



Detection of infliximab in breast milk of nursing mothers with inflammatory bowel disease

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Abstract

Introduction: Limited data suggest the absence of infliximab in breast milk, thereby implying the safety of this drug during breast-feeding. We aimed to re-evaluate the presence of infliximab in breast milk of nursing IBD patients.

Methods: Serum and breast milk were obtained post-partum from 3 breast-feeding patients with Crohn's disease before and after re-initiation of infliximab. ELISA assay was employed to measure infliximab level in maternal serum and in breast milk. The level of infliximab was also measured in breast milk of a control group of 8 nursing healthy mothers.

Results: Infliximab was undetectable in breast milk prior to the first infusion and was also not measurable in 8 lactating women not exposed to infliximab. Infliximab levels in breast milk rose up to 101 ng/ml within 2–3 days of the infusion. These levels of infliximab in breast milk were roughly 1/200th of the level in blood.

Conclusions: In contrast with prior reports, infliximab can be detected in the breast milk of nursing mothers. The miniscule amounts of infliximab transferred in breast milk are unlikely to result in systemic immune-suppression of the infant. Nonetheless, local effects of this exposure on the neonates' intestine and potential immune sensitization or tolerization towards the drug can not be excluded and merit further investigations.

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1. Introduction

The TNF antibodies – infliximab, adalimumab and certolizumab – are efficacious agents in the treatment of inflammatory bowel disease (IBD) and other disorders. A sizable portion of female patients with IBD are at child-bearing ages,

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thereby often raising questions about the safety of these drugs during pregnancy and breast feeding. The experience with the use of anti-TNF during pregnancy is rapidly increasing, and they are generally considered safe especially if withheld before the onset of the third trimester.^{1–4} However, the data regarding their use during breast feeding is much more limited. In fact, there are very few data investigating the transfer of anti-TNF agents in the breast milk. In the only 5 patients so far described, infliximab was not detected in the breast milk of nursing mothers,^{5–7} prompting several guidelines to suggest that breast-feeding is safe during lactation.^{8,9} In contrast, we have previously found that adalimumab was detectable in breast milk of a Crohn's disease patient, albeit at low concentration.¹⁰ Whether this discrepancy stems from a structural difference between adalimumab and infliximab, or does it result from the different techniques employed remains unknown.

Therefore, the aim of present study was to investigate the presence of infliximab in breast milk of nursing mothers with IBD.

2. Methods

2.1. Study population and procedures

Three female IBD patients who conceived while being treated by infliximab were enrolled. Following delivery, and before resumption of infliximab, a 5cc sample of breast milk was obtained along with serum sample for determination of infliximab. Thereafter, breast milk samples were obtained for up to 8 days after the first re-infusion of infliximab at 5 mg/kg. Because all three patients elected to stop breast feeding at initiation of infliximab, the sample collection was discontinued when milk could no more be expressed from the breast. Blood and breast milk samples were also obtained from 8 controls consisting of healthy breast-feeding mothers who were never exposed to infliximab. The study was approved by the Sheba Medical Center's ethics committee and all of the participants provided a written informed consent.

2.2. Determination of infliximab levels

Infliximab levels in serum were measured by a specifically developed ELISA assay, as previously described.¹¹ Briefly, 100 μ l of serum was added to pre-plated 500 ng/ml TNF (Peprotech Inc, New Jersey, USA) and incubated for 90 min. Following washing, HRP-goat anti-human Fc fragment antibody (MP Biomedicals, Solon, OH, USA) at a concentration of 600 ng/ml was added for 60 min. The results were then read on an ELISA reader. Quantization of the measured infliximab concentration was done by calibration to standard column in which exogenous infliximab (Schering-Plough, USA) was added at concentrations between 5 and 300 ng/ml. Infliximab levels in breast milk were similarly determined. However, to validate the measurements in this medium, a spiking experiment was done whereby the assay was employed on breast milk samples obtained before the initiation of infliximab, to which graded known concentrations of the drug were exogenously added.

3. Results

The demographics and clinical characteristics of the three patients are depicted in Table 1. Notably, 2 patients stopped their infliximab infusions before the third trimester and one patient elected to withhold infliximab upon conception. None of the patients had a flare of disease during pregnancy, but all flared after delivery (1–24 weeks post-delivery). After flaring, all three patients resumed infliximab with hydrocortisone premedication and all three preferred to stop breast feeding upon re-initiation of infliximab. No infusion reactions were apparent during re-induction. Importantly, infliximab was not resumed in any of the patients during the first 5 days after delivery, i.e. at the time of colostrum milk.

Prior to infliximab infusion, the levels of the drug were nil in both the maternal serum and in the breast milk samples (Fig. 1). However, infliximab levels rapidly rose in the breast milk and were detectable already 12 h after the infusion. Drug level in the breast milk peaked at 90–105 ng/ml on day 2–3 and seemed to plateau thereafter, although repeated measurements to determine the curve characteristics were only available from 2 patients and the plateau time-points were only reached in one patient (Fig. 1). Blood level of infliximab at the corresponding times ranged between 18 and 64 μ g/ml. Spiking experiments of adding graded exogenous infliximab at concentrations of 0–100 ng/ml to the patients' pre-infusion breast milk samples proved the assays' ability to detect concentrations as low as 10 ng/ml of infliximab in the breast milk attesting to the accuracy of the measurements (Fig. 2).

4. Discussion

Infliximab is a chimeric monoclonal antibody which, similar to all IgG antibodies, can be actively transferred by the placenta to the fetus. The rate of this active transfer exponentially increases at the late stage of the second trimester of pregnancy.² It was shown that once placental transfer occurs, the levels of infliximab in the newborn's blood are equivalent to the mother's level, but remain in circulation for up to 6 months, presumably due to slower immunoglobulin clearance mechanisms in the neonatal period.⁶ Moreover, a single case report described fatal BCGitis from live attenuated vaccine of a 3 month infant born to a mother treated with infliximab,¹² demonstrating the potential grave consequences of trans-placental transfer of infliximab. Hence, although labeled as FDA class B and generally considered safe during pregnancy in terms of mutagenesis, many authorities advocate the cessation of infliximab before the third trimester of pregnancy to reduce this potential for immune suppression of the newborn from circulating drug.^{3,4,8,9,13,14}

In contrast with the well established trans-placental transfer of anti-TNF agents, there are scarce data on their excretion in the breast milk and their safety during breast-feeding. In the only three reports hitherto published (encompassing 5 Infliximab-treated patients), no infliximab was detected in the breast milk and no adverse effects for the infants were reported.^{5–7} Human breast milk immunoglobulins are predominantly of the secretory IgA class, whereas IgG antibodies are believed to be absent beyond the first few days

Table 1 Clinical characteristics of the study patients. CD - Crohn's disease. IFX - infliximab.

Age	Year of CD diagnosis, location & phenotype	Type of IFX treatment	Duration of IFX treatment	IFX during pregnancy	Reason for re-initiation	Time of IFX level in milk measurement
34	1994, Ileum & perianal, Penetrating	Episodic (previously 2002)	8 months	No	Disease flare	4 months post partum
33	2001, colonic, obstructive	Scheduled	42 months	Until week 30	Disease flare	One week post partum
34	2007, Ileitis, inflammatory	Scheduled	12 months	Until week 28	Disease flare	6 months post partum

after delivery (the period of colostrum milk production).² Notably, there are also conflicting reports indicating the persistence of IgG antibodies in breast milk. For instance, anti-HIV-1 IgGs were detectable in breast milk for up to 18 months post-delivery in non-infected newborns, and exogenous IgG from administered IVIG preparation was similarly found in breast milk.¹⁵⁻¹⁷ As mentioned above, and in contrast with our finding, prior reports failed to recover the infliximab moiety from breast milk of treated patients.⁵⁻⁷ However, these studies all employed a commercial kit standardized according to levels in the blood. In contrast, we have used control breast milk samples for calibration of the standard curve and found that the detection level of infliximab in breast milk is significantly lower than the threshold signal in serum, thereby allowing detection of miniscule amounts in the tested milks. This method has previously enabled us to detect adalimumab in breast milk at comparable levels to those currently found for infliximab.¹⁰ Moreover, prior studies validated their negative findings by spiking experiments with exogenous drug, but have added the exogenous infliximab at concentrations comparable to 1:8 diluted sera or graded down to 1 µg/ml.^{6,7} Both these threshold concentrations are still at least 10 folds higher than those actually found in breast milk, and cannot therefore corroborate or refute the assay's accuracy for infliximab non-detection in breast milk. Indeed, when adding exogenous infliximab in concentrations comparable to those found in breast milk, we could discern an optical density (OD) reading that was clearly above the background level obtained in the control breast milk samples. These findings further corroborated that the infliximab measured in the breast milk of our patients was truly present and was not a false reading stemming from assay background noise.

What are the implications of these findings? Until the present report, most authorities contended breast-feeding to be safe during infliximab treatment mostly relying on prior drug detection studies. Thus, in the recent ECCO consensus on the management of Crohn's disease during pregnancy and nursing, it was stated that: "Infliximab cannot be detected in breast milk, so it can be considered acceptable (during breast feeding)".⁸ Similarly, the recent joint London position paper by the WCOG and ECCO endorses breast feeding during infliximab treatment, again based on its purported absence in breast milk.⁹

The present study is the first to report that infliximab, similar to adalimumab, is excreted in the breast milk. It is important to note that infliximab's level in the breast milk was low in the range of 1/200th of its level in serum. Because this miniscule quantity is also anticipated to undergo proteolysis in the stomach and intestine after ingestion, it is probably of negligible impact on the systemic immune system of the suckling infant. However, further data is pertinent to determine if this low level could still have local effects on the intestinal mucosa, especially for coping with enteric infections or in the context of immunization with orally administered live attenuated organisms such as the Rota virus vaccine. Moreover, it remains to be shown if this neonatal exposure to the drug can sensitize or tolerize the infant towards the exogenous antibody. The risk of the offspring of a Crohn's patient to develop IBD can be as high as 9%,¹⁸ and it is currently estimated that 20-30% of CD patients are or have been treated by anti-TNFs.⁸ Thus, 1 out of 37-55

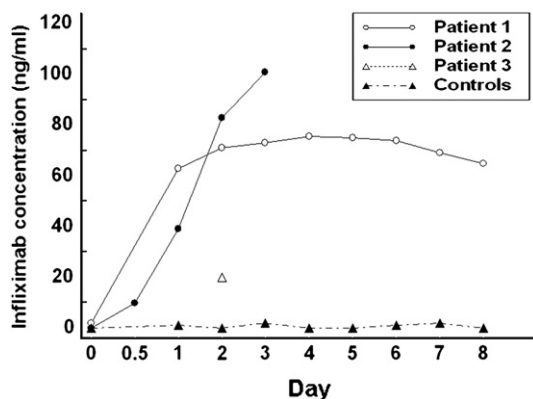


Figure 1 The levels of infliximab in the breast milk of the study patients and in 8 healthy donors. Measurements in the control healthy donors were performed at different time-points, but are depicted along the x-axis for graphical clarity purpose.

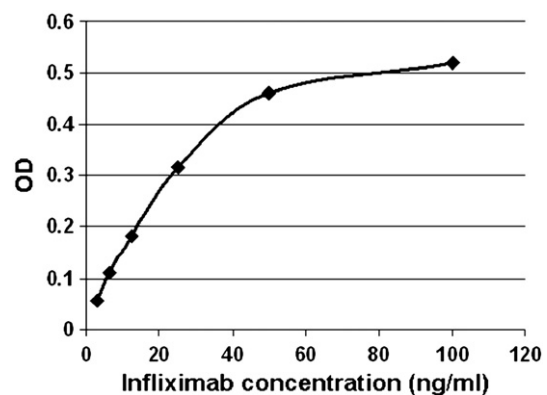


Figure 2 Spiking experiment with exogenous infliximab. OD – optical density.

suckling infants of infliximab treated mothers may require infliximab for IBD at some point throughout their life, making the question of breast-feeding mediated sensitization (or tolerization) to the drug a pertinent one. While these considerations need be borne in mind, they should also be weighed against the proven benefits of breast feeding for the newborn when discussing with patients the pros and cons of nursing with on-going infliximab therapy.

Limitations of our study should also be acknowledged. Serum samples were not collected from the infants, as in all cases the mothers stopped breast feeding after the infusion, rendering such samples non-informative. Another limitation stems from the relatively small group of nursing mothers studied. However, this group of 3 patients still equals the largest series reported so far in the literature and is comparable in size to the total number of studied patients on whom current recommendations for continued breast feeding are based.

In conclusion, the present work shows that infliximab is excreted in breast milk, albeit at miniscule amounts. Further studies are needed to elucidate the impact of such exposure, if any, on the suckling infant and to verify the safety of infliximab during breast-feeding.

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Writing assistance

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