Risk of myelotoxicity with intravenous cyclophosphamide in patients with systemic lupus erythematosus

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Abstract

Objectives. To determine the incidence of serious myelotoxicity from intravenous cyclophosphamide (IC) in systemic lupus erythematosus (SLE).

Methods. In a retrospective study, white blood cell (WBC) counts with differential and platelet counts were determined in 92 SLE patients (96 courses) given 1623 doses of IC.

Results. Only one patient developed a total leucocyte count < 1000 u/mm³, one developed a neutrophil count < 500 u/mm³, two had a lymphocyte count < 100 u/mm³ and no patients had platelet counts < 50 000 u/mm³ during follow-up. The risk of a neutrophil count < 500 u/mm³ was 0.06 per 100 visits [95% confidence interval (CI) 0.00–0.34]. Two patients discontinued IC due to neutropenia [rate of 0.12 per 100 doses (95% CI 0.01–0.44)]. No clinical consequences were recorded in conjunction with low blood cell counts. In multivariate models, both the cumulative number of IC doses and European Consensus Lupus Activity Measurement (ECLAM) score affected neutrophil and lymphocyte counts adversely. For neutrophils, lowering the ECLAM score by 1 point counteracted four additional doses of IC after adjusting for steroid dose.

Conclusions. IC and SLE disease activity have independent effects in lowering white blood cell counts, but serious myelotoxicity of IC is uncommon.

KEY WORDS: Myelotoxicity, Cyclophosphamide, Systemic lupus erythematosus, Blood cell count, Neutropenia.

Intravenous cyclophosphamide (IC) is a useful treatment for lupus nephritis [1–5] and other manifestations of systemic lupus erythematosus (SLE) [6–8]. While the response rate to IC is substantial, concern has arisen about its toxicity. Besides amenorrhoea [9, 10], myelotoxicity has been described and has also been recognized in other diseases in which IC has been used [11, 12]. Previous SLE studies [13–18] have used relatively small samples to assess the precise frequency of serious IC-related neutropenia, lymphopenia and thrombocytopenia. Most previous studies focused primarily on efficacy rather than toxicity. There is a need for exact data on toxicity [19] in order to establish the risk–benefit ratio for IC.

In this retrospective study, we evaluated IC-related myelotoxicity in a cohort of SLE patients who had received over 1600 IC doses during the last 8 yr. The large number of patients and IC doses allowed us to make precise estimates of the risk of IC myelotoxicity, treatment discontinuations and clinically significant consequences. We also investigated whether the effect of IC on blood counts is cumulative and whether there is some interplay in this regard with SLE disease activity.

Methods

Patient population

Information was obtained from the medical records of consecutive SLE patients who received pulse IC treatment between January 1992 and October 2000 at the Department of Pathophysiology, University of Athens, a referral centre for autoimmune diseases. All patients fulfilled the American College of Rheumatology criteria for SLE [20]. Indications for IC included renal disease.
Cyclophosphamide myelotoxicity in SLE

Data collection

For each patient, we collected information on the indication for starting IC, including biopsy documentation; age at the time of starting IC; European Consensus Lupus Activity Measurement (ECLAM) score for SLE disease activity [22], dose of steroids received (in mg prednisolone) and haematological parameters (total leucocyte count, neutrophil count, lymphocyte count, platelet count) at the time of the first visit (before starting IC) and at any subsequent visit made for IC administration and 1 month after the last dose. Patients with renal disease, at each visit we also recorded creatinine clearance, 24-h urinary protein and urine sediment. Urine sediment was defined as active in the presence of casts or of more than 8–10 erythrocytes per high-power field [21]. For all patients, the medical records were reviewed to collect information on serious infections that required hospitalization and serious bleeding episodes that might be related to haematological toxicity. Bladder cytology was performed every 3–9 months and cystoscopy with biopsy was performed whenever there was atypia or macroscopic haematuria. Follow-up is censored at the time of each patient’s last visit to our centre (or 1 month after the last dose, if IC was stopped), and all serious events that might have resulted in admissions at other hospitals up to that time were captured in our database.

Statistical analysis

Changes in cell counts. We examined both the time-course of absolute values for blood counts and the change in these counts compared with the baseline. For the latter evaluation, baseline values were determined as the average of the two most recent measurements preceding or coincidental with the initiation of pulse IC. Typically, one measurement had been obtained at the visit when IC was started and the other had been obtained within the previous 3 months. An average of two measurements was preferred so as to avoid the phenomenon of regression to the mean [23]. Exact numbers for total leucocyte count, differential count and platelet count were recorded in the medical records for 95.0, 79.6 and 90.4% of the pertinent measurements respectively. In the remaining cases, these counts were within normal limits but had not been recorded with exact numbers in the medical records. Missing numbers are excluded from the main analysis, but imputing normal values would not change the study inferences (not shown).

Rates of toxicity. We noted the number of subjects and the number of occasions when very low blood counts were recorded (total leucocyte count <1000 mm$^{-3}$, neutrophil count <500 mm$^{-3}$, lymphocyte count <100 mm$^{-3}$, platelet count <50 000 mm$^{-3}$). We also estimated exact 95% confidence intervals (CIs) for the proportions of subjects and measurements with such haematological toxicity.

Analysis of multiple determinants. The effect of the cumulative number of IC doses on blood counts was also examined by generalized linear model analysis of variance with or without adjustment for the ECLAM score and steroid dose. Models including the time from the initial dose of IC in addition to or instead of the number of doses did not fit the data better (not shown). In these analyses, data were censored at the 26th visit to avoid an excessive effect of the relatively few subjects who had received over 25 doses. In all models, the subject was considered a random factor and the cumulative number of IC doses, ECLAM score and the current dose of steroids were used as covariates. Separate sensitivity analyses were conducted including only patients with lupus nephritis and examining also the potential associations between blood counts and an active urine sediment, 24-h urine protein and creatinine clearance. Correlation analysis between candidate determinants of blood counts used the Spearman coefficient.

Software. Analyses were conducted in SPSS 10.0 (SPSS, Chicago, IL, USA) and StatXact3 (Cytel, Boston, MA, USA). $P$ values are two-tailed.

Results

Patient characteristics

Ninety-two patients (12 male) were treated with IC. Four patients (one male) received two courses each, typically with more than 1 yr between courses and for different indications. Therefore 96 courses were analysed: 83 given for lupus nephritis (type IIb, $n = 6$; type III, $n = 42$; type IV, $n = 17$; type V, $n = 12$; no biopsy performed, $n = 6$) and 13 for other indications [seven for central nervous system disease, two each for respiratory involvement (pulmonary hypertension) and antiphospholipid syndrome, and one each for autoimmune haemolytic anaemia and thrombocytopenia]. The total IC doses were 1623 (nephritis, $n = 1414$). Only nine patients had received more than 25 doses (maximum 54), but in 87 courses at least 1 yr of treatment was given. The mean cumulative IC dose was 19.7 g [s.d. 9.5, median 18.3, interquartile range (IQR) 13.9–25.5]. In 85 of the 96 courses, the cumulative dose exceeded 10 g.
Mean age at the first dose was 32.8 yr (s.d. 12.8, median 30, IQR 24–43). The ECLAM score at the initiation of a new course was available for 93 courses and the mean was 5.9 (s.d. 2.2, median 6, IQR 4–7.5).

The median (IQR) total leucocyte, neutrophil, lymphocyte and platelet counts at the first IC dose visit were 6000 (4600–8000), 4102 (2974–5967), 1271 (853–1995) and 240 000 (177 000–308 000) per mm$^3$ respectively. Nine patients had a leucocyte count <4000/mm$^3$ and six patients had a neutrophil count of <2000/mm$^3$ at the first visit. The respective minima were 2300 and 1316/mm$^3$.

**Evolution of haematological parameters**

As shown in Fig. 1, the median blood cell counts did not change substantially with increasing number of IC doses, and the IQRs of the changes were not very large. Nevertheless, a few outlier or extreme changes were observed, but they were equally likely to represent increases or decreases compared with the baseline.

There was one case in which the total leucocyte count decreased below 1000/mm$^3$, and it decreased below 2000/mm$^3$ in three more patients. The trajectories of the absolute counts for these four patients are shown in Fig. 2a. The neutrophil count decreased below 1000/mm$^3$ in two subjects on five occasions, and in one of them it decreased below 500/mm$^3$. Trajectories are shown in Fig. 2b. A lymphocyte count below 100/mm$^3$ was seen in two patients on one occasion each. Values below 200/mm$^3$ were seen in six patients on eight occasions. All these six patients had fewer than 1100 lymphocytes per mm$^3$ at baseline and two of them had fewer than 400/mm$^3$ at baseline. The platelet count was never lower than 90 000/mm$^3$ in any patient during follow-up. In the one patient with immune thrombocytopenia (baseline 12 000/mm$^3$) the

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**Fig. 1.** Changes compared with baseline in (a) total leucocyte count, (b) neutrophil count, (c) lymphocyte count and (d) platelet count. Data are shown for up to 25 doses. Nine patients received longer courses and did not have significant late toxicity (not shown). Also shown on the horizontal axis is the number of patients with data available at each time-point. Measurements were made typically at the time of administration of the next dose. All counts are in cells/mm$^3$. For each time-point, the boxplot shows the median and IQR and the whiskers show the highest and lowest values, with the exception of outlier measurements (>1.5 box lengths from the box margin), which are shown as circles (1.5–3.0 box lengths) or asterisks (>3.0 box lengths). All counts are in cells/mm$^3$. 

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platelet count was corrected promptly after the first IC dose. Five other patients with platelet counts less than 100 000 u/mm³ at baseline also improved promptly during follow-up.

**Discontinuations and changes in dose due to myelotoxicity**

Only two patients discontinued IC therapy due to neutropenia (trajectories are shown in Fig. 2b). One of them had persistently low neutrophil counts, with values less than 1000/mm³ on four occasions, and she discontinued after myelotoxicity was documented on bone marrow biopsy. In this case, the total leucocyte count was not affected. The other patient had been referred to us and baseline blood counts were not available, but reportedly he had normal values prior to referral. He developed severe myelotoxicity with total leucocyte count 500/mm³, neutrophil count 305/mm³ and lymphocyte count 85/mm³, and bone marrow biopsy was consistent with a drug toxicity effect. The neutropenia reversed promptly in both patients upon IC discontinuation. No patients discontinued IC due to isolated lymphopenia or thrombocytopenia.

**Potential consequences**

A total of 15 infections requiring hospitalization were recorded during follow-up among 12 patients (skin and soft tissue infections, n = 7; acute pyelonephritis, n = 3; hidradenitis, n = 1; Pseudomonas corneal ulcer, n = 1; endophthalmitis, n = 1; epididymitis, n = 1; cytomegalovirus retinitis, n = 1), but no patient had severe cytopenia at the time of diagnosis. With the exception of one patient with a total leucocyte count of 2600/mm³, all other cases had leucocyte counts of 3500/mm³ or higher. On admission, all patients had over 1500/mm³ neutrophils and over 500/mm³ lymphocytes. Another five patients had required hospitalization for an infection at the time it was decided to initiate IC. No patients developed serious bleeding episodes during follow-up. One patient had macroscopic haematuria and two more had minor atypia on cytology. All three had cystoscopy with biopsies negative for haemorrhagic cystitis or cancer.

**Frequency of serious haematological toxicity**

The estimated proportions (and 95% CIs) of patients with significant haematological toxicity, as defined in the Methods section, were 1.0% (0.03–5.7%) for total leucocyte count <1000/mm³, 1.0% (0.03–5.7%) for neutrophil count <500/mm³, 2.1% (0.25–7.3%) for lymphocyte count <100/mm³ and 0.0% (0.0–3.8%) for platelet count <50 000/mm³ during any IC course. When expressed as a percentage of visits with documented significant haematological toxicity, the respective proportions were 0.06% (0.00–0.34%) for leucocyte count <1000/mm³, 0.06% (0.00–0.34%) for neutrophil count <500/mm³, 0.12% (0.01–0.43%) for lymphocyte count <100/mm³ and 0% (0.00–0.23%) for platelet count <50 000/mm³ at any time-point during an IC course. The discontinuation rate due to IC-related myelotoxicity was 0.12 per 100 doses (95% CI 0.01–0.44). We can thus be 95% certain that the discontinuation rate due to myelotoxicity is less than 1 in 220 doses.

**Effects of IC, steroids, disease activity and renal parameters on WBC counts**

In univariate models, IC had a highly statistically significant cumulative effect on the total leucocyte, neutrophil and lymphocyte counts, but the absolute magnitude of the effect was modest. There was no apparent adverse effect on the platelet count. The dose of corticosteroids was also a strong predictor of cell counts, while no clear effect was seen for the ECLAM score in univariate analyses. The univariate analyses should be interpreted cautiously as the various potential predictors could be confounding the effects of each.
other. The ECLAM score was positively correlated with the dose of prednisolone used \((r = 0.43, P < 0.001)\) and negatively correlated with the number of prior IC doses \((r = -0.37, P < 0.001)\), while the number of prior IC doses was negatively correlated with the prednisolone dose \((r = -0.64, P < 0.001)\). In multivariate modelling (Table 1), the ECLAM score was also a significant predictor, with higher ECLAM scores clearly related to lower cell counts. After adjusting for ECLAM score and the dose of steroids, 10 doses of IC were estimated, on average, to decrease the total leucocyte count by 680/mm\(^3\), neutrophils by 430/mm\(^3\) and lymphocytes by 240/mm\(^3\). The effect of 1 point in the ECLAM score on the total leucocyte and neutrophil counts counteracted approximately the effect of four doses of IC (Table 1). One dose of IC was counteracted by approximately a 1–2 mg higher dose of prednisolone (Table 1).

Analyses limited to patients with lupus nephritis yielded qualitatively similar results for all haematological parameters. In addition, higher levels of proteinuria tended to be associated with a higher total leucocyte count and a higher neutrophil count \((P < 0.001\) for both) in univariate analyses, while no significant effect was seen for an active urine sediment or creatinine clearance. Again, the univariate association might simply have reflected confounding with other predictors, because the level of 24-h urinary protein was positively correlated with the ECLAM score \((r = 0.48, P < 0.001)\) and the dose of prednisolone \((r = 0.34, P < 0.001)\) and negatively correlated with the number of prior IC doses \((r = -0.18, P < 0.001)\). No renal parameters were found to have a significant effect on WBC counts after adjusting for steroid dose, number of IC doses and ECLAM score. In multivariate modelling, on average, the total leucocyte count decreased by 52/mm\(^3\) with each IC dose and by 217/mm\(^3\) for each additional ECLAM score point, whereas it increased by 569/mm\(^3\) for each 10 mg increase in the prednisolone dose \((P = 0.002, P < 0.001\) and \(P < 0.001\) respectively). On average, the neutrophil count decreased by 29/mm\(^3\) for each IC dose and by 156/mm\(^3\) for each additional ECLAM score point, and increased by 475/mm\(^3\) for each 10 mg increase in the prednisolone dose \((P = 0.064, P < 0.001\) and \(P < 0.001\) respectively).

**Discussion**

Serious myelotoxicity is rather uncommon in SLE patients receiving IC for nephritis or other indications. We observed a discontinuation rate due to myelotoxicity of 1 in 800 doses. The data offer 95% certainty that this rate should be less than 1 in 220 doses. None of the 92 patients experienced any serious clinical consequences from cytopenia. The recorded infections during follow-up were not atypical in frequency or type for SLE patients. One other study of more limited sample size [17] reported 1.8 hospitalizations due to infection per 100 IC pulses, an estimate slightly higher than but not incompatible with the rate that we observed. Importantly, none of the infection-related hospitalizations occurred in conjunction with manifest leucopenia. Myelotoxicity should not be a major concern in the use of IC.

IC does have an effect on WBC counts that reflects the cumulative dose received, while no such effect is exerted on the platelet count. On average, 10 doses of IC are expected to decrease the total leucocyte count by approximately 680 cells/mm\(^3\), and most of them would be neutrophils. This average estimate should be viewed with the caveat that the substantial between-subject variability reflected both true variability and laboratory measurement error. For most patients, such decreases should not cause a clinical problem. Disease activity acts as an independent predictor in modulating blood counts after adjustment for steroid dose, which is an additional strong modulator of neutrophil counts. A lowering of 1 point in ECLAM score may counteract the decrease associated with approximately four IC doses. Thus, since IC has a beneficial effect on disease activity, the net effect on cell counts may be even less detrimental. Moreover, the neutrophilic effect of an IC dose seems small compared with the neutrophilic effect of the adjunctive steroids.

The results were similar when limited to patients with nephritis. Renal parameters did not affect the cell counts once other predictors had been accounted for. The other medical conditions that we considered in this study were each represented by only a few cases, but there is no reason to believe that IC tolerance might have been different in these patients. All patients with thrombocytopenia at baseline promptly improved after starting IC and thrombocytopenia was never seen during follow-up. Thus, there should be no concern about IC-related megakaryocyte toxicity.

The current safety evaluation is the largest in the SLE literature in terms of the total number of IC doses. We are aware of at least eight other reports that each included more than 120 doses of IC in SLE patients, using a regimen of 0.5–1.0 mg/m\(^2\) [1–4, 16, 17, 24, 25]. None had apparently included more than a total of 500 IC doses. Data on myelotoxicity were not described in four reports [1–3, 24]. In the remaining studies, neutropenia and myelotoxicity were also infrequent occurrences. Martin et al. [17] describe one case of neutropenia <300/mm\(^3\) accompanied by septic shock.
among 75 patients receiving IC for various connective tissue diseases, mostly SLE. In Gourley et al. [4], two of 55 SLE patients given IC developed neutropenic fever. Valeri et al. [25] describe one patient with transient leucopenia among 20 treated SLE patients, and Omdal et al. [16] describe one discontinuation in the fifth month due to leucopenia among 25 treated patients with various connective tissue diseases, 12 of whom had SLE. Taking the results of previous studies together, there was only one discontinuation of the regimen due to myelotoxicity for a total of almost 1000 doses given to SLE patients. These data are very consistent with the estimates that we have obtained with a larger number of IC doses.

Some investigators have suggested that low-dose IC may be better tolerated [14, 16, 18]. The largest series [14] includes the experience from a total of 883 IC doses given to 90 subjects, including 43 SLE patients. One SLE patient developed fatal sepsis in conjunction with neutropenia and a patient with Wegener’s granulomatosis died with sepsis and neutropenia. From this evidence, the low-dose regimen does not seem to offer a clear advantage in terms of reduced myelotoxicity, although the patient groups in various studies are not directly comparable. Advantages may exist in terms of other toxicities, in particular amenorrhoea, which is a major concern for women of reproductive age [14]. Amenorrhoea may be a more important consideration than myelotoxicity in assessing the risk–benefit ratio of IC in this group. Nevertheless, indirect comparisons should be undertaken cautiously in the absence of evidence from randomized controlled trials [26].

The WBC count nadir often occurs about 2 weeks after IC administration. In our patients, we have examined blood counts at longer intervals. When we started using IC in our centre, we recorded 2-week values in a few patients but no major leucopenias were seen, and this policy was quickly abandoned. A few more cases of leucopenia might have been recorded with routine 2-week measurements, but would have been transient and of no clinical significance. In our opinion, extra visits for blood tests 2 weeks after IC are unlikely to be useful or cost-effective.

Although this is a retrospective study, our patients were enrolled consecutively and followed in a referral centre. It is unlikely that patients with more favourable blood cell count responses were selected. Nevertheless, only nine patients started IC with a baseline leucocyte count below 4000/mm³ and only six patients started IC with a baseline neutrophil count below 2000/mm³. Referring clinicians may have been reluctant to start IC in their SLE patients if the blood cell counts had been relatively low, although this has not been a concern in our practice. We have not delayed or denied IC to patients with lupus nephritis because of low WBC counts. In our experience, fear of myelotoxicity should not necessarily prevent the use of this regimen in patients with moderately depressed WBC counts. The expected average decreases are not such that would cause problems, while an improvement in disease activity may counteract the cumulative myelotoxic effect of IC. Nevertheless, more data are needed for patients who start IC while they have low WBC counts, and until then such patients may still need continuous monitoring for serious myelotoxicity.

References

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