCP-1 [CCL2], and -3 [CCL7]) alter hippocampal proliferation, and influence development of offspring spatial memory and cognitive function. In short, reduced maternal TNFα and accompanying lower levels of various chemokines in milk of Tnf-gene knockout mice are associated with enhanced spatial memory and cognitive function of their offspring.[24, 25] Importantly, administration of infliximab to the wild-type lactating mice had effects similar to those seen in the offspring of Tnf-knockout mice (i.e., improved memory). In contrast, feeding these pups with the exogenous chemokines via gavage negated the effects. These findings indicate an intricate function of TNFα-dependent 'lactocrine' pathway in the developmental program of offspring brain and memory capacity. In addition, orally administered large molecules such as chemokines clearly confer biological function in the neonates, [25] challenging the widespread notion that consequence of orally ingested protein drugs is insignificant due to gastrointestinal degradation. Thus, even if it is not absorbed, TNFmAb may exert its function by inhibiting endogenous milk TNFα, thereby reducing milk levels of TNFα-dependent chemokines. Although mouse biology is not directly applicable to humans for various reasons, the findings are intriguing.

TNFmAb may suppress hyperactive TNFα-dependent lactocrine pathway in IBD

As described earlier, serum TNFα levels in IBD patients are 500-fold higher than healthy controls. [40] Although there is no data available yet, the TNFα-dependent lactocrine pathway in the mammary gland of women with IBD may be highly activated as well. It is enticing to speculate that TNFmAb suppresses the hyperactive TNFα-dependent lactocrine pathway in women with IBD, reducing milk chemokines to a normal level, and thereby restoring a normal developmental program of offspring hippocampal proliferation. On the other hand, complete absence of TNFα may have a detrimental effect on defense against infection in the breastfed infant. However, milk disposition of TNFmAb is poorly understood, and there is no data on milk TNFα and chemokines in patients with IBD.

Measurement of TNFmAb in milk must take into account high levels of milk TNFα

To quantify TNFmAb, most previous studies used exogenous TNFα to capture the drug for detection. Clearly, these methods detect free TNFmAb in serum, which are not bound to TNFα. Given that endogenous TNFα in serum of IBD patients is about 10 ng/L, [40] measurement of serum infliximab at 10^{-3} g/L (4 weeks post infusion) [19] is unlikely to be influenced by endogenous TNFα. In other words, serum TNFmAb is mostly unbound. However, use of a TNFα-based (or similar) method is problematic in measuring TNFmAb in milk. As described above, endogenous milk TNFα levels are extremely high in healthy individuals when compared to serum (~7,500- fold higher), [42, 43] and may be even higher in patients with IBD. Moreover, because milk level of TNFmAb such as infliximab is lower than serum, significant portions may be bound to milk TNFα. Therefore, previous reports may have overlooked a significant fraction of TNFmAb, which is bound to endogenous milk TNFα.

Population Pharmacokinetic (popPK) approach is effective in a real world setting

PK data is the first line of evidence for safety assessment of drug excretion into milk. A population PK (popPK) approach is a powerful tool, which is based on variable-timed (i.e., no need to use the same time points among subjects) and sparse per-individual sampling from a relatively large pool of individuals. The term "population" refers to the simultaneous estimation of the model parameters, i.e. typical and variance parameters using data from all patients at once.

When the number of samples per individual is limited or sampling times differ among subjects, conventional PK methods would fail, but popPK approach can provide accurate estimates and reliable predictions.[46] Furthermore, using the popPK model, one may perform simulation by randomly introducing parameter variations, and obtain population-level pictures of drug disposition, which are otherwise impossible to experimentally obtain. Although PK analyses usually deal with serum drug disposition, we have reported the first successful application of popPK modeling and simulation of drug disposition in breast milk.[47, 48]

HYPOTHESES AND OBJECTIVES

Overarching Hypothesis

TNFmAb excreted into milk normalizes the hyperactive state of the TNFα-dependent lactocrine pathway in women with IBD

Specific Hypotheses

Primary

Concentrations of endogenous TNFα in milk are higher in IBD women than in healthy controls

Exploratory

- TNFα-dependent chemokine (CXCL10, CCL2, 4, and 7) levels in milk are higher in IBD women than in healthy controls
- TNFα-dependent chemokine levels in milk (CXCL10, CCL2, 4, and 7) are low in IBD women receiving TNFmAb, compared to women with IBD, who are not receiving TNFmAb

Rationale for Selection of Cytokines

Informed by the mouse study[25] described above on page 3, we will investigate TNFα and four TNFα-dependent chemokines in milk of women with IBD. We chose these chemokines because they are implicated in compromising the brain development of the offspring.[25]

Primary Objective

1. To compare milk levels of TNFα between women with IBD and healthy controls

Secondary Objectives

2. To compare milk levels of CXCL10, CCL2, 4, and 7 between women with IBD and healthy controls
3. To describe pharmacokinetics of TNFmAb in milk of women with IBD, separating TNFα-bound and unbound fractions
4. To predict milk profiles of TNFmAb at a population level using model-based simulation
(The primary focus is infliximab; the secondary target is adalimumab)
5. To determine serum levels of TNFmAB in infants of mothers with IBD who have received these medications (during pregnancy and/or lactation)
6. To describe developmental milestones and their differences among the infants of the IBD groups (±TNFmAb) and the healthy control.

METHODOLOGY

Study Design (Figure 1): Observational cohort study with a comparison group
Endogenous TNFα and TNFα-dependent chemokines (CXCL10, CCL2, 4, and 7) will be measured in milk of healthy women and IBD patients. A popPK study of TNFmAb in milk will be conducted in a subset of IBD

patients who receive infliximab or adalimumab. Serum levels of TNFmAB in infants of mothers with IBD, participating in the nested study, will also be measured as an optional part of the study. The optional assessment of developmental milestones (cognitive and language scales) in infants of healthy breastfeeding mothers and mothers with IBD will be conducted by a psychometrist at two time-points (12 and 18 month-old).

Note: Due to the COVID-19 restrictions of face-to-face encounter starting from March 14 2020, we add ASQ questionnaire for both groups (IBD cohorts and control) as a mandatory component of the developmental assessment (see page 10: the second paragraph of **Prospective Assessment of Infant Development**). Face-to-face examination remains to be optional.

IBD Cohort and Healthy Controls (Figure 1)

The study population is composed of lactating women aged over 18 years in their first 6-month postpartum period who reside in the Greater Toronto Area, are able to communicate in English and provide the informed consent. The participants will be recruited in two arms:

1) IBD arm: the lactating women who are diagnosed with UC or CD will be recruited in this arm regardless of severity and activity of disease, unless they meet the exclusion criteria (see below). The participants will be asked to take part in the PK study, provided that they receive infliximab or adalimumab.
2) Healthy Control arm: The participants in this arm will be recruited according to the eligibility criteria.

The infants of the participants in both arms will be evaluated for cognitive and language development longitudinally.

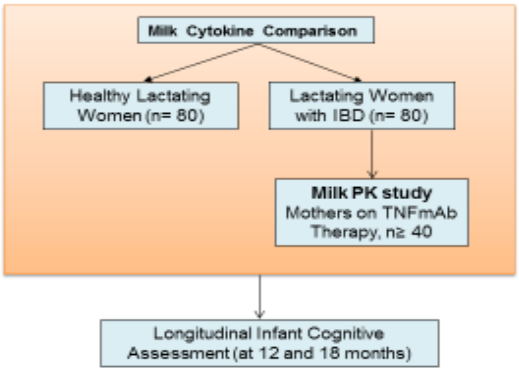


Figure 1. Study structure

Enrollment Criteria

Inclusion criteria

- Age over 18 years old
- Pregnant women with IBD and Lactating women* with IBD** during the first 4-month postpartum period (Arm 1)
- Healthy pregnant OR lactating women during the first 4-month postpartum period (Arm 2)
- Living in Greater Toronto Area
- Signed consent to participation

*Eligible women may or may not continue breastfeeding their infants, depending on advice from their

physicians; ** Those who receive TNFmAb will be offered to participate in the PK component of the study, as well.

Exclusion criteria

- Unable to communicate in English
- Present illness of chronic inflammatory conditions (except IBD)
- Mastitis
- Acute or chronic infection
- Use of a different TNFmAb within the last 2 months
- Living outside Greater Toronto Area

Sample Size Consideration

To test milk TNFα difference between healthy and IBD women (Specific hypothesis: primary), we aim to detect 80-fold difference. This arbitrary effect size is based on the study, [40] which reported that serum TNFα levels were 380 to 630-fold higher in IBD patients than healthy subjects. We chose "80-fold" because the 75th percentile value of serum TNFα in healthy subjects was 0.036 ng/L and the 25th percentile values in IBD patients ranged from 2.8 to 7.5 ng/L in the study, [40] with the IBD/healthy subject ratio ranging from about 80- to 200-fold. Assuming that relative standard deviation is 200% to account for large variances in the existing data (milk TNFα mean ±SD: 7.8 ± 15.8 ng/L[49]), we will require at least 65 subjects in each group (alpha=0.05, beta=0.8, minimal important difference of milk TNFα levels as a ratio between IBD and healthy groups to be 80; two-sided). Assuming 20% dropout, we will enroll 80 women in each group. For chemokine levels in mature milk of healthy individuals, mean/median values and variations in reported studies are[41]: CXCL10, 518 ng/L (range: 81-1162 ng/L); and CCL2, 362 ng/L (SD: 3592 ng/L). However, no data is available for CCL4, and 7 in both healthy women and IBD patients, and no profile of all of these chemokines has been reported for women with IBD. Because data structures of these observations are difficult to predict, we consider this specific hypothesis as exploratory. Similarly, impact of milk TNFmAb on the chemokine levels will be examined as exploratory investigation.

Our primary target for the nested milk PK study is infliximab. Robust parameter estimates from popPK modeling usually require 30-50 data points (from about 20 individuals), [46-48] depending on inter-subject variability. We set the minimum target sample size for the 3-yr term to be 40 women for infliximab (≥200 data points: ≥5 samples x 40 patients). We anticipate that patients on adalimumab will be fewer than infliximab, but we plan to collect the samples to maximize information value of the study.

Enrollment

<u>Arm 1: Lactating women with IBD</u>

The potential participants in this arm (n=80) will mainly be identified through the IBD clinic at Mount Sinai Hospital (MSH), where the study collaborator, Dr. [redacted] is a Clinician Scientist. After obtaining MSH-REB approval, the pregnant women with IBD or lactating mothers with IBD in their first 6-months postpartum period, will be identified by a research coordinator at MSH, who will notify the study coordinator (from SickKids: SK) to attend the clinic on their next clinic visit, when a person in the circle of care (the clinic nurse) will ask the patients' permission for being approached by the study coordinator regarding participation in the study. We will also advertise the study through the study website and promotional material (posters and pamphlets), distributed at both SK and MSH, so that the pregnant or lactating women with IBD who are interested in participation, can contact the research team by email or phone. The study will be explained to the potential participants, their questions (if any) will be answered, and informed consent will be obtained. Those participants, who receive infliximab or adalimumab, will

be offered to opt for the PK component of the study in the consent form. All participants in this arm may also opt for infant blood sampling at SK. The study visits will take place at the participant's home and will be scheduled as per their convenience after obtaining the informed consent.
The pregnant women who consent to participate in the study will receive a letter by mail or email (according to the participant's choice) in about 2 weeks after their due date to ascertain their continuing consent. In the letter, they will be instructed to respond by an email with "Not available to participate" in the subject line, should they choose to not participate for any reason (including an unfortunate event to the fetus or baby). Otherwise, a member of the research team will contact the participant by a reminder email or mail about a week after the date of sending the first letter to schedule the study visit. If no response to the reminder email/mail is received, the participant will be considered as "withdrawn from the study" and no further follow up will take place.

Arm 2: Healthy breastfeeding mothers

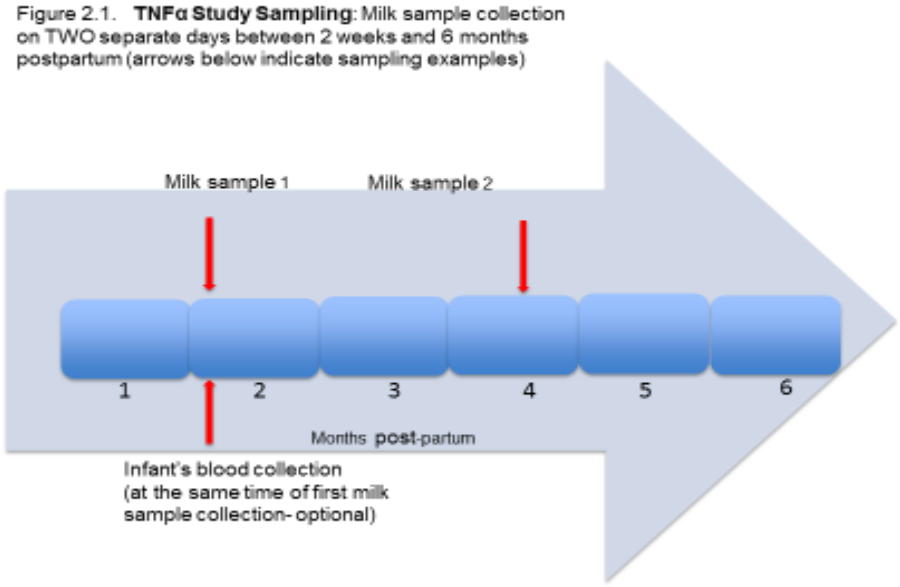
We will use the study website, promotional materials, posted at SK, MSH and breastfeeding clinics, and social media (SK Twitter) to advertise the study for recruitment of this group. The healthy pregnant or breastfeeding mothers during their first 4-month postpartum period (Gestational age and postpartum age matched) (n= 80) will be provided with the study contact information (email, phone number) if willing to volunteer for the study. Upon the potential participants' contact, the study will be explained and their questions (if any) will be answered. The study visits will take place at the participant's home and will be scheduled as per their convenience after their verbal consent. The informed consent form may be sent to the participants by email, mail, or provided for them at the first study visit (as per the participant's choice) to read and sign.
The pregnant women who consent to participate in the study will receive a letter by mail or email (according to the participant's choice) in about 2 weeks after their due date to ascertain their continuing consent. In the letter, they will be instructed to respond by an email with "Not available to participate" in the subject line, should they choose to not participate for any reason (including an unfortunate event to the fetus or baby). Otherwise, a member of the research team will contact the participant by a reminder email or mail about a week after the date of sending the letter to schedule the study visit. If no response to the reminder emails/mails is received, the participant will be considered as "withdrawn from the study" and no further follow up will take place.

Milk sampling for TNF measurement (Fig 2.1)

The study home visits (for Arms 1 and 2) will be conducted by the SK coordinator on two separate days between 2 weeks and 4 months postpartum (to ensure mature milk stage and minimize the milk stage-dependent variation of TNFα[41]). The first sample will be collected between 2-6 weeks postpartum and the second sample collection will take place between 11-16 weeks postpartum. The study visits will be scheduled during morning hours (to avoid potential diurnal variations of milk chemokines), at a convenient date for the participants. Alternatively, for those participants who opt for optional infant blood sampling, the first study visit will be scheduled at SK in order to collecting the first milk sample from mother, and infant blood (5 ml or a teaspoonful) simultaneously during their hospital visit. Upon consenting process, all participants will be instructed not to breastfeed the infant from one breast for two hours before the scheduled visit (to minimize intra-feed variations), and 5-10 ml of the expressed milk will be collected into a sterile container. A breast milk pump will also be provided for the participants. The samples will immediately be placed inside a cooler box with ice packs and transferred to the analysis core (Dr. Ito's lab) for centrifugation and storage at -80°C by the study coordinator.

If the participants (Arm 1) provide consent for optional infant blood sampling concurrently with the first

milk collection, they will be asked to travel to SickKids for the study procedures, and blood collection will take place in the phlebotomy unit at the hospital. A volume of 5 ml (a teaspoonful) of blood will be drawn from infant.



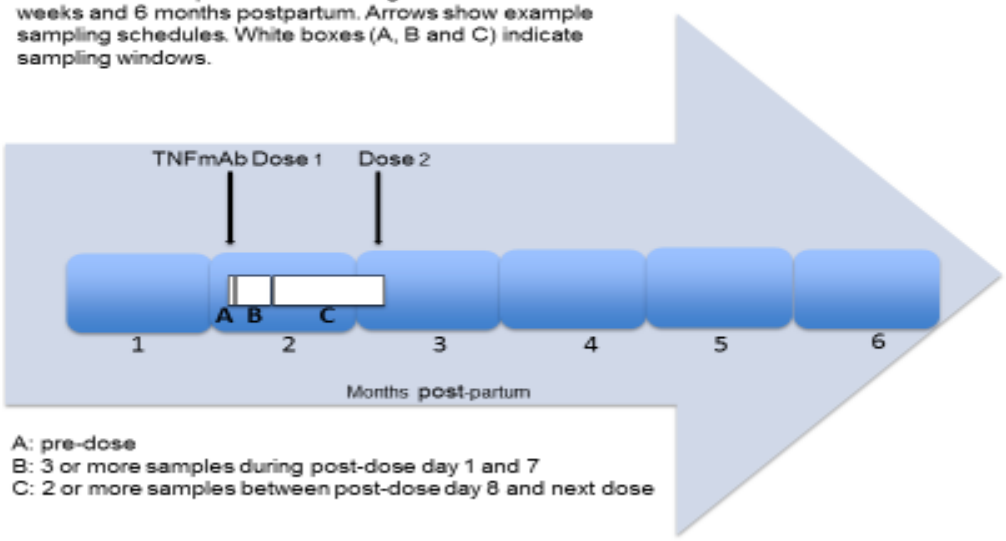
Population PK study (Figure 2.2)

Those participants, who consent to take part in the TNFmAb PK study, will receive a study package by mail or courier service. The package will include: a thermal box, milk containers, ice packs, detailed instructions for sample collection and a form to record the specifics of milk samples. The participants will be asked to collect several milk samples (5-10 ml or 1-2 teaspoonful each, preferably hind milk) between two doses of their medication as follows:

- One sample pre-dose
- Three or more samples during post-dose day 1 and 7
- Two or more samples between post-dose day 8 and the next dose

Collection of hind milk is to avoid potential interference with infant feeding. However, if the participant is willing, we will collect foremilk, and use the feeding phase as a covariate in the modeling analyses. Because popPK approach benefits from data with varying time points among individuals, there is no need to define strict sampling time-points except for daytime sampling. The participants will be advised to collect the samples as per their convenience between 2 doses of their drug, store them in freezer immediately after collection, and contact the study coordinator by email or phone when the study procedure is completed. The pickup delivery by a courier service from the participant's address will be arranged at their convenience. The frozen samples will be delivered to the study lab (inside a thermal box, on ice packs) for processing and analysis.

Figure 2.2. **TNFmAb PK study milk sampling**: a total of 5 or more milk samples within a dosing interval between 2 weeks and 6 months postpartum. Arrows show example sampling schedules. White boxes (A, B and C) indicate sampling windows.



Data Collection

At enrollment (Mt Sinai)

- Participants' name, phone number, email address and mailing address will be collected upon recruitment.
- In order to address the issue of heterogeneity of IBD patient population, participant's disease phenotype (Ulcerative Colitis-UC or Crohn's Disease-CD) will be determined from the data recorded in the patient's chart.

These data will be transferred to SK through Research Information Technology (RIT) File Transfer Portal (RITFTP), which is a web-based, secure file transfer service. All data transferred through this service is encrypted. Sensitive data (Identifiers) will be sent through the "Sensitive Data" option, which will allow the transmission to include a field for a second password where the recipient must obtain from the sender directly. This information will be used for future contacts with participants in order to scheduling further research procedures and follow up visits (including infant development assessments), and delivery of study packages. Each participant will be assigned a study ID, and a password-protected Masterfile will be created to connect the study ID and identifying data. Only the PI and study coordinator will have access to the Masterfile.

At Study Visits (SickKids)

During the study visits a questionnaire will be administered to the participants to collect the following data:

- Demographic: date of birth (Month-Year format), ethnicity, and infant sex
- Pregnancy/Delivery data: length of pregnancy, date of delivery, and mode of delivery
- Birth/Medical history (Infant): Apgar score (if known), neonatal problems /hospital stay, health conditions, infections, medications
- Medical history (Mother): presence of other medical conditions e.g. diabetes, concomitant medications e.g. anti-diabetics, lipid-lowering medications, other immunosuppressants, history

of exposure to TNFmAbs during pregnancy and breastfeeding

- Weight and height/length (mother and infant) will be recorded and disease severity will be evaluated by administering the standardized Patient Reported Outcome measures (6-point Mayo score for UC and Harvey-Bradshaw Index- HBI for CD) [57, 58] at each study visit.
- Sample collection data: date and time of milk sample collection, time of last breastfeeding, type of sample (fore- or hind milk); and date, time and route of medication administration (for nested popPK study) will be collected on data collection forms.

Prospective Assessment of Infant Development

The infants of mothers with IBD (with or without taking TNFmAb) and healthy controls will be given the option of being evaluated for neurocognitive development by means of Bayley Scales of Infant Development (Version III) cognitive and language subtests, [50] prospectively. The longitudinal design has been chosen to capture more robust measurements of infant cognitive development. The assessment test administration will be initiated at 12 months because of better-reported test validity at this age[60], and the time-point of 18 months is selected due to the limited time frame of the study. If choosing to participate in the assessment of infant development, the follow up visits will take place at 12 and 18 months old and will be scheduled after the second sample collection visit. A reminder contact will be made 2 weeks in advance by phone, email or mail (as per the participants' preference). A psychometrist at SickKids will perform the assessment and scores will be calculated and recorded. The participants will receive an evaluation report, which will be prepared under supervision of a psychologist. The PI will make an appropriate referral upon recommendation by the psychologist.

In addition, subjects (i.e., mothers) will be asked to complete for their infants the "Communication" and "Problem-Solving" subscales of the The Ages & Stages Questionnaires®, Third Edition (ASQ®-3) at 12- and 18-months of age (ASQ-12 month, ASQ-18 month) either in person (via mail or hand delivered at visit to HSC) or over the telephone. This supplementary measure is intended to provide additional data under circumstances which preclude participant completion of the Bayley Scales of Infant Development-III (e.g COVID-19 related policies, infant illness, family unable to travel to HSC).

REIMBURSEMENT AND COMPENSATION

The participant will be provided with a $20 Shopper's Drug Mart gift card upon completion of study home visits. If the participant chooses to participate in the assessment of infant development, they will be provided with a second $20 Shopper's Drug Mart gift card upon completion of the two hospital visits. In addition, the participants will be reimbursed up to $20 for parking and transportation expenses for each visit to SickKids (optional blood sampling, infant development assessment).

DATA MANAGEMENT

Study data will be collected and managed using REDCap electronic data capture tools hosted at SickKids. REDCap (Research Electronic Data Capture) is a secure, web-based application designed to support data capture for research studies, providing: 1) an intuitive interface for validated data entry; 2) audit trails for tracking data manipulation and export procedures; 3) automated export procedures for seamless data downloads to common statistical packages; and 4) procedures for importing data from external sources.[59] To ensure accuracy and completeness of entered data, multiple features of REDCap including validation and flagging the required fields will be used. Only the PI and designated study team members will have

access to REDCap database by username and password. The Masterfile will be kept on a password-protected computer in a locked office. The consent forms and hard copies of data will be maintained inside a locked file cabinet, in a locked office, accessible only by the PI and study coordinator.

SAMPLE ANALYSIS

Chemokine/Drug Analysis Core: The analysis core is located within the Therapeutic Drug monitoring (TDM) laboratory, the Division of Clinical Biochemistry, Department of Pediatric Laboratory Medicine, at the Hospital for Sick Children in Toronto. The TDM laboratory, directed by the co-investigator (Dr. Colantonio), is an accredited clinical testing laboratory involved in patient testing. Although the analysis core is located at a fully certified clinical laboratory, clinical services for individual patients will not be the proposed research mandate.

Sample preparation: In order to minimize milk fat interference of immune assay, a fraction of milk whey will be obtained by temperature-controlled centrifugation (4°C) as previously described.[43] For this reason, we will collect the samples from patients' homes immediately after the collection as we did for other drugs.[53]

Assay methods: TNFα will be measured in whey fraction of milk using a commercially available Enzyme-Linked Immune Assay (ELISA) assay kit (R&D systems). Although no interference from co-existing TNFmAb has been reported, we will perform a series of experiments using a combination of low pH[54] and size exclusion (TNFα soluble trimeric form: 55kDa vs. TNFmAb: ~150kDa) to confirm the notion. We will use ELISA kits for the planned quantification of TNFα and the chemokines (CXCL10, CCL2, CCL4 and CCL7). We will use ELISA with a microtiter plate coated with human antibodies against TNFmAb from AbD Serotec: infliximab (HCA213 for unbound infliximab; and HCA215 for total infliximab); and adalimumab (HCA202 for unbound adalimumab, and HCA231 for bound adalimumab). Initial assay validation will follow our protocol for clinical test development. In order to address endogenous milk TNFα, our blank milk standard will be freshly obtained milk from healthy lactating women, instead of pooled milk as usual.

DATA ANALYSIS

To test the primary hypothesis, we will compare the mean values of TNFα milk levels between the 2 groups. Because each subject will provide 2 separate milk samples, an average value will be assigned to each subject. We are aware that data may need to be log-transformed before parametric comparison with unpaired Student's t-test ($p<0.05$ as statistical significance). If necessary, we will use non-parametric Mann-Whitney test for the comparison.

We will first examine if the ELISA method for TNFα is interfered by the presence of infliximab or adalimumab. If interference is detected, then we will use TNFα level data obtained after sample treatment to dissociate antigen-antibody binding for all samples (see above). Influences of disease type/severity/TNFmAb on milk levels of cytokines will be assessed in a subgroup analysis.

Other potential confounders including co-morbidities and concomitant medications will be subjected to post-hoc subgroup analyses for their effects on milk levels of cytokines. Bayley scale scores of the infants will be compared between the 3 groups (Healthy mothers, mothers with IBD and no TNFmAb treatment, mothers with IBD and TNFmAb treatment) by ANOVA.

For exploratory hypotheses, chemokine levels will be summarized using appropriate descriptive

statistics, depending on the dataset structure. Comparisons between the two groups will follow the method described above. In parallel, we will analyze correlation between the chemokine levels and the unbound fractions of TNFα to infer the functional consequences.

A popPK modeling will be done according to our protocol with regard to drugs in milk, [47,48] in which population parameters were estimated. Model selection will be based on the likelihood ratio test, PK parameter point estimates and confidence intervals (CI), goodness-of-fit plots, and visual predictive checks. On the basis of the final popPK model, drug concentration profiles in milk will be simulated at steady state for a large population, as we showed for other drugs.[47, 48] Briefly, a set of breastfeeding frequency and feeding times will be randomly generated to simulate drug concentration at various time intervals after the last dose administered. A truncated normal distribution of daily breastfeeding frequency mimicking values will be used.[48, 55] Modeling and simulation processes will be carried out using NONMEM and R (version 2.5; http://www.R-project.org). [47,48] In a subset of IBD women and their infants, we will examine correlation between milk levels of TNFmAb and infant serum levels corrected for gestational exposures. Dosing of TNFmAb, concomitant medications, demographic data and sample type (fore- or hind milk) will be evaluated as covariates in the final popPK modeling.

MAIN STUDY OUTCOMES

Comparison of milk TNFα between healthy individuals and those with IBD
This data will be the first of its kind, providing insight into the differences (or similarities) of milk TNFα environment in the IBD disease state, compared to healthy women. As an additional exploratory analysis, infant neurocognitive development (Bayley scale) will be also compared.

Description of TNFα-dependent chemokine profiles in milk of healthy and IBD women
This novel data will inform us of the potential impact of TNFmAb therapy on milk chemokines in function.

Observed time–milk concentration profiles of TNFmAb in lactating women with IBD
We will obtain a full picture of bound and unbound TNFmAb disposition in milk, and construct a popPK model for simulation.

Simulated/predicted profiles of TNFmAb in milk in a large population of lactating women with IBD
Population-level datasets of drug levels in milk are almost impossible to obtain experimentally at a large scale in a population. Our model simulation will provide an estimate of the population distribution of TNFmAb in milk of women with IBD, and infant exposure levels. This will provide a foundation of a risk assessment for women with IBD receiving TNFmAb. Moreover, coupled with milk TNFα disposition data, we will be able to speculate potential clinical implications of concentration data of TNFmAb in milk.

PITFALLS AND CONTINGENCY

- Subject enrollment may be slower than we expect. If so, we will expand subject catchment base if enrollment is <20 in each group during the first 12 months.
- We are aware that TNFmAb binding to TNFα may interfere with the TNFα assay, underestimating TNFα levels in milk. To circumvent this, as described above, we will pretreat the samples to dissociate antigen (TNFα)-antibody (TNFmAb) binding, and quantify TNFα in an unbound form.
- If there are unforeseen difficulties in TNFmAb detection, we will have an option to test other sets of antibodies, which are available as well.

KNOWLEDGE TRANSLATION PLAN

The results will be incorporated into a research paper(s) for submission. Also, we will share the data with nutritional scientists in the field of human milk nutrition as knowledge users, and further present the updated views on the implications of the data at IBD-focused scientific meetings.
popPK model-based predictions will be summarized as population distribution of milk levels, and a full account of the study will be submitted to a peer-reviewed journal. The data will be also shared with IBD experts. In addition, the data will be forwarded to the LactMed center[19] for review and consideration for posting. LactMed is a freely accessible web-based database on drug excretion into human milk, which is considered the most updated and reliable information source in the field of drug safety during breastfeeding.[56]

SIGNIFICANCE AND FUTURE POTENTIAL

Our focus on postpartum women with IBD is logical and important as this population of mother and infant is often excluded from systematic research effort for better clinical care.
Circumventing difficulties of conducting conventional PK studies in breastfeeding women, our popPK approach with refined measurement of TNFmAb will produce the first comprehensive picture on TNFmAb disposition in human milk, informing the risk assessment for women with IBD receiving the drug. Moreover, results generated in this project will lay a solid foundation for future research of the impact of TNFα-dependent chemokines in milk on infant developmental programs, particularly those born to the mother with IBD receiving TNFmAb.

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Research Consent Form

Healthy Breastfeeding Mothers

Study Title

Assessing drug exposure risk of infants breastfed by women with inflammatory bowel disease

Stud Investigators

Principal Investigator

Dr. Shinya Ito Division of Clinical Pharmacology & Toxicology, SickKids

[redacted]

Co-Investigators

[redacted]

Collaborator

[redacted]

Team members

[redacted]

Study Coordinator/Research Contact

[redacted]

Study funder

This study is funded by the Crohn's and Colitis Foundation of America.

Conflict of Interest

The PI and the other research team members have no conflict of interest to declare related to this study.

Introduction

We would like to invite you to take part in our research study. This consent describes the research study and what it means to participate. Before deciding to take part, please take as much time as you need to ask any questions you have. You are encouraged to discuss with family, friends, your personal physician or other health professionals. Participation in any research study is voluntary (You do not have to participate if you don't want to). If you decide to participate now, you can change your mind later.

Why is this study being done?

The health benefits of breastfeeding to both mother and infant are widely accepted. However, many breastfeeding women experience chronic conditions and need to take various medications during breastfeeding. Inflammatory Bowel Disease (IBD) is a chronic, complex digestive tract disorder that disrupts body's ability for normal break down of food, absorption of nutrients, and removing the waste products. IBD can happen at any age, but most commonly starts during the childbearing years (before the age of 35). Like many other chronic inflammatory conditions, IBD is associated with an abnormal function of immune system in body and increased levels of certain proteins (e.g. cytokines) that promote inflammatory reactions. Therefore, biological products that target and block these proteins are widely used for treatment of IBD. We are conducting this study to examine the levels of some cytokines and the biologics (drugs that are prescribed to block these cytokines), in breast milk of mothers with IBD. However, some of these cytokines can also be present in breast milk of healthy women in variable levels (depending on the infant age); and their presence may be related to development of infant's immune system and memory function. The purpose of this study is to create knowledge about the cytokine levels in breast milk, as well as cytokine-drug interaction in breast milk of mothers with IBD. We examine how the infants are exposed to these substances through breast milk, and explore the potential effects on infant's memory and learning function.

We are recruiting participants in two groups: 1) mothers with Inflammatory Bowel Disease, and 2) healthy pregnant or breastfeeding mothers as controls. We are going to analyze breast milk samples from both groups and a psychometrist will examine the infants' cognitive and language development at ages 12 and 18 months using a questionnaire (provided via phone or email/mail) and/or a standardized face-to-face test named the "Bayley Scales of Infant and Toddler Development".

Why am I being asked to participate?

You are being invited to participate in this research study because you are a healthy pregnant woman or a healthy breastfeeding mother and you child is less than 6 months old. If you agree to participate, you will be enrolled in the healthy control group.

How many participants will be in this study?

It is anticipated that about 160 participants will be recruited for this study (80 women with IBD and 80 healthy pregnant or breastfeeding mothers).

What are the study procedures?

The study procedure will include two home visits by the study personnel to collect milk samples and two optional hospital visits. During the visits the following will be done (Table 1):

- ***MANDATORY: Your role (home visits 1 & 2):***

  These visits will be scheduled to take place between 2-6 weeks AND 11 weeks-16 weeks after delivery. Because the composition of milk before and after feeding (fore-milk and hind-milk) can be different, at the time of scheduling the study visits you will be asked not to feed your baby from one breast for 2 hours before sample collection, in order to avoiding the potential variations in milk composition. A female study coordinator will visit you at your home during the morning hours to drop off sample

collection supplies at your doorstep. You will be asked to pump 5-10 ml of milk in a sterile container from the same breast, using a breast pump that will be provided in the sample collection supplies. You will also be asked to fill out a short questionnaire, which will take about 10 minutes.
If you are pregnant and consent to take part in this study, you will receive a letter in email or mail in about 2 weeks after your due date to determine if you are willing to move forward with participating in the study. You will have the option to continue or withdraw from the study after delivery.

- ***OPTIONAL: Your child's role (Study visits 3 & 4):***

Your infant's cognitive and language development will be evaluated twice, at the ages of 12 and 18 months old. You will receive a reminder call or email two weeks before each visit and will be invited to visit the Hospital for Sick Children (SickKids). During the visit, a psychometrist will conduct the assessment that takes about one hour. An evaluation report will be prepared and reviewed by a psychologist at the Hospital for Sick Children and mailed to you in few months after completion of infant assessments. If recommended by the psychologist, the principal investigator of the study (Dr. Shinya Ito) will refer your child to a pediatrician for a more comprehensive assessment and follow up.

- ***MANDATORY: Your role (response to questionnaire)***

We will also ask that you complete a questionnaire report about your child's communication and problem-solving abilities at 12- and 18-months of age. This will be done by phone or via email/mail.

Table 1- Study procedures

Who is involved?	Study Visits	When	Where	How long (approximately)	What happens?
Mother (MANDATORY)	Visit 1	Between 2-6 weeks after childbirth Morning hours	Home visit by the study coordinator	One hour	a) One milk sample will be collected (5-10ml)
	Visit 2	Between 11 weeks-16 weeks after childbirth Morning hours	Home visit by the study coordinator	One hour	b) You will be asked to fill out a questionnaire (about 10 min)
Infant (OPTIONAL)	Visit 3	12 month-old	SickKids	One hour	Your child's cognitive and language development will be assessed by a psychometrist, using a standardized test.
	Visit 4	18 month-old	SickKids	One hour	
	N/A	12 month-old	Home	10 minutes	You will be asked to fill out a questionnaire

Mother (MANDATORY)	N/A	18 month-old	Home	10 minutes	about your child's development either by phone or via email/mail.

What are the risks, harms or discomfort of the study?

We know of no harm that taking part in this study could cause you. There may be discomfort associated with milk collection using a pump. There is an inconvenience of time and the inconvenience of the study coordinator visiting your home. There is a total of about 4 hours required for this study. There will be an inconvenience of visits 3 and 4 where you will have to bring your child to SickKids.

Are there any benefits from being in the study?

You and your child will not directly benefit from participating in this study.
We hope that the results of this study will provide drug safety information for breastfeeding mothers with IBD and their infants. The findings of this research will also lay a foundation for future research about role of inflammatory proteins in development of brain in infants of mothers with IBD.

Can I choose to leave the study?

You can change your mind at any time during the research study. You do not need to give a reason to withdraw from the study. Withdrawal from the study will not have any effect on the care your or your family will receive at SickKids.

How the samples will be identified?

To protect your identity, we will de-identify the milk samples and label them with a unique study number.

Can I withdraw these samples?

If you no longer want your samples to be used in this research, you should tell the study coordinator, who will ensure the samples are withdrawn and destroyed. If the tests have already been done on your samples, it will not be possible to withdraw those results. However, no further testing will be done on your samples, and all of your samples will be destroyed.

How will your privacy be protected?

We respect your privacy. The funder of the study is also committed to respecting your privacy. No information about you and your child will be given to anyone or be published without your permission, unless the law requires us to do this.

The SickKids study staff (study investigators and coordinator) will collect personal health information about you and your child. This includes things learned from the study procedures described in the consent form. They will only collect the information they need for this study and access will take place under supervision of the study doctor. Any information about you/your child will be kept in a secure and confidential location

for 7 years and then destroyed according to SickKids policy.

All personal health information or personal information collected about you/your child will be "de-identified" by replacing your identifiable information (i.e. name) with a study number. The SickKids study staff are in control of the study code key, which is needed to connect your personal health information/ personal information to you. The link between the study number and your identity will be safeguarded by the SickKids study staff and will not be available to the funding agency. SickKids guideline include the following:

- The study will collect personal information that could identify you such as: name, address, and phone number in order to contact you for the study procedures. All information that identifies you, both paper copy and electronic information will be kept confidential and stored and locked in a secure place that only the study staff will be able to access.
- Electronic files will be stored securely on hospital or institutional networks or securely on any portable electronic devices.
- No information identifying you will be allowed off site in any from without your consent.

Representatives of the SickKids Research Ethics Board and/or Research Quality and Risk Management team may look at your personal health information to check that the information collected for the study is correct and to make sure the study followed the required laws and guidelines.

Will I be paid and/or reimbursed if I join this study?

We will provide you with a $20 Shopper's Drug Mart gift card upon completion of Study Visit 1 & 2. If you choose to participate in Study Visits 3 & 4, we will also provide you with a second $20 Shopper's Drug Mart gift card upon completion.

In addition, we will reimburse you for parking and transportation expenses to a maximum of $20 for each visit to SickKids (Study Visit 3 & 4).

What if I am injured during/in this study?

If you or your child suffer an injury from participation in this study, medical care will be provided to you in the same manner as you would ordinarily obtain any other medical treatment. In no way does signing this consent form waive you or your legal rights nor release the study doctor, sponsors or involved institutions from their legal and professional responsibilities.

How will I be informed about new information?

We may learn new information during the study that you may need to know. We can also learn about things that might make you want to stop participating in the study. If so, you will be notified about any new information in a timely manner, and we will inquire if you are still interested in being part of this study.

What are your rights when participating in a research study?

You have the right to receive all information that could help you make a decision about participating in this

study. You also have the right, throughout the study, to ask questions about this study and to have them answered to your satisfaction.
During this study we may create new tests, new medicines, or other things that may be worth some money. Although we may make money from these findings, we cannot give you any of this money now or in the future because you and/or your child took part in this study.

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If you have any questions about this study, please call Dr. Shinya Ito at [redacted]

The study protocol and consent form have been reviewed by the SickKids Research Ethics Board (REB). If you have any questions about your rights as a research participant, you may contact the Office of the Research Ethics Board at [redacted] during business hours.

Consent to Participate in a Research Study

Study Title: Assessing drug exposure risk of infants breastfed by women with inflammatory bowel disease

By signing this research consent form, I understand and confirm that:

1. The study has been explained to me and all of my questions have been answered.
2. I have the right not to take part in the study.
3. I can stop participating or withdraw from the study at any time without affecting the quality of care my child or my family receives at SickKids.
4. The possible harms and benefits (if any) of this study have been explained to me.
5. I have been told that my medical records will be kept private except as described to me.
6. I know that no identifying information about me will be given to anyone or be published without first asking permission, unless required by law.
7. I have been told that I have not waived my legal rights nor released the investigators, sponsors, or involved institutions from their legal and professional responsibilities.
8. I have been given sufficient time to read and think about the information in this consent form.
9. I know that I may ask now, or in the future, any questions I have about the study.
10. I have been told I will be given a signed and dated copy of this consent form.

I consent to participate in this study:

☐ I would like to participate in Study Visits 3 & 4, the Infant Assessment. I understand I will be contacted when my infant is 12months and 18months old to come into SickKids Hospital for the cognitive and language assessment and/or to complete a developmental questionnaire.

Printed Name of Participant	Participant's signature & date
Printed Name of person who explained consent & date	Signature of Person who explained consent

MOUNT SINAI HOSPITAL
Joseph and Wolf Lebovic Health Complex

CONSENT TO PARTICIPATE IN A RESEARCH STUDY

Group: Breastfeeding Mothers with IBD

Title Assessing drug exposure risk of infants breastfed by women with inflammatory bowel disease

Investigator [redacted]

Shinya Ito MD, FRCPC
Principal Investigator (Hospital for Sick Children)
[redacted]

Co-Investigators [redacted]

24 Hour Phone Number Not Applicable

Sponsor Crohn's and Colitis Foundation of America

Introduction

You are being asked to take part in a research study. Please read this explanation about the study and its risks and benefits before you decide if you would like to take part. You should take as much time as you need to make your decision. You should ask the study doctor or study staff to explain anything that you do not understand and make sure that all of your questions have been answered before signing this consent form. Before you make your decision, feel free to talk about this study with anyone you wish. Participation in this study is voluntary.

Background and Purpose

You are being invited to participate in this research study because you are a breastfeeding mother with Inflammatory Bowel Disease (IBD) and your child is less than 6 months old. The health benefits of breastfeeding to both mother and infant is widely accepted. However, many breastfeeding women experience chronic conditions and need to take various medications during breastfeeding. IBD is a chronic, complex digestive tract disorder that disrupts body's ability for normal break down of food, absorption of nutrients, and removing the waste products. Like many other chronic inflammatory conditions, IBD is associated with an abnormal function of immune system in body that causes increased levels of inflammatory mediators (chemicals that promote inflammatory reactions).

Therefore, biological products that target and block these chemicals are widely used for treatment of IBD. We are conducting this study to examine the levels of some specific cytokines (cell signalling molecules that aid cell to cell communication in immune responses and stimulate the movement of cells towards sites of inflammation, infection and trauma) and the biologics (drugs that are prescribed to block these cytokines), in breast milk of mothers with IBD. It is thoughtthat these cytokines and biologic products may alter the development of infant's immune system and memory function. As such, the purpose of this study is to create knowledge about the cytokine levels in breast milk, as well as cytokine-drug interaction in breast milk of mothers with IBD. We will also examine how the infants are exposed to these substances through breast milk, and explore the potential effects on infant's memory and learning function.We are recruiting participants in two groups: 1) mothers with Inflammatory Bowel Disease as intervention patients, and 2) healthy breastfeeding mothers as control patients. If you agree to participate, you will be enrolled in the "Intervention" group. We are going to analyse breast milk samples and a psychometrist will examine the infants' cognitive and language development at ages 12 and 18 months using an optional standardized test named the "Bayley Scales of Infant and Toddler Development".

Study Design

This is a multi-centre study being conducted across Mount Sinai Hospital (MSH) and the Hospital for Sick Children (SK). You will be approached to participate in the study, at any one of your regularly scheduled IBD related clinic visits to Mount Sinai Hospital. During this process, a SK study coordinator will explain the study to you, address any questions/concerns you may have, and gauge your willingness to participate. If you choose to participate in the study, you will be asked to sign an informed consent form. All patients who consent to participate in this study will be enrolled in a prospective observational study until their child is 18 months of age.

Study Visits and Procedures

1.0. Study Procedures: By enrolling in this study you agree to the following study procedures and associated study visits outlined below:

1.1. Enrollment Visit - During the enrollment visits, when you're consented into the study, you permit the MSH research coordinator to collect the following identifying information from you: Name; Phone Number; Mailing Address; E-mail Address. This information will be used to contact you for future follow-up study visits and procedures. Additionally, you permit the MSH research coordinator to review your medical record and collect information on your IBD diagnosis.

1.2. Milk Sampling Visits - By enrolling in this study you agree to two home visits, at 2-6 weeks AND 11 weeks-16 weeks postpartum, for the collection of milk samples. A (female) SK research coordinator will visit you at your home during the morning hours. You will be asked not to feed your baby from one breast for 2 hours before the visit and pump milk from the same breast at the visit. A manual breast pump and a sterile container for milk collection will be provided. The SK research coordinator will collect

5-10 ml of the pumped milk for analysis. You will also be asked to fill out a short questionnaire at these visits which will take less than 10 minutes.

1.3. Optional: Infant Cognitive Assessment Visits - By enrolling in this portion of the study you agree for your infant language development and memory function to be evaluated twice, at the ages of 12 and 18 months. You will receive a reminder call or email two weeks before each visit and will be invited to visit the Hospital for Sick Children. During the visit, a psychometrist will conduct the assessments, which are approximately 1 hour in duration. An evaluation report will be prepared and reviewed by a psychologist at the Hospital for Sick Children and mailed to you in a few months after completion of the infant assessments. If recommended by the psychologist, the principle investigator of the study (Dr. Shinya Ito) will refer your child to a pediatrician for a more comprehensive assessment and follow up.

1.4. Questionnaire over the phone or email/mail. **We will ask that you complete a brief questionnaire report about your child's communication and problem-solving abilities at both 12- and 18-months of age.**

2.0. Other Optional Study Procedures: If you receive treatment with a prescription biologic, either Infliximab or Adalimumab, you will be offered to participate in the optional Population PK Component of the study. Additionally, all breastfeeding mothers with IBD, may opt for Infant Blood Sampling at SK. These additional procedures will be used to further tease out variation in cytokines levels in milk between healthy mothers and mothers with IBD; in addition to measuring how prescription biologics impact these cytokine levels. These additional procedures and any associated visits are outlined below:

2.1. Infant Blood Sampling - If you agree to the optional infant blood sampling, you will be contacted by the SK research coordinator prior to your first milk sampling visit. You will be asked to travel to the hospital for Sick Children at the time of your first milk sample visit (2 to 6 weeks post-partum); where the first milk sample and the infant blood sampling will occur concurrently. A 5ml (i.e. teaspoonful) infant blood sample will be obtained by the phlebotomy unit.

2.2. Population PK Component - If you are receiving treatment with either Infliximab or adalimumab, and agree to participate in the population PK component of this study, then the SK research coordinator will send you a study package by mail or courier service. The package will include: a thermal box, milk containers, ice packs, detailed instructions for sample collection and a form to record the specifics of milk samples. Participants will be asked to collect a minimum 5 milk samples between two doses of your prescription biologic (one sample pre-dose; three or more samples during post-dose day 1 and 7; and two or more samples post-dose day 8 and the second dose). These samples can be collected at your convenience, between 2 weeks to 6 months post-partum. After these samples have been collected, the SK research coordinator will arrange for pickup of these samples.

Calendar of Visits

Table 1. Study Procedures

	Enrollment Visit	Visit 1 (2- 6 Weeks Post-Partum)	Visit 2 (11- 16 Weeks Post-Partum)	Visit 3 (Infant Age-12 Months)	Visit 4 (Infant Age-18 Months)	ASQ-3 Questionnaire at 12 and 18 months
Informed Consent	X					
Identifying Information Collection	X					
Demographic Disease Data Collection	X					
Milk Sampling		X	X			
Questionnaire		X	X			
Infant Blood Sampling		X^{o} (optional)				
Infant Language Development and Cognitive Assessment				X (optional)	X (optional)	X

- Boxes marked 'X' Designates Study Procedures During Visits
- Boxes marked 'X^{o}' Designate Optional Study Procedures Occurring Concurrently with Study Visit

Table 2. Logistics of Study Visits

	Enrollment Visit	Visit 1 (2- 6 Weeks Post-Partum)	Visit 2 (11 - 16 Weeks Post-Partum)	Visit 3 (Infant Age-12 Months)	Visit 4 (Infant Age-18 Months)	ASQ-3 Questionnaire at 12 and 18 months
Time	30 Min	1 hour	1 hour	1 hour	1 hour	10 minutes
Location	Mount Sinai Hospital	Home Visit or Sick Kids Hospital	Home Visit	Sick Kids Hospital	Sick Kids Hospital	Sick Kids Hospital or Home
Study Subject Involved	Mother	Mother	Mother	Infant	Infant	Infant

Research Coordinator Involved	MSH and SK Coordinator	SK Coordinator	SK Coordinator	SK Coordinator	SK Coordinator	SK Coordinator

Reminders

- Tell study staff anything about your health that has changed.
- Tell your study team if you change your mind about being in this study.

Risks Related to Being in the Study

There are no medical risks if you take part in this study, but being in this study may make you feel uncomfortable. You may refuse to answer questions or stop the interview at any time if there is any discomfort.

Benefits to Being in the Study

You may or may not/will not receive any direct benefit from being in this study. Information learned from this study may help other people with your condition in the future.

Voluntary Participation

Your participation in this study is voluntary. You may decide not to be in this study, or to be in the study now and then change your mind later. You may leave the study at any time without affecting your care.

We will give you new information that is learned during the study that might affect your decision to stay in the study.

Alternatives to Being in the Study

You do not have to join this study to receive treatment for your condition.

Confidentiality

<u>Personal Health Information</u>

If you agree to join this study, the study doctor and his/her study team will look at your personal health information and collect only the information they need for the study. Personal health information is any information that could be used to identify you and includes your:

- name,
- address,
- date of birth,
- new or existing medical records, that includes types, dates and results of medical tests or procedures.

The information that is collected for the study will be kept in a locked and secure area by the study doctor for 10years. Only the study team or the people or groups listed

below will be allowed to look at your records. Your participation in this study also may be recorded in your medical record at this hospital.

The following people may come to the hospital to look at the study records and at your personal health information to check that the information collected for the study is correct and to make sure the study followed proper laws and guidelines:

- The study sponsor or its representatives/partner companies.
- Representatives of the Mount Sinai Hospital Research Ethics Board.
- Representatives of the Sick Kids Hospital Research Ethics Board.

Study Information that Does Not Identify You

Some study information will be sent outside of the hospital to Sick Kids Hospital.. Any information about you that is sent out of the hospital will have a code and will not show your name or address, or any information that directly identifies you.

All information collected during this study, including your personal health information, will be kept confidential and will not be shared with anyone outside the study unless required by law.

You will not be named in any reports, publications, or presentations that may come from this study.

If you decide to leave the study, the information about you that was collected before you left the study will still be used. No new information will be collected without your permission.

In Case You Are Harmed in the Study

If you become ill, injured or harmed as a result of taking part in this study, you will receive care. The reasonable costs of such care will be covered for any injury, illness or harm that is directly a result of being in this study. In no way does signing this consent form waive your legal rights nor does it relieve the investigators, sponsors or involved institutions from their legal and professional responsibilities. You do not give up any of your legal rights by signing this consent form.

Expenses Associated with Participating in the Study

You will not have to pay for any of the procedures involved with this study. We will provide you with a $20 Shopper's Drug Mart gift card upon completion of Study Visits 1&2. If you choose to participate in Study Visits 3&4, we will also provide you with a second $20 Shopper's Drug Mart gift card upon completion. In addition, you will be reimbursed up to $20 for transportation and parking expenses associated with study visits that require transport to the Hospital for Sick Children..

Conflict of Interest

The investigators do not have any conflicts of interest to declare.

Questions About the Study

If you have any questions, concerns or would like to speak to the study team for any reason, please call: Dr. [redacted], Dr. Shinya Ito at [redacted], or [redacted]

If you have any questions about your rights as a research participant or have concerns about this study, call the Chair of the Mount Sinai Hospital Research Ethics Board (REB) or the Research Ethics office number at [redacted]. The REB is a group of people who oversee the ethical conduct of research studies. These people are not part of the study team. Everything that you discuss will be kept confidential.

Consent

This study has been explained to me and any questions I had have been answered. I know that I may leave the study at any time. I agree to take part in this study and to the use of my personal health information as described above.

☐ I would like to participate in Study Visits 3 & 4, the Infant Assessment portion. I understand I will be contacted when my infant is 12months and 18months old to come into SickKids Hospital for the cognitive and language assessment.

Print Study Participant's Name	Signature	Date

(You will be given a signed copy of this consent form)

My signature means that I have explained the study to the participant named above. I have answered all questions.

Print Name of Person Obtaining Consent	Signature	Date

I also agree to participate in the following, optional, components of the study:

☐ Infant Blood Sampling - To occur as the same time as the first milk sample visit (2-6weeks post-partum).

☐ Population PK - Collection of additional 5 milk samples (2 weeks to 6 months postpartum) for patients on either Infliximab or Adalimumab.