

# Corticosteroid-induced leukocytosis in pregnancy: A prospective observational study

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## ABSTRACT

**Background:** In the course of managing preterm labour, increasing trends of total white cell count raises concern for the obstetrician, suggesting a possible underlying infectious aetiology. Although mild leukocytosis is expected in pregnancy, the patterns of increment after corticosteroid administration are not well described beyond animal models and in a small number of human studies.

**Methods:** Seventy-three consecutive patients who required antenatal corticosteroids for either preterm labour or prelabour caesarean section were recruited and given a standard course of 12mg dexamethasone phosphate, twelve hours apart. Venous blood samples were taken before administration, at six hours and 36 hours after the first dose of dexamethasone.

**Results:** The total white count trend was  $10.31 \pm 2.62$  at baseline,  $11.44 \pm 3.05$  at six hours and  $12.20 \pm 3.49$  at 36 hours. Neutrophil-lymphocyte ratio was  $3.60 \pm 1.31$ ,  $8.73 \pm 3.63$  and  $3.24 \pm 1.49$  respectively, reflecting relative neutrophilia and lymphopenia which normalised by 36 hours.

**Conclusion:** In contrast to previous studies, we found only a slight increment in total white cell count of about 10%. The marginal changes described in our study would not normally raise any clinical concern, although vigilance should be exercised if higher levels were observed.

## KEY WORDS:

*Corticosteroid, dexamethasone, leukocytosis, neutrophil-lymphocyte ratio, pregnancy, preterm labour*

## INTRODUCTION

Leukocytosis has been observed in both animals and humans after administration of parenteral corticosteroid.<sup>1,2</sup> Dexamethasone and betamethasone are amongst the corticosteroids known to cross the placenta and are widely used in women with preterm labour.<sup>3</sup> On the other hand, intrauterine infection, a known cause of preterm labour in up to 40% of cases, may also result in leukocytosis.<sup>4</sup> As a consequence, fortuitous leukocytosis secondary to corticosteroid administration may prompt unnecessary use of antibiotics and influence the decision to expedite delivery.

Another contemporary indication for corticosteroid use is in women with prelabour caesarean section prior to 39<sup>0</sup> weeks,

which approximately halves the incidence of combined neonatal respiratory morbidity.<sup>3</sup> A large, single-centre, prospective study had previously demonstrated respiratory morbidity of approximately 40-70 in 1000 newborns, when elective caesarean section was carried out between 37<sup>0</sup> and 38<sup>6</sup> weeks of gestation.<sup>5</sup> Delaying elective caesarean sections may on the other hand, result in an increase in emergency surgeries. Undoubtedly then, the use of corticosteroid has risen substantially because of this balancing act.

Studies using methylprednisolone in patients with multiple sclerosis have demonstrated that a three-fold increment in leukocyte count occurs as early as six hours post administration. There is evidence that the leukocytosis is transient, with a two-fold increment by 24 hours, normalising by 48 hours post-delivery.<sup>1</sup> The mechanism by which leukocytosis occurs involves mainly a reduction in neutrophil adhesion molecules, resulting in sequestration of leukocytes within the intravascular compartment. A second postulated mechanism is by increasing production and release of neutrophils via induction of granulocyte-colony stimulating factor.<sup>1,2</sup>

To date, there are few studies looking at this phenomenon specifically in the context of pregnancy and after the use of dexamethasone. Available evidence showed a transient rise in leukocyte count and return to baseline within 24 hours, although they were largely based on betamethasone.<sup>6-8</sup> The authors believe that this study can further confirm the magnitude of leukocytosis in both women presenting with preterm labour and electively planned for prelabour caesarean section, after a common intervention in pregnancy, i.e., corticosteroids. This understanding would aid the judicious use of antibiotics and prevent unnecessary early intervention in the preterm subgroup. Therefore, the risk-benefit ratio of this study is favourable.

## MATERIALS AND METHODS

### Design and subject recruitment

This was a prospective observational study which involved women who required antenatal corticosteroids for either preterm labour or prelabour caesarean section. We designed this study to observe a continuous response variable of total white blood count (WBC) among pregnant women, before and after treatment. Prior data suggested that if the true difference in the mean response of matched pairs was estimated at  $5.0 \times 10^9/L$ , thirty-three pairs of subjects would be required to reject the null hypothesis that this response

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difference was zero, with a probability (power) 0.8.<sup>6</sup> The Type I error probability associated with the testing of this null hypothesis was 0.05. The sample size was calculated using PS software version 3.0.12.<sup>9</sup> An additional seven patients were recruited allowing a 10% drop-out rate, bringing the total sample size to 73.

The participants were divided into two groups, the first involving patients who presented with preterm labour while the second group included patients planned for caesarean section prior to 39<sup>0</sup> weeks, who were given dexamethasone prophylactically to reduce the likelihood of neonatal respiratory morbidity. This has been the standard practice in our unit for the past few years, hence did not involve additional intervention. This latter group were considered to be at low risk of infection and any degree of leukocytosis would likely be attributable to corticosteroid treatment.

Recruitment took place in the patient admission centre, where the indication of dexamethasone, route of administration and timing of blood investigation were explained. A patient information leaflet containing the above was provided in both English and Malay language. A translator was used when patients did not speak one of the two aforementioned languages. Written informed consent was provided by women who agreed to participate.

#### Inclusion criteria

All women with singleton pregnancies beyond 24<sup>0</sup> weeks of gestation at the time of presentation were invited to participate. Preterm labour was defined as the onset of regular uterine contractions, increasing in frequency, duration and occurring at least once in ten minutes in women between 24<sup>0</sup> to 35<sup>0</sup> weeks of gestation. Prelabour caesarean section was defined as women who required elective caesarean section <39<sup>0</sup> weeks of gestation.

#### Exclusion criteria

Women with multiple pregnancy, preceding fever (axillary temperature >37.5°C on two or more occasions at least one hour apart or temperature >38°C on one occasion), active systemic lupus erythematosus (SLE), retroviral disease, women on oral or parenteral steroids, lithium or beta agonist within the past seven days. Any patients with a history of haematopoietic disease or receiving other forms of immunosuppressant, including chemotherapy within the last one year were excluded.

Patients who delivered within six hours of the first dose of dexamethasone or had leaking liquor were excluded.

#### Study protocol and laboratory analysis

Three to five millilitres of venous blood was drawn from women prior to dexamethasone administration, at six hours and 36 hours later. Two doses of 12mg (3mls) of dexamethasone phosphate (Duopharma, Selangor, MY) were given intramuscularly, 12 hours apart. C-reactive protein (CRP) was used as an adjunct to exclude concurrent infection and was expected to be useful only in cases with equivocal clinical findings.<sup>10</sup> A qualitative CRP was used with a cut-off point of ≥6mg/L interpreted as a positive result. The

biospecimen collected was stored in an Ethylenediaminetetraacetic acid (EDTA) tube and a plain collection tube, then sent to the respective laboratories. Full blood count and differential count (FBC+DC) was analysed using Sysmex XS 1000i (Sysmex, Lincolnshire, IL) from the Sarawak General Hospital Haematology Laboratory while CRP was analysed in the Biochemistry laboratory using a rapid latex agglutination test kit Avitex (Omega Diagnostics, Reinbek, Germany).

Axillary temperature was monitored at presentation, then four-six hourly in all patients. Fever was defined as above and if clinical suspicion of chorioamnionitis was present, patients would receive treatment as per local protocol with broad spectrum intravenous antibiotics.

#### Statistical analysis

Data were collected using a standardised data collection sheet and entered into SPSS 19 (IBM, Chicago, IL). Descriptive data were tabulated and comparison of means between subgroups were analysed using Chi-square test. Changes in the serial pre- and post-dexamethasone haematological parameters were analysed using analysis of variance (ANOVA) for repeated measures. In both tests, P<0.05 was considered statistically significant.

Ethical approval for the study was obtained from the Medical Research and Ethics Committee (NMRR ID 15-1172-26851).

## RESULTS

Of the 73 women who agreed to participate in the study, 67 were available for analysis after exclusion of women who withdrew consent or did not fulfil the prespecified criteria. Thirty-four women were given corticosteroid for preterm labour while 33 were for planned elective caesarean section prior to 39<sup>0</sup> weeks, giving a total of 201 data points for analysis.

The mean age of women receiving corticosteroid was 29.45±6.37 years, body mass index (BMI) of 25.20±5.57 kg/m<sup>2</sup> and at a gestation of 34.21±3.74 weeks. Women who received corticosteroid due to preterm labour had a lower gestational age and a higher proportion were primiparous compared to the prelabour caesarean subgroup (35.3% vs, 15.2%). There was no statistically significant difference in age, BMI or parity comparing the two groups (Table I).

The serial total WBC, neutrophil, lymphocyte and neutrophil-lymphocyte ratio changes prior and subsequent to corticosteroid administration are shown in Table II. The overall trend showed a significant, albeit marginal rise, in total WBC (10.9%), a more marked neutrophilia (37.9%) and relative lymphopenia (-40%) 6 hours after administration of corticosteroid. While the total WBC continued to rise at a lower rate, neutrophil and lymphocyte count showed a degree of normalization at 36 hours.

This trend was maintained even when the prelabour caesarean and preterm subgroup were analysed separately. Total WBC on admission were (9.96±2.94 vs 10.65±2.27;

Table I: Demographics of patients receiving antenatal corticosteroids

| Patient characteristics  | Indication                 |                       | P-value |
|--------------------------|----------------------------|-----------------------|---------|
|                          | Prelabour caesarean (n=33) | Preterm labour (n=34) |         |
| Age (years)              | 32.52±5.20                 | 26.47±6.04            | P=0.17  |
| Parity                   | 1.97±1.78                  | 1.24±1.20             | P=0.10  |
| BMI (kg/m <sup>2</sup> ) | 26.07±5.52                 | 24.3±5.56             | P=0.42  |
| Gestation (weeks)        | 36.40±3.03                 | 32.08±3.09            | P=0.02* |

(Values represent mean and standard deviation, BMI: Body mass index, \*statistically significant)

Table II: Serial haematological parameters

|                                  | All indications for corticosteroid (n=67) |            |            | P-value |
|----------------------------------|---|------------|------------|---------|
|                                  | Adm                                       | 6hrs       | 36hrs      |         |
| WBC(10 <sup>9</sup> /L)          | 10.31±2.62                                | 11.44±3.05 | 12.20±3.49 | P<0.01* |
| Neutrophils (10 <sup>9</sup> /L) | 7.25±2.24                                 | 10.00±3.04 | 8.49±3.19  | P<0.01* |
| Lymphocytes (10 <sup>9</sup> /L) | 2.13±0.63                                 | 1.28±0.57  | 2.70±0.69  | P<0.01* |
| NLR                              | 3.60±1.31                                 | 8.73±3.63  | 3.24±1.49  | P<0.01* |
| Platelets(10 <sup>9</sup> /L)    | 258±63                                    | 260±63     | 256±61     | P=0.73  |

(Adm:admission, hrs:hours, WBC: total white blood count, NLR: Neutrophil-Lymphocyte ratio, \* statistically significant)

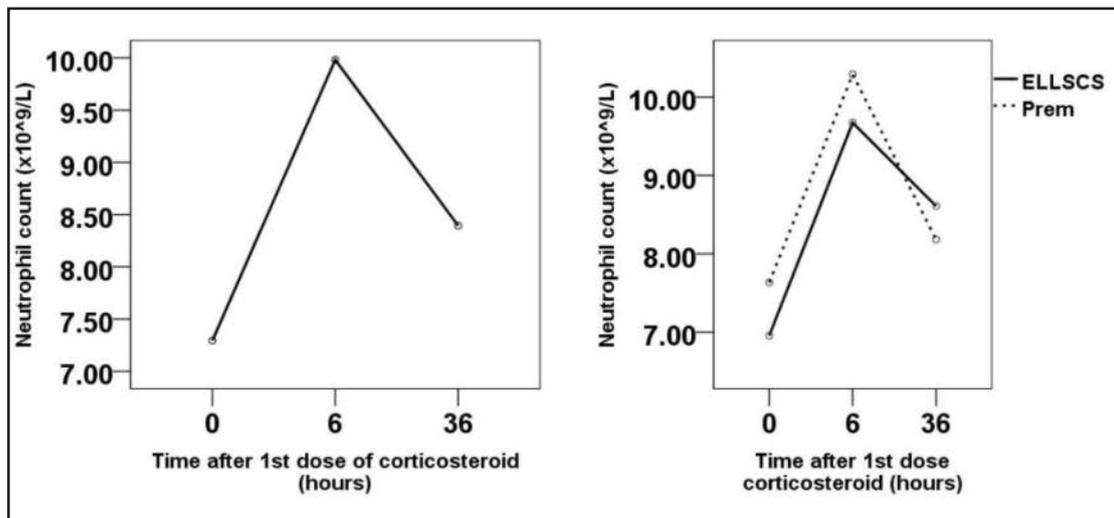


Fig. 1: Changes in neutrophil count after corticosteroid.

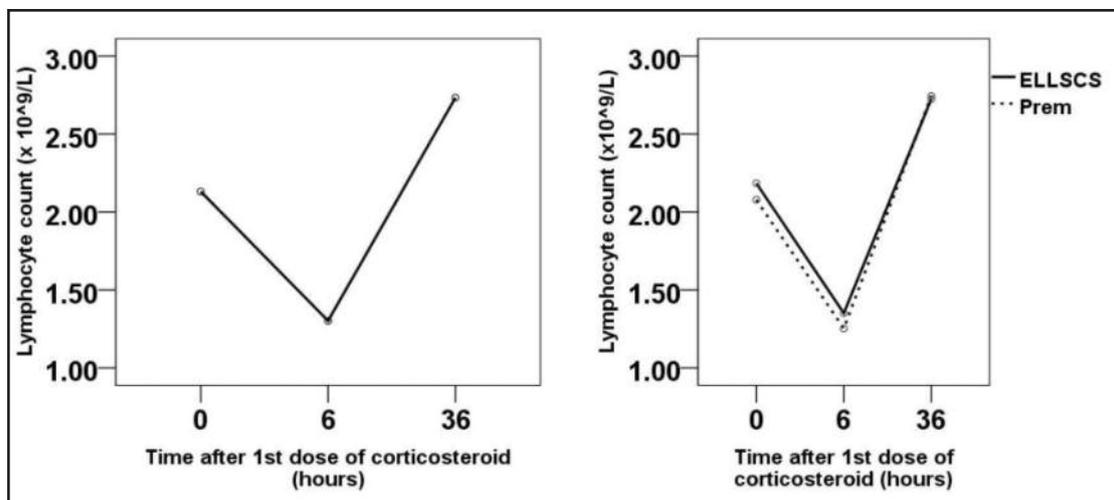


Fig. 2: Changes in lymphocyte count after corticosteroid.

$P=0.07$ ), six hours ( $11.15\pm 3.53$  vs  $11.72\pm 2.52$ ;  $P=0.09$ ) and 36 hours ( $12.42\pm 4.32$  vs  $11.99\pm 2.49$ ;  $P=0.69$ ) for prelabour caesarean and preterm respectively. The neutrophil and lymphocyte trends are shown in Figures 1 and 2. Another form of reporting this was by using the neutrophil-lymphocyte ratio (NLR), which increased from a baseline of  $3.60\pm 1.31$ , peaking at  $8.73\pm 3.63$  at six hours before normalizing to  $3.24\pm 1.49$  at 36 hours.

Four of the 34 preterm patients had CRP positive on admission but only one patient (25%) went on to deliver within the same admission. None of them developed fever or chorioamnionitis. Of the 30 patients with negative CRP, four (13.3%) went on to deliver within the same admission.

## DISCUSSION

Corticosteroid therapy is arguably one of the most common form of non-surgical prenatal therapy. Increasing trends of white cell count after the administration of corticosteroid is an interesting phenomenon which has been previously reported in both animals models and humans.<sup>2,6</sup> Despite its lack of sensitivity as a marker of infection, a full blood count remains one of the least invasive and most basic investigations available to health care personnel managing women in resource-poor or geographically-removed units worldwide.<sup>11</sup>

Baseline total WBC in pregnancy has been reported to be higher than in their non-pregnant counterparts.<sup>12</sup> Similarly, the baseline total WBC in our study was higher at  $10.31\pm 2.62 \times 10^9$  cells/L.

We observed a lower margin of rise in total WBC at two separate intervals after dexamethasone, which despite its statistical significance, would not have been noticeable in clinical practice. The lack of intergroup difference between the preterm labour and prelabour caesarean group showed that regardless of ongoing labour or the possibility of underlying infection, white cell and differential count changes were attributable to corticosteroid administration. Previous studies have demonstrated a larger margin of increment from  $9.8 \times 10^9$  to  $14.2 \times 10^9$  cells/L and  $11.3 \times 10^9$  to  $16.2 \times 10^9$  cells/L which may have caused more of a concern.<sup>6,8</sup> Despite excluding women who were given dexamethasone for prelabour caesarean section, the extent of leukocytosis was minimal. This may be explained by the fact that the prior studies recruited preterm women, including those with prelabour rupture of membranes rather than preterm labour per se.

The underlying mechanism of corticosteroid-induced leukocytosis is most likely multifaceted. Corticosteroids exercise their anti-inflammatory properties by inhibiting phospholipase action but paradoxically, causes an increase in circulating polymorphonuclear leukocytes, ready to participate in an inflammatory response. Demargination and sequestration of leukocytes within the intravascular compartment due to a reduction in neutrophil adhesion molecules has been proposed as the primary mechanism. An increased production and release of marrow neutrophil precursors has also been observed, secondary to the effect of granulocyte-colony stimulating factors. Delayed apoptosis of

neutrophils in the circulation may also contribute to this phenomenon, to a certain extent.<sup>1,2</sup>

Although four of the 30 patients with negative CRP delivered during the same admission, we were unable to draw any conclusions from this study due to the relatively low event rate.

The strength of the study is the prospective design of the study with a larger number of women recruited than previously reported.

Although non-labouring preterm patients who require antenatal corticosteroids would have been an ideal control group, the authors opined that in the majority of such instances, caesarean delivery would be performed more expediently, for example in the context of severe preeclampsia or foetal growth restriction, negating the interval required for completion of blood taking. Obtaining ethical approval for repeated blood sampling in pregnant women who did not require any form of intervention, solely for the purpose of control was difficult to justify. This was the limitation in our design of the study, in addition to the observational nature and single-centre recruitment.

On the other hand, inclusion of women planned for prelabour caesarean section prior to 39<sup>0</sup> also allowed a larger population of women requiring corticosteroid to be sampled.

The discrepancy in gestation between both groups would not have contributed significantly to baseline total white cell count, since values were shown to be stable from midtrimester onwards.<sup>12</sup>

Interestingly, we also reported the neutrophil-lymphocyte ratio as it has received some attention in recent years as a marker of inflammation, including in preterm labour and sepsis. A NLR of more than five increases the likelihood of underlying local infection and more than ten in systemic infections. NLR did not differ between term and preterm patients.<sup>13,14</sup>

In summary, transient leukocytosis, relative neutrophilia and lymphopenia are expected after a course of corticosteroids, although the level of rise is lower than demonstrated in previous studies. Knowledge of the pattern of total white cell and differential count, especially in the context of potential intrauterine infection such as preterm labour can be reassuring to the clinician. There should still be increased vigilance when marked ( $>16.0k \times 10^9$  cells/L; two standard deviation from mean) or sustained leukocytosis is encountered, since its significance cannot be ascertained from this paper.

## CONFLICTS OF INTEREST

The authors have no conflicts of interest to declare. The study was sponsored by the Sarawak General Hospital.

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