Analyzing cord blood levels of TNF inhibitors to validate the EULAR points to consider for TNF inhibitor use during pregnancy

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ABSTRACT
Background To minimise placental transfer of tumour necrosis factor inhibitors (TNFi), the European League Against Rheumatism (EULAR) created points to consider (PtC) for the use of TNFi during pregnancy. We are the first to validate the EULAR-PtC by analysing TNFi concentrations in cord blood.

Methods Patients were derived from the Preconceptional Counselling in Active Rheumatoid Arthritis Study. TNFi was stopped at the time points recommended by the EULAR. Maternal blood and cord blood were collected and analysed for the concentration of TNFi.

Results 111 patients were eligible for the analysis. Median stop time points were gestational age (GA) 37.0 weeks for certolizumab pegol, GA 25.0 weeks for etanercept, GA 19.0 weeks for adalimumab and GA 18.4 weeks for infliximab. Certolizumab pegol (n=68) was detectable in 5.9% of cord blood samples, with a median concentration of 0.3 µg/mL (IQR: 0.2–1.3) and a median cord/maternal concentration ratio of 0.010. Etanercept (n=30) was not detected in any cord blood samples. Adalimumab (n=25) was detectable in 48.0% of cord blood samples, with a median concentration of 0.5 µg/mL (IQR: 0.2–0.7) and a median concentration ratio of 0.062 (IQR: 0.018–0.15). Infliximab (n=14) was detectable in 57.1% of cord blood samples, with a median concentration of 0.4 µg/mL (IQR: 0.1–1.2) and a median concentration ratio of 0.012 (IQR: 0.006–0.081).

Conclusion Compliance with the EULAR-PtC results in absence or low levels of TNFi in cord blood.

INTRODUCTION
Tumour necrosis factor inhibitors (TNFi) have become an important component of the treatment of rheumatic diseases during pregnancy.1 A drawback of prescribing TNFi during pregnancy is active transport of these drugs across the placenta mediated by neonatal Fc receptors (FcRn).2 Placental transfer starts around gestational week 20, and the rate of transfer increases throughout pregnancy.2 The extent of placental transfer depends on the molecular structure of the drug. Adalimumab and infliximab are whole anti-TNF antibodies and have a strong affinity for the FcRn.3 Etanercept is a fusion protein that comprises a TNF receptor and the Fc domain of human IgG1. Its affinity for the FcRn is lower than that of adalimumab and infliximab.4 Certolizumab pegol is a PE-Glyated Fab fragment of an anti-TNF monoclonal antibody. Because certolizumab pegol lacks the Fc domain, it is not actively transported across the placenta.5

The European League Against Rheumatism (EULAR) created points to consider (PtC) for the use of TNFi during pregnancy.6 These PtC recommend discontinuation of treatment at gestational age (GA) 20 weeks for adalimumab and infliximab, GA 30–32 weeks for etanercept and conditional continuation of certolizumab pegol.7 The EULAR-PtC are based on limited evidence; only for certolizumab pegol, it has been demonstrated that cord blood concentrations are minimal when treatment is continued throughout pregnancy.

What does this study add?
This study demonstrates that stopping TNF inhibitor treatment according to the EULAR-PtC results in undetectable or low levels of TNF inhibitor in cord blood.

What is already known about this subject?
✓ Tumour necrosis factor (TNF) inhibitors can be actively transported across the placenta as early as week 20 of gestation, mediated by fetal Fc receptors and dependent on TNF inhibitor structure.
✓ European Alliance of Associations for Rheumatology (EULAR) points to consider (PtC) recommend to stop adalimumab and infliximab at gestational age (GA) 20 weeks, etanercept at GA 30–32 weeks and conditional continuation of certolizumab pegol.

How might this impact on clinical practice or future developments?
✓ Compliance with the EULAR-PtC results in absence or low concentration of TNF inhibitors in cord blood, indicating that the children are most likely not immunologically compromised.
METHODS

Patients
Patients were derived from the Preconceptional Counselling in Active Rheumatoid Arthritis (PreCARA) cohort at Erasmus Medical Center in Rotterdam, the Netherlands (ClinicalTrials.gov registration: NCT01345071). The PreCARA cohort is an ongoing, prospective cohort study on inflammatory rheumatic diseases and pregnancy. Patients whose cord blood was collected at birth were used for the current analysis.

PreCARA treatment protocol
Patients in the PreCARA cohort were treated according to a modified treat-to-target approach. Details on the PreCARA treatment protocol have been previously described. Patients were allowed to get pregnant on the TNFi used at enrolment. TNFi were discontinued at the GAs advised by the EULAR, and a switch to certolizumab pegol and/or prednisone was considered. Certolizumab pegol was discontinued at GA 38 weeks to prevent maternal infections during delivery, based on expert opinion.

Data collection
Information on diagnosis and previous medication use was collected at the first visit. Maternal blood was collected in each trimester, at moments unrelated to the administration of TNFi. At birth, cord blood was collected by the patient’s midwife or gynaecologist. Blood samples were clustered and subsequently sent to Sanquin Laboratory (Amsterdam) for analysis (online supplemental appendix).

Statistical analysis
Descriptive statistics on clinical characteristics and TNFi use are presented as mean (SD), median (IQR) or number (%). Differences in GA at stopping TNFi treatment between patients with and without measurable TNFi levels in cord blood were assessed with the two-sample Wilcoxon rank-sum test. P values <0.05 were considered significant. Stata software V.16.0 was used for all statistical analyses.

RESULTS
Data from 111 patients were used for the analysis (table 1). During some pregnancies, the use of etanercept, adalimumab or infliximab was switched to certolizumab pegol. Therefore, in the cord blood of those pregnancies, the concentration of two TNFi was to be determined, resulting in a total of 137 cord blood measurements. Most patients stopped treatment before the recommended GA (table 2). Etanercept (n=30) was stopped before GA 30 weeks by 29 (96.7%) patients, adalimumab (n=25) was stopped before GA 20 weeks by 20 (80.0%) patients and infliximab (n=14) was stopped before GA 20 weeks by 10 (71.4%) patients. For certolizumab pegol, the median GA at stopping treatment was GA 37.0 weeks (IQR: 34.1–38.1 weeks), and the median time between last dose and delivery was 15 days (IQR: 2–34 days).

Certolizumab pegol (n=68) was detected in 5.9% of cord blood samples; the median level of certolizumab pegol was 0.3 µg/mL (IQR: 0.2–1.3). The maximum concentration (2.3 µg/mL) was measured in a patient that stopped treatment at 26 days before delivery and received 200 mg every other week. The concentration ratio of cord blood to maternal blood for certolizumab pegol was 0.010 (IQR: 0.007–0.066). Etanercept was not detected in any of the cord blood samples, including the sample of one patient who stopped after GA 30 weeks (GA 36.7 weeks).

Adalimumab and infliximab were detected in 12 (48.0%) and 8 (37.1%) cord blood samples, respectively. The median cord blood concentrations were 0.5 µg/mL (IQR: 0.2–0.7) for adalimumab and 0.4 µg/mL (IQR: 0.1–1.2) for infliximab. The median concentration ratios of cord blood to maternal blood were 0.062 (IQR: 0.018–0.15) for adalimumab and 0.012 (IQR: 0.006–0.081) for infliximab. The maximum concentration for adalimumab (2.1 µg/mL) was measured in a patient who stopped treatment at GA 19.4 weeks and received 40 mg every other week. For infliximab, the maximum concentration (4.5 µg/mL) was measured in a patient who stopped treatment at GA 21.1 weeks and received 400 mg every 5 weeks (online supplemental appendix).

Differences in GA at stopping adalimumab and infliximab between patients with and without detectable TNFi in the cord blood are shown in table 3.

DISCUSSION
In the current study, we show that stopping TNFi around the GA recommended by the EULAR-PtC results in no detectable or low levels of TNFi in the cord blood.

Most patients in our study used certolizumab pegol during pregnancy. We observed certolizumab pegol in 5.9% of the cord blood samples. In comparison, a study by Mariette et al observed certolizumab pegol in 20% of the umbilical cord samples. The lower limit of quantification was higher in our study (0.1 µg/mL vs 0.032 µg/mL), which might explain the observed difference. Furthermore, there was one patient with a certolizumab pegol concentration of 2.3 µg/mL in our study; this was an outlier. In this particular case, placental blood sample contamination with mother’s blood cannot be excluded.

The use of etanercept during pregnancy has not been investigated on a large scale before. Etanercept has a low affinity for the FcRn. Our study shows that stopping treatment with etanercept before GA 30 weeks results in absence of etanercept in the cord blood. Interestingly, the patient that stopped after the recommended GA (at GA...
continued treatment beyond GA 30 weeks, considerably higher \( \mu g/mL \) for adalimumab and 10.0 \( \mu g/mL \) for infliximab in patients who used etanercept until 4 days before delivery. This might be explained by the shorter half-life of etanercept (circa 3 days) compared with other TNFi (8–10 days for infliximab and 14 days for certolizumab pegol and adalimumab). Both these observations might indicate that etanercept could be used beyond GA 30–32 weeks if necessary.

We detected adalimumab and infliximab in about half of the patients’ cord blood samples, however in low concentrations. A study by Julsgaard et al reported median concentrations of 2.5 \( \mu g/mL \) for adalimumab and 10.0 \( \mu g/mL \) for infliximab in patients who continued treatment beyond GA 30 weeks, considerably higher than the respective 0.5 \( \mu g/mL \) and 0.4 \( \mu g/mL \) in patients from our study, who stopped around GA 20 weeks. These discrepancies might be the result of different indication groups included in the study by Julsgaard et al, which were mainly patients with inflammatory bowel diseases and have continued infliximab and adalimumab until a higher GA period during pregnancy.

The effects of low TNFi concentrations in the fetal circulation are unknown. Previous research shows that a TNFi concentration as low as 0.1 \( \mu g/mL \) is sufficient to bind all circulating TNF. Therefore, clinical relevance cannot be excluded. Nevertheless, the concentrations are only a few percent of those found in the mothers during active use. Intrauterine exposure to TNFi can have major consequences in infants exposed to high levels of TNFi have been pathogenic in infants with a suppressed immune system. In one case, a Bacillus Calmette-Guérin (BCG) vaccination after intrauterine exposure to infliximab resulted in neonatal death after a disseminated BCG infection. It can be concluded from the results of our study that, if PtC recommendations are followed, intrauterine exposure to certolizumab pegol or etanercept will not result in placental transfer and future recommendations for attenuated live vaccination could be less restrictive. If for adalimumab and infliximab minimal or absence of TNFi concentrations in cord blood are aimed, these should be withdrawn even earlier than week 20 of gestation (eg, week 15 of gestation) (online supplemental appendix). A possible consequence of TNFi in the infant’s circulation is an increased risk for infections during the first months of life. However, literature reports both increased and non-increased risk for infections and therefore remains inconclusive.

Our study has several strengths. It is the first large study to evaluate the EULAR-PtC for the use of TNFi during pregnancy. All 111 patients included in the current analysis were treated at the same hospital, so differences between physicians were minimal. Patient data were retrieved directly from the patient; therefore, the risk for biases, like misclassification bias, was minimal.

A limitation of our study is that we did not measure trough and peak values of maternal TNFi concentrations. The concentration ratios we calculated are therefore less accurate than those calculated in a pharmacokinetic study. Another limitation is that the majority of patients using etanercept stopped or switched their TNFi quite earlier than the recommended stop time point of GA 32 weeks.

In conclusion, compliance with the EULAR-PtC results in undetectable levels or absence of TNFi in cord blood in most patients that use certolizumab pegol or etanercept. For adalimumab and infliximab, TNFi was detectable in cord blood in about half of the patients. The detected concentrations of TNFi in cord blood were far lower than the maternal levels during active use. The potential harmful effects of these low concentrations of TNFi in cord blood are unknown and require further investigation. If these concentrations of TNFi were

<table>
<thead>
<tr>
<th>Table 2</th>
<th>TNF inhibitor (TNFi) use during pregnancy and TNFi concentrations in maternal blood and cord blood. Values are expressed as median (IQR) unless indicated otherwise</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Certolizumab pegol (n=68)</td>
</tr>
<tr>
<td>Stop time point as recommended by EULAR-PtC, weeks</td>
<td>N/A</td>
</tr>
<tr>
<td>Gestational age (GA) at time of stopping TNFi, weeks</td>
<td>36.7 (34.8–38.6)</td>
</tr>
<tr>
<td>Maternal concentration of TNFi in the 1st trimester, ( \mu g/mL )</td>
<td>26.4 (19.0–31.0)</td>
</tr>
<tr>
<td>Maternal concentration of TNFi in the 2nd trimester, ( \mu g/mL )</td>
<td>22.5 (13.0–30.72)</td>
</tr>
<tr>
<td>Maternal concentration of TNFi in the 3rd trimester, ( \mu g/mL )</td>
<td>20.5 (13.0–29.6)</td>
</tr>
<tr>
<td>Concentration of TNFi in the cord blood if measurable, ( \mu g/mL )</td>
<td>0.3 (0.2–1.3)</td>
</tr>
<tr>
<td>Concentration ratio cord blood to maternal blood*</td>
<td>0.010 (0.007–0.066)</td>
</tr>
</tbody>
</table>

*Concentration ratios of cord blood to maternal blood were calculated with the maternal concentrations during active use of TNFi (trimester 3 for certolizumab pegol and trimester 1 for adalimumab and infliximab).

EULAR, European League Against Rheumatism; PtC, points to consider; TNF, tumour necrosis factor.

Table 3 | Stop time points of TNFi for patients with and without detectable TNFi in the cord blood |
<table>
<thead>
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<tbody>
<tr>
<td></td>
<td>Cetolizumab pegol (n=68)</td>
</tr>
<tr>
<td>Stop time point if TNFi was detectable, GA, weeks</td>
<td>36.9 (34.8–38.6)</td>
</tr>
<tr>
<td>Stop time point if TNFi was undetectable, GA, weeks</td>
<td>–</td>
</tr>
<tr>
<td>P value for difference</td>
<td>0.82</td>
</tr>
</tbody>
</table>

*Etanercept was not detectable in any of the cord blood samples.

GA, gestational age; TNF, tumour necrosis factor.
to be clinically relevant, stopping infliximab and adalimumab at an earlier GA than the EULAR-Pc recommend may be appropriate.

Contributors All authors met the authorship criteria; they had a substantial contribution to the concept or design of the work (HTWS and RiemDM) or the acquisition (RJEMD), analysis (NG, EK, HTWS, RiemDM, GW and TR) or interpretation of data for the work (all authors) and were involved in revising a draft of this work, gave final approval of this version to be published and are accountable for all aspects of the work in ensuring accuracy and integrity.

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Competing interests None declared.

Patient consent for publication Obtained.

Ethics approval Approval obtained (MEC-2011-032).

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REFERENCES

Supplement Appendix for:
Ghalandari N. et al., Analysing cord blood levels of TNF inhibitors to validate the EULAR points to consider for TNF inhibitor use during pregnancy

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- Assessment of TNFi levels
- Patient involvement and ethics statement
- Supplementary table 1: Dosage of TNFi during pregnancy
- Supplementary figure 1: Association of stop time points and detectability of TNFi

Supplementary text
Assessment of TNFi levels
Cord blood samples and maternal blood samples acquired during pregnancy were analysed with Enzyme-Linked Immunosorbent Assay (ELISA) by Sanquin Laboratory (Amsterdam)[13-16]. The lower limit of detection of the test was 0.1 µg/mL for certolizumab-pegol and etanercept, 0.01 µg/mL for adalimumab and 0.03 µg/mL for infliximab. TNFi-concentration ratios of cord blood to maternal blood were calculated with the cord blood concentration and the maternal concentration during active use of TNFi.

Patient involvement and ethics statement
Patients were involved in the design of the PreCARA-cohort. The burden on participating patients was carefully assessed. Study results will be shared with participating patients. Informed consent was obtained from all participating patients. Approval by the medical ethics committee (METC Erasmus MC) was obtained (MEC-2011-032).
Supplementary table 1: Dosage of TNFi during pregnancy and clinical diagnosis of those patients with detectable TNFi levels in cord blood

<table>
<thead>
<tr>
<th>TNF inhibitor and dosage*</th>
<th>Number (%)</th>
<th>Patients with detectable TNFi levels in cord blood</th>
<th>Clinical diagnosis in patients with detectable TNFi levels in cord blood (number)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Certolizumab-pegol</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>200 mg every 2 weeks</td>
<td>59 (86.8%)</td>
<td>4 (6.7%)</td>
<td>RA(2), PsA(1), SpA(1)</td>
</tr>
<tr>
<td>200 mg every 3 weeks</td>
<td>7 (10.3%)</td>
<td>0 (0.0%)</td>
<td></td>
</tr>
<tr>
<td>200 mg every 4 weeks</td>
<td>2 (2.9%)</td>
<td>0 (0.0%)</td>
<td></td>
</tr>
<tr>
<td>Etanercept</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>50 mg every week</td>
<td>27 (90.0%)</td>
<td>-</td>
<td></td>
</tr>
<tr>
<td>25 mg every week</td>
<td>1 (3.3%)</td>
<td>-</td>
<td></td>
</tr>
<tr>
<td>50 mg every 2 weeks</td>
<td>1 (3.3%)</td>
<td>-</td>
<td></td>
</tr>
<tr>
<td>50 mg every 3 weeks</td>
<td>1 (3.3%)</td>
<td>-</td>
<td></td>
</tr>
<tr>
<td>Adalimumab</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>40 mg every 2 weeks</td>
<td>23 (92.0%)</td>
<td>10 (43.5%)</td>
<td>RA(2), PsA (3), SpA(4), JIA(1)</td>
</tr>
<tr>
<td>40 mg every 3 weeks</td>
<td>2 (8.0%)</td>
<td>2 (100%)</td>
<td>PsA(1), SpA(1)</td>
</tr>
<tr>
<td>Infliximab</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>300 mg every 4 weeks</td>
<td>1 (7.1%)</td>
<td>1 (100%)</td>
<td>SpA (1)</td>
</tr>
<tr>
<td>500 mg every 6 weeks</td>
<td>1 (7.1%)</td>
<td>0 (0.0%)</td>
<td></td>
</tr>
<tr>
<td>400 mg every 5 weeks</td>
<td>1 (7.1%)</td>
<td>1 (100%)</td>
<td>PsA (1)</td>
</tr>
<tr>
<td>400 mg every 6 weeks</td>
<td>2 (14.3%)</td>
<td>2 (100%)</td>
<td>RA(2)</td>
</tr>
<tr>
<td>300 mg every 6 weeks</td>
<td>9 (64.3%)</td>
<td>4 (44.4%)</td>
<td>RA(3), SpA(1)</td>
</tr>
</tbody>
</table>

RA= rheumatoid arthritis; PsA= psoriatic arthritis; SpA= spondyloarthropathies; JIA= juvenile idiopathic arthritis. *

Last dosage before discontinuation or delivery of TNF inhibitor among 111 patients included in the current analysis.
A. Adalimumab detectible in cord blood per patient

B. Adalimumab cumulative detectability

C. Infliximab detectible in cord blood per patient

D. Infliximab cumulative detectability

**Supplementary figure 1**: association of stop time points and detectability of TNFi in cord blood. X-axis: gestational week of pregnancy when TNFi was stopped. Y-axis in A (adalimumab) and C (infliximab): detectible in cord blood per patient (positive/negative), Y-axis in B (adalimumab) and D (infliximab) cumulative curves. Cumulative curves are calculated based on proportion of positive samples from total numbers in each group at each stop time point (based on gestational age in weeks).