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RESEARCH ARTICLE

Biochemical and Haematological Predictors of Reduced Neutrophil Granulocyte Count associated with Intravenous Ceftriaxone Treatment

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ARTICLE HISTORY

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Abstract: ***Background*:** Intravenous treatment with ceftriaxone, a commonly used third-generation cephalosporin, is associated with a risk of the potentially fatal side-effect of neutropenia.

***Objective*:** The first systematic study to determine whether six to 12 days' intravenous ceftriaxone treatment is associated with a reduction in the neutrophil count and the extent to which biochemical and/or haematological parameters routinely measured at baseline predict such a fall.

***Method*:** Baseline and follow-up haematological and biochemical blood indices were measured in 86 patients (mean age 39.4 years; 55 female) receiving 2 g intravenous ceftriaxone daily.

***Results*:** At follow-up, the mean (standard error) neutrophil count had fallen from 3.93×10^9 (0.16×10^9) L^{-1} to 3.15×10^9 (0.15×10^9) L^{-1} ($p < 0.000001$). This reduction was predictable according to the following multifactor linear regression model: (baseline neutrophil count ($\times 10^9$ L^{-1})) – (follow-up neutrophil count ($\times 10^9$ L^{-1})) = 76 + 159.2(baseline haematocrit) – 14.5(baseline red blood cell count ($\times 10^{12}$ L^{-1})) – 0.724(baseline mean corpuscular volume (fL)) + 0.474(baseline neutrophil count ($\times 10^9$ L^{-1})) + 0.0448(baseline total iron binding capacity (μM)) + 7.15(baseline calcium ion concentration (mM)) – 13.2(baseline corrected calcium ion concentration (mM)) + 0.0166(baseline alkaline phosphatase (IU L^{-1})). The residuals were normally distributed and model testing by random partition of the original data into two parts, with training of the model using the first part and model testing with the second part, gave highly satisfactory results.

***Conclusion*:** Intravenous ceftriaxone treatment is associated with a fall in neutrophils, which can be predicted by routine baseline blood indices.

Keywords: Biochemistry, ceftriaxone, haematology, multifactor linear regression modelling, neutropenia, neutrophil count.

1. INTRODUCTION

Following the discovery of the fungal species *Cephalosporium acremonium* in seawater close to a sewage outlet in Sardinia by Professor Giuseppi Brotzu in 1945, the first types of cephalosporin β-lactam antibiotics were isolated from this fungus by researchers at the University of Oxford in 1948 from samples sent to them by Brotzu [1, 2]. Since then, numerous cephalosporins have been synthesised; the most widely used clinical classification of them is *via* 'generations', which is essentially based on general aspects of antimicrobial activity [3, 4].

In adult humans, bone marrow-derived polymorphonuclear leukocytes, also known as neutrophil granulocytes on account of the fact that their granules do not stain strongly with either acidic or basic dyes, comprise the largest proportion of leukocytes, with a normal count of 2 to 7.5×10^9 L^{-1} (normally higher in women than in men), which usually rises in response to an acute bacterial infection [5-10]. In turn, this elevated neutrophil count helps defend the body against bacterial infection through mechanisms such as phagocytosis, the production of reactive oxygen species (the 'oxygen burst') and the elaboration of web-like neutrophil extracellular traps [11]. Ideally, therefore, following the advent of pharmacotherapy with a cephalosporin for a bacterial infection, one would wish any elevated neutrophil count to be sustained. Unfortunately, however, there is evidence that the opposite might actually occur, with 17 cases reported, at the time of writing, of intravenous treatment with ceftriaxone (a third-generation cephalosporin) being associated with the serious, and potentially fatal, side-effect of neutropenia [12]. Uy and colleagues have reported that in 14 of these cases the neutrophil count dropped to below 0.5×10^9 L^{-1}, with the time taken to reach this level of neutropenia being as few as five days in two cases; in 11 cases the daily dose of ceftriaxone being administered was 2 g, with 1 g daily being given in two cases and 4 g daily in the remaining case [12]. While the incidence of neutropenia associated with ceftriaxone therapy, or with treatment with cephalosporins generally, is

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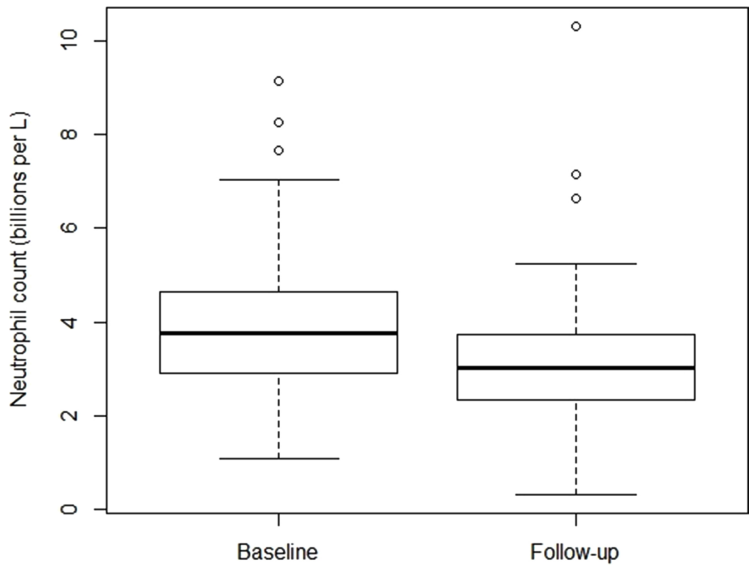

Fig. (1). Boxplot showing the baseline and follow-up neutrophil counts.

not known, the incidence of neutropenia induced by non-chemotherapy drugs is estimated to range between 1.6 and 15.4 cases per million of the population per year [13-15]. Cephalosporins are amongst the drugs which relatively commonly induce neutropenia [16]. Indeed, in their 2007 systematic review of 980 cases of agranulocytosis induced by non-chemotherapy medication, Andersohn and colleagues reported two definite cases of a definite relationship between cephalosporin treatment and neutropenia (level 1 evidence) and 18 cases of a probable such relationship (level 2 evidence) [14]. While for a small number of non-chemotherapy drugs specific risk factors, such as histocompatibility antigens, have been found for the development of neutropenia, to date no such risk factors have been identified for cephalosporins [16].

In light of the seriousness of the side-effect of neutropenia, we carried out the first systematic study to determine whether six to 12 days' intravenous ceftriaxone treatment is associated with a reduction in the neutrophil count and, if so, the extent to which any biochemical and/or haematological parameters routinely measured as blood indices at baseline might be predictive of such a fall.

2. METHODS

2.1. Patients and Methods

The medical records from a two-and-a-half year period were accessed of 86 patients receiving 2 g intravenous ceftriaxone daily for the treatment of Lyme borreliosis at a British medical centre specialising in environmental (functional) medicine; the stage of infection was well past that of early localised infection or early disseminated infection but, rather, consisted of late disseminated infection. Baseline and follow-up haematological and biochemical blood indices were independently assessed at The Doctors' Laboratory, London, UK; the follow-up measures used in this study were all carried out within 12 days of starting this treatment. The AONMREC approved this audit. The study was carried out in accordance with the Declaration of Helsinki. The STROBE (STrengthening the Reporting of OBservational studies in Epidemiology) checklist for this study can be found in Appendix 1 [17].

2.2. Statistical Analyses

After checking for normality, the pre- and post-treatment mean values of the dependent variable were compared using the Student *t*-test. A corresponding boxplot was constructed. Multifactor linear regression modelling was carried out using backward elimination, with the *p*-value threshold for elimination set at 0.05 in the context of the overall change in the value of r^2 and the adjusted value of r^2 for a given model [18]. The validity of the assumptions used in the modelling was checked by plotting the residuals against the fitted model [19], and the model itself was tested by random partition of the original data [20]. These statistical analyses were carried out using the software R version 3.0.1, running on an x86_64-w64-mingw32/x64 (64-bit) platform [21].

3. RESULTS

The mean (standard error, s.e.) age of the 86 patients was 39.4 (1.7) years. 31 patients were male and the remaining 55 female. At the time of follow-up, five of the patients had received six days' intravenous ceftriaxone treatment; one had received seven days' treatment; the remaining 80 patients had received the intravenous treatment for 12 days. The laboratory serum creatinine level normal range was 49 to 92 μM. One patient had a baseline value lower than this range (45 μM), while in seven cases the value was higher (up to 154 μM); in all these seven cases the estimated glomerular filtration rate was below 90 mL/min/1.73 m^2.

The baseline mean (s.e.) neutrophil count was 3.93×10^9 (0.16×10^9) L^{-1}. At follow-up, this was highly significantly reduced to 3.15×10^9 (0.15×10^9) L^{-1} ($t = 5.483$, $df = 85$, $p = 4.215 \times 10^{-7}$). These data are illustrated in the boxplot shown in Fig. (**1**).

Table 1. Final multifactor regression model.

	Coefficient	S.e.	*t*	*p*
Intercept	75.953	25.894	2.933	0.004
Haematocrit	159.245	64.780	2.458	0.016
Red blood cell count	-14.539	5.711	-2.546	0.013
Mean corpuscular volume	-0.724	0.291	-2.486	0.015
Neutrophil count	0.474	0.074	6.385	< 0.000 000 1
Total iron binding capacity	0.045	0.014	3.132	0.002
Calcium ion concentration	7.150	2.048	3.492	< 0.001
Corrected calcium ion concentration	-13.153	2.716	-4.844	< 0.000 01
Alkaline phosphatase	0.017	0.007	2.408	0.018

To evaluate the baseline predictors of the reduced neutrophil count, a multifactor linear regression model was developed using backward elimination and starting with the following factors: age, sex, haemoglobin, haematocrit, red blood cell count, mean corpuscular volume, mean corpuscular haemoglobin, mean corpuscular haemoglobin concentration, red cell distribution width, platelet count, mean platelet volume, white blood cell count, neutrophil count, lymphocyte count, monocyte count, eosinophil count, basophil count, erythrocyte sedimentation rate, sodium ion concentration, potassium ion concentration, chloride ion concentration, bicarbonate ion concentration, urea concentration, creatinine concentration, estimated glomerular filtration rate, bilirubin concentration, alkaline phosphatase, aspartate transferase, alanine transferase, lactate dehydrogenase, creatine kinase, gamma glutamyl transferase, total protein, albumin, globulin, calcium ion concentration, corrected calcium ion concentration, phosphate ion concentration, uric acid, glucose, triglycerides, total cholesterol, iron concentration, total iron binding capacity and transferrin saturation. The *p*-value threshold used for retaining potential predictors was set at 0.05 in the context of the overall change in the value of r^2 and the adjusted value of r^2 for a given multiple regression model. The coefficients for the final model are shown in Table **1**, with the final column representing the probability, for each coefficient, of a value greater than the modulus of the corresponding value of *t*. This model had a residual s.e. of 0.9267 (df = 77); multiple r^2 = 0.5506, adjusted r^2 = 0.504; F = 11.79, df = 8,77; $p = 8.292 \times 10^{-11}$.

The validity of the assumptions used in the modelling was checked by plotting the residuals against the fitted model. They were relatively uniformly scattered about zero, as shown in Fig. (**2**). The corresponding Q-Q normality plot is shown in Fig. (**3**) and shows that the residuals were aligned with the line indicated.

Finally, the regression model was tested by random partition of the original data into two parts, with training of the model using the first part and model testing with the second part. Defining delta, δ, for each patient, i, as δ_i = (*predicted value*)$_i$ – (*actual measured value*)$_i$, and taking the mean of all the δ_i values, a one-sample *t*-test verified that this mean was not significantly different from zero (mean = 0.071, t = 0.5722, df = 42, p = 0.5702; 95% confidence interval = –0.181 to 0.323).

4. DISCUSSION

The present study, the largest systematic study of its kind, has confirmed that daily intravenous ceftriaxone treatment for under a fortnight is indeed associated with a significant reduction in the neutrophil count. Furthermore, this reduction appears to be predictable according to the following linear regression model:

(Baseline neutrophil count ($\times 10^9$ L^{-1})) – (follow-up neutrophil count ($\times 10^9$ L^{-1}))

= 76 + 159.2 (baseline haematocrit) – 14.5 (baseline red blood cell count ($\times 10^{12}$ L^{-1}))

– 0.724 (baseline mean corpuscular volume (fL))

+ 0.474 (baseline neutrophil count ($\times 10^9$ L^{-1}))

+ 0.0448 (baseline total iron binding capacity (μM))

+ 7.15 (baseline calcium ion concentration (mM))

– 13.2 (baseline corrected calcium ion concentration (mM))

+ 0.0166 (baseline alkaline phosphatase (IU L^{-1})).

From this model, it is seen that higher levels of the following baseline factors are associated with a greater reduction in the neutrophil count whilst being treated with intravenous ceftriaxone: haematocrit, neutrophil count, total iron binding capacity and alkaline phosphatase. The calcium ion concentration appears to influence the neutrophil count in both directions, with a higher baseline calcium being associated with a greater reduction in the neutrophil count, but the opposite being true for the baseline corrected calcium ion concentration; elimination of either measure led to a reduction in the adjusted value of r^2 for the multiple regression model. Higher baseline values of the red blood cell count and mean corpuscular volume are associated with a diminished

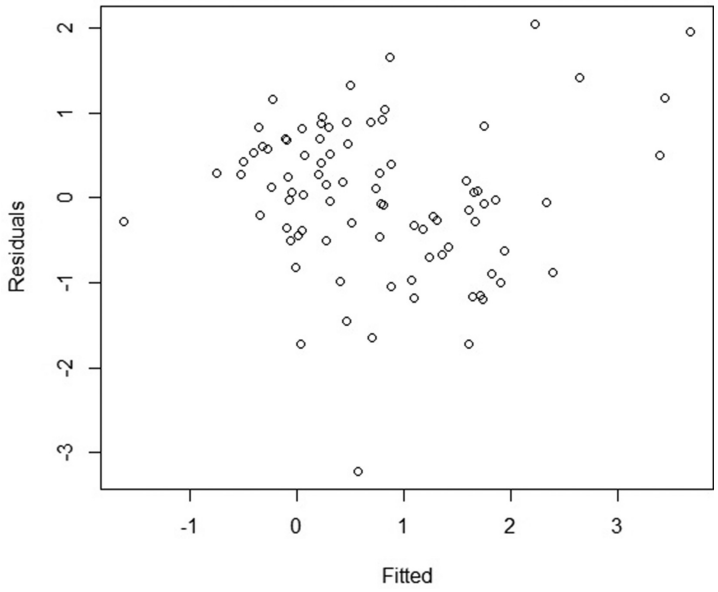

Fig. (2). Plot of residuals versus fitted values for the final multifactor regression model.

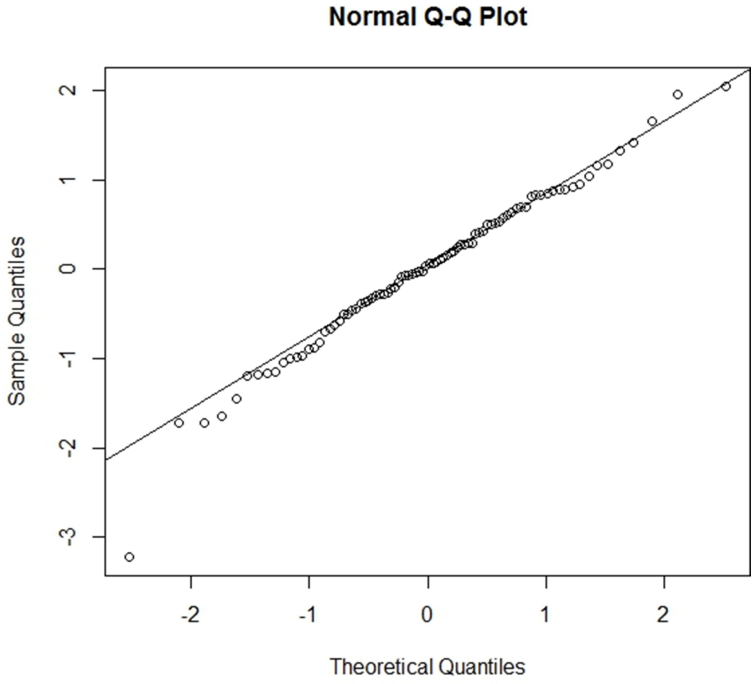

Fig. (3). Q-Q plot for the final multifactor regression model.

reduction in the neutrophil count; that is, they are protective factors. These findings readily give rise to testable hypotheses. For example, given that there is little binding of ceftriaxone to erythrocytes (*e.g.* only 5% in whole blood with a haematocrit of 0.5) [22], it could be hypothesised that as the haematocrit rises, so the plasma concentration of ceftriaxone rises, which might, in turn, be associated with an increased risk of a fall in the neutrophil count. The findings also show the importance of taking measures to ensure that the alkaline phosphatase level does not rise during ceftriaxone treatment; our group have previously shown the value of co-administration of alpha-lipoic acid and reduced glutathione in this regard [23].

The testing of the above multiple regression model yielded satisfactorily small values of δ_i with a relatively tight 95% confidence interval around the mean value of this parameter. A major limitation of the present study is its single centre design. It is recommended that the present systematic study should be repeated in a different setting, preferably in a study approximately as large as the present one, and that the above model should be tested with the new data.

Finally, it is good to note that the model developed here accounts for a reasonable proportion of the variance of the change in neutrophil count, based entirely on baseline measures which are readily available from routine haematology and biochemistry investigations.

ETHICS APPROVAL AND CONSENT TO PARTICIPATE

This study has received approval from AONMREC.

HUMAN AND ANIMAL RIGHTS

No animals were used in this research. All research procedures followed were in accordance with the ethical standards of the committee responsible for human experimentation (institutional and national), and with the *Helsinki Declaration* of 1975, as revised in 2008 (http://www.wma.net/en/20activities/10ethics/10helsinki/).

CONSENT FOR PUBLICATION

Not applicable.

CONFLICT OF INTEREST

The authors declare no conflict of interest, financial or otherwise.

ACKNOWLEDGEMENTS

Declared none.

Appendix 1. STROBE Checklist.

	Item No.	Recommendation
Title and abstract	1	(*a*) The study's design is indicated in the abstract
		(*b*) The abstract provides an informative and balanced summary of what was done and what was found. In particular, the objective is stated to be the first systematic study to determine whether six to 12 days' intravenous ceftriaxone treatment is associated with a reduction in the neutrophil count and the extent to which biochemical and/or haematological parameters routinely measured at baseline predict such a fall. The abstract also makes it clear that multifactor linear modelling was utilised.
Introduction		
Background/ rationale	2	The scientific background and rationale for the investigation being reported are given, and the seriousness of the side-effect of neutropenia associated with intravenous treatment with ceftriaxone is noted.
Objectives	3	The first systematic study to determine whether six to 12 days' intravenous ceftriaxone treatment is associated with a reduction in the neutrophil count and the extent to which biochemical and/or haematological parameters routinely measured at baseline predict such a fall.
Methods		
Study design	4	Key elements of study design early in the paper in the objectives (in the introduction to the paper) and in the methods section.
Setting	5	The medical records from a two-and-a-half year period were accessed of 86 patients receiving 2 g intravenous ceftriaxone daily for the treatment of Lyme borreliosis at a British medical centre specialising in environmental (functional) medicine, namely the Breakspear Medical Group, Hertfordshire House, Wood Lane, Paradise Industrial Estate, Hemel Hempstead HP2 4FD, United Kingdom. Baseline and follow-up haematological and biochemical blood indices were independently assessed at The Doctors' Laboratory (also known as TDL or DL), London, United Kingdom, the main laboratory of which is based at The Halo Building, 1 Mabledon Place, London WC1H 9AX, United Kingdom; the follow-up measures used in this study were all carried out within 12 days of starting this treatment.
Participants	6	Eligibility criteria included being a patient under treatment at the index medical centre during the two-and-a-half year period with 2 g ceftriaxone daily for Lyme borreliosis. Both males and females were eligible for inclusion and there was no age cut-off exclusion criterion.
		The patients acted as their own controls.
Variables	7	The dependent variable was the neutrophil count. All patients in this study were exposed to intravenous ceftriaxone at a dose of 2 g daily. The potential predictors considered were age, sex, haemoglobin, haematocrit, red blood cell count, mean corpuscular volume, mean corpuscular haemoglobin, mean corpuscular haemoglobin concentration, red cell distribution width, platelet count, mean platelet volume, white blood cell count, neutrophil count, lymphocyte count, monocyte count, eosinophil count, basophil count, erythrocyte sedimentation rate, sodium ion concentration, potassium ion concentration, chloride ion concentration, bicarbonate ion concentration, urea concentration, creatinine concentration, estimated glomerular filtration rate, bilirubin concentration, alkaline phosphatase, aspartate transferase, alanine transferase, lactate dehydrogenase, creatine

		kinase, gamma glutamyl transferase, total protein, albumin, globulin, calcium ion concentration, corrected calcium ion concentration, phosphate ion concentration, uric acid, glucose, triglycerides, total cholesterol, iron concentration, total iron binding capacity and transferrin saturation.
Data sources/ measurement	8	Age was calculated for each patient from the person's date of treatment and his or her date of birth. The calculation was performed using R version 3.0.1. The sex of each patient was obtained from his or her records. Assessments, carried out in a routine manner, by The Doctors' Laboratory (see above) were used to ascertain the values of the following variables: neutrophil count, haemoglobin, haematocrit, red blood cell count, mean corpuscular volume, mean corpuscular haemoglobin, mean corpuscular haemoglobin concentration, red cell distribution width, platelet count, mean platelet volume, white blood cell count, neutrophil count, lymphocyte count, monocyte count, eosinophil count, basophil count, erythrocyte sedimentation rate, sodium ion concentration, potassium ion concentration, chloride ion concentration, bicarbonate ion concentration, urea concentration, creatinine concentration, estimated glomerular filtration rate, bilirubin concentration, alkaline phosphatase, aspartate transferase, alanine transferase, lactate dehydrogenase, creatine kinase, gamma glutamyl transferase, total protein, albumin, globulin, calcium ion concentration, corrected calcium ion concentration, phosphate ion concentration, uric acid, glucose, triglycerides, total cholesterol, iron concentration, total iron binding capacity and transferrin saturation. There was just one group of patients and therefore comparability of assessment methods did not need to be formally assessed.
Bias	9	The validity of the assumptions used in the modelling was checked by plotting the residuals against the fitted model.
Study size	10	There were no previous such systematic studies of this nature. Therefore, it was not possible formally to carry out a sample size calculation. The results of this study, however, should enable future study size calculations to be performed by other groups who seek to replicate and build upon the present findings.
Quantitative variables	11	After checking for normality, the pre- and post-treatment mean values of the dependent variable were compared using the Student *t*-test. A corresponding boxplot was constructed. Multifactor linear regression modelling was carried out using backward elimination. These statistical analyses were carried out using the software R version 3.0.1, running on an x86_64-w64-mingw32/x64 (64-bit) platform.
Statistical methods	12	(*a*) The pre- and post-treatment mean values of the dependent variable were compared using the Student *t*-test. A corresponding boxplot was constructed. Multifactor linear regression modelling was carried out using backward elimination, with the *p*-value threshold for elimination set at 0.05 in the context of the overall change in the value of r^2 and the adjusted value of r^2 for a given model. The validity of the assumptions used in the modelling was checked by plotting the residuals against the fitted model, and the model itself was tested by random partition of the original data.
		(*b*) No subgroups were formally examined, but the overall regression model was tested by random partition of the original data.
		(*c*) It was pre-determined that any missing data would lead to the exclusion of the corresponding patient's overall data from this study.
		(*d, e*) Not applicable.
Results		
Participants	13	(*a*) The number of patients who were examined for eligibility, confirmed eligible, included in the study, completed follow-up, and analysed were 86 at each stage.
		(*b*) Not applicable.
		(*c*) Not applicable.
Descriptive data	14	(*a*) The mean (standard error) age of the patients was 39.4 (1.7) years. 31 patients were male and the remaining 55 female. At the time of follow-up, five of the patients had received six days' intravenous ceftriaxone treatment; one had received seven days' treatment; the remaining 80 patients had received the intravenous treatment for 12 days.
		(*b*) There were no missing data for each variable of interest.
		(*c*) As above.
Outcome data	15	As above.
Main results	16	(*a*) The baseline mean (standard error)

		neutrophil count was 3.93×10^9 (0.16×10^9) L^{-1}. At follow-up, this was 3.15×10^9 (0.15×10^9) L^{-1} ($t = 5.483$, $df = 85$, $p = 4.215 \times 10^{-7}$).
		(*b*) Continuous variables were not categorised.
		(*c*) Not applicable.
Other analyses	17	To evaluate the baseline predictors of the reduced neutrophil count, a multifactor linear regression model was developed using backward elimination and starting with the following factors: age, sex, haemoglobin, haematocrit, red blood cell count, mean corpuscular volume, mean corpuscular haemoglobin, mean corpuscular haemoglobin concentration, red cell distribution width, platelet count, mean platelet volume, white blood cell count, neutrophil count, lymphocyte count, monocyte count, eosinophil count, basophil count, erythrocyte sedimentation rate, sodium ion concentration, potassium ion concentration, chloride ion concentration, bicarbonate ion concentration, urea concentration, creatinine concentration, estimated glomerular filtration rate, bilirubin concentration, alkaline phosphatase, aspartate transferase, alanine transferase, lactate dehydrogenase, creatine kinase, gamma glutamyl transferase, total protein, albumin, globulin, calcium ion concentration, corrected calcium ion concentration, phosphate ion concentration, uric acid, glucose, triglycerides, total cholesterol, iron concentration, total iron binding capacity and transferrin saturation. The *p*-value threshold used for retaining potential predictors was set at 0.05 in the context of the overall change in the value of r^2 and the adjusted value of r^2 for a given multiple regression model. The final multifactor linear regression model was as follows: (baseline neutrophil count ($\times 10^9$ L^{-1})) – (follow-up neutrophil count ($\times 10^9$ L^{-1})) = 76 + 159.2(baseline haematocrit) – 14.5(baseline red blood cell count ($\times 10^{12}$ L^{-1})) – 0.724(baseline mean corpuscular volume (fL)) + 0.474(baseline neutrophil count ($\times 10^9$ L^{-1})) + 0.0448(baseline total iron binding capacity (μM)) + 7.15(baseline calcium ion concentration (mM)) – 13.2(baseline corrected calcium ion concentration (mM)) + 0.0166(baseline alkaline phosphatase (IU L^{-1})). This model had a residual standard error of 0.9267 ($df = 77$); multiple $r^2 = 0.5506$, adjusted $r^2 = 0.504$; $F = 11.79$, $df = 8,77$; $p = 8.292 \times 10^{-11}$. The validity of the assumptions used in the modelling was checked by plotting the residuals against the fitted model. They were relatively uniformly scattered about zero. The corresponding Q-Q normality plot showed that the residuals were aligned with the line indicated in Fig. (**3**) of the paper. The regression model was tested by random partition of the original data into two parts, with training of the model using the first part and model testing with the second part. Defining delta, δ, for each patient, i, as $\delta_i = (predicted\ value)_i - (actual\ measured\ value)_i$, and taking the mean of all the δ_i values, a one-sample *t*-test verified that this mean was not significantly different from zero (mean = 0.071, $t = 0.5722$, $df = 42$, $p = 0.5702$; 95% confidence interval = –0.181 to 0.323).
Discussion		
Key results	18	At follow-up, the mean (standard error) neutrophil count had fallen from 3.93×10^9 (0.16×10^9) L^{-1} to 3.15×10^9 (0.15×10^9) L^{-1} ($p < 0.000001$). This reduction was predictable according to the following multifactor linear regression model: (baseline neutrophil count ($\times 10^9$ L^{-1})) – (follow-up neutrophil count ($\times 10^9$ L^{-1})) = 76 + 159.2(baseline haematocrit) – 14.5(baseline red blood cell count ($\times 10^{12}$ L^{-1})) – 0.724(baseline mean corpuscular volume (fL)) + 0.474(baseline neutrophil count ($\times 10^9$ L^{-1})) + 0.0448(baseline total iron binding capacity (μ M)) + 7.15(baseline calcium ion concentration (mM)) – 13.2(baseline corrected calcium ion concentration (mM)) + 0.0166(baseline alkaline phosphatase (IU L^{-1})). The residuals were normally distributed and model testing by random partition of the original data into two parts, with training of the model using the first part and model testing with the second part, gave highly satisfactory results.
Limitations	19	The study was carried out in a single centre. It requires repeating in a different setting.
Interpretation	20	From the results of this study, it appears that intravenous ceftriaxone treatment is associated with a fall in neutrophils, which may be predictable by routine baseline blood indices.
Generalisability	21	It is difficult to comment formally on the generalisability (external validity) of the study results as this is the first systematic study in this area. This study clearly needs to be repeated, as mentioned above.
Other information		
Funding	22	None.

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