



# A Randomized Open Label Parallel Design Clinical study of Rosastim, the Biosimilar Granulocyte Colony-stimulating Factor, for Chemotherapy-induced Neutropenia

Mehdi Mohammadzadeh<sup>1</sup>, Tayebeh Ghari\*<sup>2</sup>, Hamid Rezvani<sup>3</sup>, Babak Salimi<sup>3</sup>, Najva Kashani<sup>4</sup> and Mahindokht Sayadinia<sup>5</sup>

<sup>1</sup>Department of Pharmaco-economic and Pharmacy management, School of Pharmacy, Shahid Beheshti University of Medical Sciences, Tehran, Iran

<sup>2</sup>Department of Pharmaceutics, Faculty of pharmacy, Alborz University of Medical Sciences, karaj, Iran

<sup>3</sup>Department of Hematology and Oncology, Taleghani General Hospital, Shahid Beheshti University of Medical Sciences, Tehran, Iran

<sup>4</sup>Research and Development Department, Behin Tamin Rosamed Company, Tehran, Iran

<sup>5</sup>Department of Hematology and Oncology, Taleghani General Hospital, Tehran, Iran

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## Address for Correspondence

Department of Pharmaceutics,  
Faculty of pharmacy,  
Alborz University of Medical Sciences,  
karaj, Iran  
E-mail: [tayebehghari@gmail.com](mailto:tayebehghari@gmail.com)

## ABSTRACT

**Background:** There are little differences between biosimilars and reference products. Therefore, these products must be compared to the reference medicinal products in terms of safety and efficiency.

**Aims:** This open label study has been done to compare adverse drug reaction and clinical efficiency of Rosastim and Neupogen in order to show the same efficiency and safety in the gastric cancer through two parallel groups.

**Study Design:** Our research is a randomized open-label parallel design clinical study

**Methods:** 5 µg/kg subcutaneously G-CSF therapy were randomly injected to eligible consenting subjects (60 patients) the day after chemotherapy for five days. All patients were followed up every three weeks. neutrophil and lymphocyte counts and adverse drug reactions (bone pain, nausea, fever, allergy, liver enzyme increasing) were measured in those patients according to a protocol.

**Results:** Neutrophil and lymphocyte counts did not have significant differences in two groups (P-value > 0.05). Furthermore, according to homogeneity chi-square test, frequency of adverse drug reactions in two groups in all cycles did not have significant differences (P-value > 0.05).

**Conclusion:** Our study suggested that Rosastim is as same as Neupogen in case of effectiveness and tolerance when used as an

adjuvant in patients with gastric cancer. Therefore, this biosimilar is a suitable and an alternative option for patients need G-CSF.

**Keywords:** Biosimilar, Clinical study, Rosastim, Neupogen.

## INTRODUCTION

Over the past 70 years, mortality and gastric cancer incidence have reduced<sup>1</sup>. Nevertheless, the second cause of cancer death and the fourth most common cancer is gastric cancer<sup>2,3</sup>. Gastric tumor types dominate in groups with different races, geographic locations and socio-economic groups. It is also common in populations with different genetic and pathologic history<sup>4-7</sup>. *Helicobacter pylori* infections<sup>8,9</sup>, dietary factors<sup>10,11</sup>, tobacco<sup>12,13</sup>, obesity<sup>14</sup> and oxidative stress<sup>15</sup> are the main reason of gastric cancer. Symptoms of gastric cancer are including gastric pain, blood in stool, vomiting, weight loss for no reason, trouble swallowing and constipation or diarrhea<sup>16</sup>. Chemotherapy can be used in different ways to treat gastric cancer. The most prevalent side effects of cytotoxic chemotherapy are neutropenia and myelosuppression. In cancer patients, neutropenia (less than 500 neutrophils/microL) is the most important risk factor for infections. The incidence and severity of infectious complications are related to depth and duration of neutropenia, with the highest risk if neutrophils are less than 100/microL for more than a week<sup>17</sup>. Serious gastrointestinal and pulmonary infections as well as sepsis are the inevitable results of severe neutropenia which can cause a delay in subsequent chemotherapy cycles. Granulocyte colony-stimulating factor, G-CSF, is the most important recovery factor of neutrophils. It has been shown to help prevent neutropenia in certain subgroups of cancer patients undergoing chemotherapy<sup>18</sup>.

It acts in different stages of myeloid cell development as a positive

granulopoiesis regulator. G-CSF increases phagocytosis, chemotaxis and oxidative metabolism. The selective effects of G-CSF in comparison with many other cytokines is via a high-affinity G-CSF-specific receptor<sup>19</sup>. The natural human G-CSF is a glycoprotein with a single polypeptide chain with 174 or 177 amino acids. The use of G-CSF in combination with chemotherapy drugs reduces period of treatment and duration of neutropenia. Filgrastim, as a non-glycosylated recombinant methionyl human G-CSF, is composed of 175 amino acids and expressed in *E. coli*. It is the first FDA approved granulocyte colony-stimulating factor under the trade name of Neupogen (sourced from Amgen Inc, USA). Depending on the indication, it is administered with dose of 1 to 10 µg/kg/day via SC or IV routes. It has some side effects including bone pain, fever, allergic response, nausea and liver enzyme increasing specially LDH and ALP. For medical uses, a lot of Filgrastim biosimilars have been currently approved or are in the development. If these products have the same quality, safety and efficiency in comparison to Neupogen, they could be a suitable alternative.

Considering the above-mentioned, G-CSF is the most recovery factor of neutropenia caused by chemotherapy in patients who are suffering from cancer. Since, the mortality of stomach cancer is the first cause of death due to cancer in both sexes in Iran, G-CSF is the best formulation to reduce mortality rate of gastric cancer. Because of high cost of Neupogen, our researcher team has developed a newly

generic formulation of G-CSF with the name of Rosastim in Iran. The aim of this generic formulation is to reduce cost of treatment by offering lower-cost alternatives to high-priced Neupogen. Rosastim could cause delivery of drugs to the patients with lower cost and higher availability in comparison to Neupogen. It is prepared in pre-filled syringes composed of sterile and preservative-free solution for injection consisting of 300 µg Filgrastim, D-sorbitol, tween 20, sodium acetate buffer and water for subcutaneous or IV injections.

There are minor differences between biosimilars and reference products. Therefore, these products must be compared to the reference medicinal products in terms of safety and efficiency. In our previous study, physiochemical and biological activities of Rosastim were evaluated. Rosastim has similar physiochemical and biological activities in comparison with Neupogen. The aim of our present study is to conduct the comparable study to specify the clinical efficiency and adverse effects of Rosastim and Neupogen. In order to show the biosimilarity between Rosastim and Neupogen, the clinical study is also required. This clinical study between Rosastim and Neupogen has not been done before. In order to do this, the clinical program which composed of an open-label clinical trial on patients who suffer from gastric cancer was done. Patients were under treatment of Ajani modified chemotherapy treatment were chosen because of the heavy and long period of their treatments. In our clinical study, frequency of adverse drug reactions like fever, bone pain, nausea, allergy and liver enzyme increasing, determination of neutrophil and lymphocyte counts and amounts of neutropenic days (neutrophil counts below 1500/mm<sup>2</sup>) were evaluated. If Rosastim has comparable clinical efficiency and adverse drug

reactions to Neupogen, it could be an effective alternative.

## MATERIALS AND METHODS

### Patients

Patients with gastric cancer attending the hematology and oncology department at Taleghani General Hospital (Tehran, Iran) were screened for eligibility of this study. If the patients have the following characteristics, they will be eligible: age between 18 and 70 years, gastric cancer defined by biopsy and pathology, filling out the consent form and possibility of patient compliance. Patients who have one of the following problems were excluded from the study: inaccessibility for follow up, impaired initial CBC, sensitivity to any of the G-CSF products, history of malignancy, cancer relapsing or resistance, depression or mental illness.

### Procedures

This study was a randomized open-label parallel design clinical trial. For comparing the efficiency of two very similar treatments, an open-label trial is required. An Open-label trial is a kind of clinical trials in which the administered treatment has been known for both the investigators and patients. It may still be randomized. In a parallel study, one group of treatment receives one drug and another group receives a different drug.

This clinical trial is also an Equivalence /noninferiority trial which means that new drug effect is equal or even better than that of a control drug. Our study protocol is based on 1975 declaration of Helsinki ethical guidelines. 5 µg/kg subcutaneously G-CSF therapy was randomly injected to the eligible consenting subjects the day after chemotherapy for five days. Test product samples were Rosastim 300 µg/0.5 ml (with 24 months expiry date at the time of administration) and reference

product samples were Neupogen (administrated 24 months before expiry). Patients were divided into two groups, by use of a permuted balance block randomized method. Chemotherapy protocol was modified Ajani including 5-FU 750 mg/m<sup>2</sup> Cisplatin, 75 mg/m<sup>2</sup> and Taxoter 75 mg/m<sup>2</sup> administration every 3 weeks. In order to reduce the adverse drug reactions, 8 mg Dexamethasone was administrated twice a day for three days. Neutrophil and lymphocyte counts were measured on day five from samples blood until normalization of their numbers. After that, patients without any clinical problems were discharged. All patients were followed up every three weeks. They were evaluated based on a protocol which included neutrophil and lymphocyte counts measurement, amounts of neutropenic days and adverse drug reactions (bone pain, nausea, fever, allergy, liver enzyme increasing).

#### Statistical analysis

Analysis was done after completion of six-month follow up. SPSS statistical package, version 21.0 (SPSS, Chicago, IL, USA) was used to analyze the differences between Rosastim and Neupogen groups with different outcome measures. The P-value < 0.05 is considered as significant difference in this study.

## RESULTS

At this study, 60 patients who were found eligible were randomly assigned to Rosastim and Neupogen. There were 29 patients in Neupogen group and 31 patients in Rosastim group. Numbers of patients according to the gender were 8 and 7 females and 21 and 24 males for Neupogen and Rosastim respectively. As shown in Table 1, no significant difference was seen in two groups according to the gender with person Chi-Square tests (P-value > 0.05).

The difference between patients age has been investigated in order to continue the study. Mean and standard deviation of age were 54.06 and 6.49 for Neupogen group and 54.35 and 11.36 for Rosastim group. Standard errors of mean were 1.20 and 2.04 for Neupogen and Rosastim group respectively. Based on these data, effect of age in two groups was evaluated by independent sample t test (Table 2). According to the test, there was no significant difference between two groups according to age (P-value > 0.05).

In this clinical trial, 60 patients in two groups were treated with Neupogen and Rosastim for six cycles. In each cycle, neutrophil and lymphocyte counts and amounts of neutropenic days were measured for five days. The results of cycle one, two, three and four for neutrophil and lymphocyte counts have been shown in Fig. 1 and Fig. 2 (the results for other cycles have not been shown). Independent t-student test was used for evaluating the effects of these two drugs on the neutrophil and lymphocyte counts and there was no significant difference in two groups according to neutrophil and lymphocyte counts in all cycles (P-value > 0.05). For analysis of the neutropenic days, Mann-Whitney test was used. There was no significant difference in two groups regarding to neutropenic days (P-value > 0.05).

In another part of this study, increasing or decreasing in some adverse drug reactions of chemotherapy agents in two groups was evaluated. In order to determine the homogeneity of two groups, homogeneity chi-square test was used. Frequency of fever, bone pain, nausea allergy and liver enzyme increasing in two groups in cycle 1 have been shown in Fig. 3 (Data for other cycles have not been shown). According to homogeneity chi-square test, frequency of adverse drug reactions does not

have any significant differences in two groups in all cycles (date have not been shown) (P-value > 0.05).

## DISCUSSION

Biosimilars are a new class of drugs intended to offer comparable safety and efficacy to the reference, off-patent biological<sup>20</sup>. A generic drug is a copy that is the same as a branded drug, in dosage, safety, strength, how it is taken, quality, performance and intended use<sup>21</sup>. For a generic version of a small molecule, a single bioequivalence study is mostly enough to determine the safety and efficiency of the products. But this single study is not sufficient in the case of biopharmaceuticals. Because of the different manufacturing processes, complex structure and process related impurities, biosimilar cannot be replicated. Secondary to complex protein structure, they are also more prone to acute and chronic immune responses<sup>22</sup>. Therefore, they may not have the same characteristics with the reference product. As a result, generic form of biopharmaceuticals does not exist, but development of similar biological medicinal products has been enabled by legislation. Over the next few years, , field of biosimilars is expected to rapidly expand because of patent expiry of a number of important biopharmaceuticals<sup>23,24</sup>.

Our previous study has been demonstrated that Rosastim has the similar physiochemical and biological activities in comparison to Neupogen. In this study, a randomized open-label parallel design clinical trial was used to show the clinical similarity of these two drugs in patients who suffer from gastric cancer. Based on the previous randomized trials, single agent chemotherapy in gastric cancer did not lead to suitable results regarding efficiency. Therefore, it provided the reason for the development of combination regimens. In several clinical trials, response rates were

increased in combination regimens in comparison to the single therapy. Furthermore, consistent survival benefit was seen in the combination therapy<sup>25, 26</sup>. For these reasons, in our study a combination chemotherapy protocol was selected. This protocol was modified Ajani including 5-FU 750 mg/m<sup>2</sup>, Cisplatin 75 mg/m<sup>2</sup> and Taxoter 75 mg/m<sup>2</sup> administration every 3 weeks. Neutrophil and lymphocyte counts were selected as two major criteria for evaluation of the efficiency of Rosastim. In the past decade, potential advantages of adjunctive therapy with G-CSF has been proved to ameliorate or prevent severe neutropenia and its potentially life threatening outcomes with many clinical trials. The most common dose limiting side effects of cytotoxic chemotherapy is neutropenia<sup>27</sup>. Therefore, we decided to study the effect of these two products on neutrophil and lymphocyte counts in our patients coming to the hospital. Based on this study, neutrophil and lymphocyte counts did not have any significant difference in two groups. It means that Rosastim has the same efficiency as Neupogen.

For evaluation of safety, common adverse drug reactions of G-CSF were studied. Regarding to the previous study, the most common adverse reactions of G-CSF are fever, bone pain, nausea, allergy and liver enzyme increasing<sup>28</sup>. Therefore, we chose them and evaluated the effects of Rosastim and Neupogen on their frequency. According to this study, there was no significant difference in frequency of adverse drug reactions in two groups. According to the above mentioned results, it seems that Rosastim is as same sa Neupogen in case of effectiveness and tolerance when used as an adjuvant in patients with gastric cancer. Therefore, this biosimilar could be used as a suitable and an alternative option for patients need G-CSF.

## CONCLUSION

According to our study, there was no significant difference in two groups according to neutrophil and lymphocyte counts and frequency of adverse drug reactions. Therefore, Our study suggested that Rosastim is as effective and well tolerated as Neupogen when used as adjuncts in patients with gastric cancer. This biosimilar could be used as a suitable and an alternative option for patients need G-CSF.

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## REFERENCES

- Parkin D, Pisani P, Ferlay J. Estimates of the worldwide incidence of eighteen major cancers in 1985. *Int J Cancer*. 1993;54:594-606.
- Parkin D, Bray F, Devesa S. Cancer burden in the year 2000. The global picture. *Eur J Cancer*. 2001;37 Suppl 8:S4-S66.
- Brown L, Devesa S,;. Epidemiologic trends in esophageal and gastric cancer in the United States. *Surg Oncol Clin N Am*. 2002;11:235-56.
- Parkin D, Whelan S, Ferlay J. Cancer Incidence in Five Continents. France 1997.
- Nomura A. Cancer Epidemiology and Prevention. 2nd ed ed. Schottenfeld D, Fraumeni J, editors. New York: Oxford University Press; 1996.
- El-Serag H, Mason A, Petersen N, Key C. Epidemiological differences between adenocarcinoma of the oesophagus and adenocarcinoma of the gastric cardia in the USA. *Gut*. 2002;50:368-72.
- Powell J, McConkey C. The rising trend in oesophageal adenocarcinoma and gastric cardia. *Eur J Cancer Prev*. 1992;1:265-69.
- Buckley M, O'Shea J, Grace A, English L, Keane C, Hourihan D, et al. A community-based study of the epidemiology of Helicobacter pylori infection and associated asymptomatic gastroduodenal pathology. *Eur J Gastroenterol Hepatol*. 1998;10:375-79.
- Parsonnet J, Friedman G, Vandersteen D, Chang Y, Vogelman J, Orentreich N, et al. Helicobacter pylori infection and the risk of gastric carcinoma. *N Engl J Med*. 1991;325:1127-31.
- O'Connor H, Schorah C, Habibzedah N, Axon A, Cockel R. Vitamin C in the human stomach: relation to gastric pH, gastroduodenal disease, and possible sources. *Gut*. 1989;30:436-42.
- Lee S, Kang D, Shim K, Choe J, Hong W, Choi H. Effect of diet and Helicobacter pylori infection to the risk of early gastric cancer. *J Epidemiol*. 2003;13:162-68.
- Chao A, Thun M, Henley S, Jacobs E, McCullough M, Calle E. Cigarette smoking, use of other tobacco products and stomach cancer mortality in US adults: The Cancer Prevention Study II. *Int J Cancer*. 2002;101:380-89.
- Koizumi Y, Tsubono Y, Nakaya N, Kuriyama S, Shibuya D, Matsuoka H, et al. Cigarette smoking and the risk of gastric cancer: a pooled analysis of two prospective studies in Japan. *Int J Cancer* 2004; 112:1049-55.
- Calle E, Rodriguez C, Walker-Thurmond K, Thun M. Overweight, obesity, and mortality from cancer in a

- prospectively studied cohort of U.S. adults. *N Engl J Med.* 2003;348:1625-38.
15. RI S, B A. Oxidative Stress and Cancer: Role of Anti Carcinogenic Herbs and Spices. *American Journal of Phytomedicine and Clinical Therapeutics.* 2013;3:351-69.
  16. Kasper D, Fauci A, Hauser S, Longo D, Jameson JL, Loscalzo J. Harrison, s Principales of Internal Medicine. New York: *McGraw-Hill*; 2012.
  17. Montemurro F, Gallicchio M, Aglietta M. Prevention and treatment of febrile neutropenia. *Tumori.* 1997;83(2 Suppl) :S15-9.
  18. García-Carbonero R, Mayordomo J, Tornamira M, López-Brea M, Rueda A, Guillem V, et al. Granulocyte colony-stimulating factor in the treatment of high-risk febrile neutropenia: a multicenter randomized trial. *J Natl Cancer Inst.* 2001;93(1):31-8.
  19. Robert AW. G-CSF: a key regulator of neutrophil production, but that's not all! *Growth Factors.* 2005;23(1):33-41.
  20. G D, E A, K D. Biosimilars of biological drug therapies: regulatory, clinical and commercial considerations. *Drugs.* 2011 Aug 20;71(12):1527-36.
  21. Rohilla S, Marwaha RK, Singal GL, Nanda A. Quality of branded-generics versus branded products for Amoxicillin trihydrate and Potassium clavulanate tablets. *International Research Journal of Pharmaceutical Sciences.* 2011;02(01).
  22. G D, K D, E H, E. A. Clinical trial design in biosimilar drug development. *Invest New Drugs.* 2013;31(2):479-87.
  23. European Medicines Agency (EMA) Committee for Medicinal Products for Human Use (CHMP). Guideline on similar biological medicinal products containing biotechnology-derived proteins as active substance: non-clinical and clinical issues." from 2006; Available from: <http://www.ema.europa.eu/pdfs/human/biosimilar/4283205en.pdf>.
  24. Ajani J. Optimizing docetaxel chemotherapy in patients with cancer of the gastric and gastroesophageal junction: evolution of the docetaxel, cisplatin, and 5-fluorouracil regimen. *Cancer.* 2008;113(5):945-55.
  25. Murad A, Santiago F, Petroianu A, Rocha P, Rodrigues M, Rausch M. Modified therapy with 5-fluorouracil, doxorubicin, and methotrexate in advanced gastric cancer. *Cancer.* 1993;72(1):37-41.
  26. Pyrhonen S, Kuitunen T, Nyandoto P, Kouri M. Randomized comparison of fluorouracil, epidoxorubicin and methotrexate (FEMTX) plus best supportive care alone in patients with non-resectable gastric cancer. *Br J Cancer.* 1995;71:587-91.
  27. Ghalaut P, Sen R, Dixit G. Role of granulocyte colony stimulating factor (G-CSF) in chemotherapy induced neutropenia. *J Assoc Physicians India.* 2008;56:942-4.
  28. Harada M, Nagafuji K, Fujisaki T, Kubota A, Mizuno S, Takenaka K, et al. G-CSF-induced mobilization of peripheral blood stem cells from healthy adults for allogeneic transplantation. *J Hematother.* 1996;5(1):63-71.

**Table 1.** Person Chi-Square tests for statistical analysis of gender effects in two groups

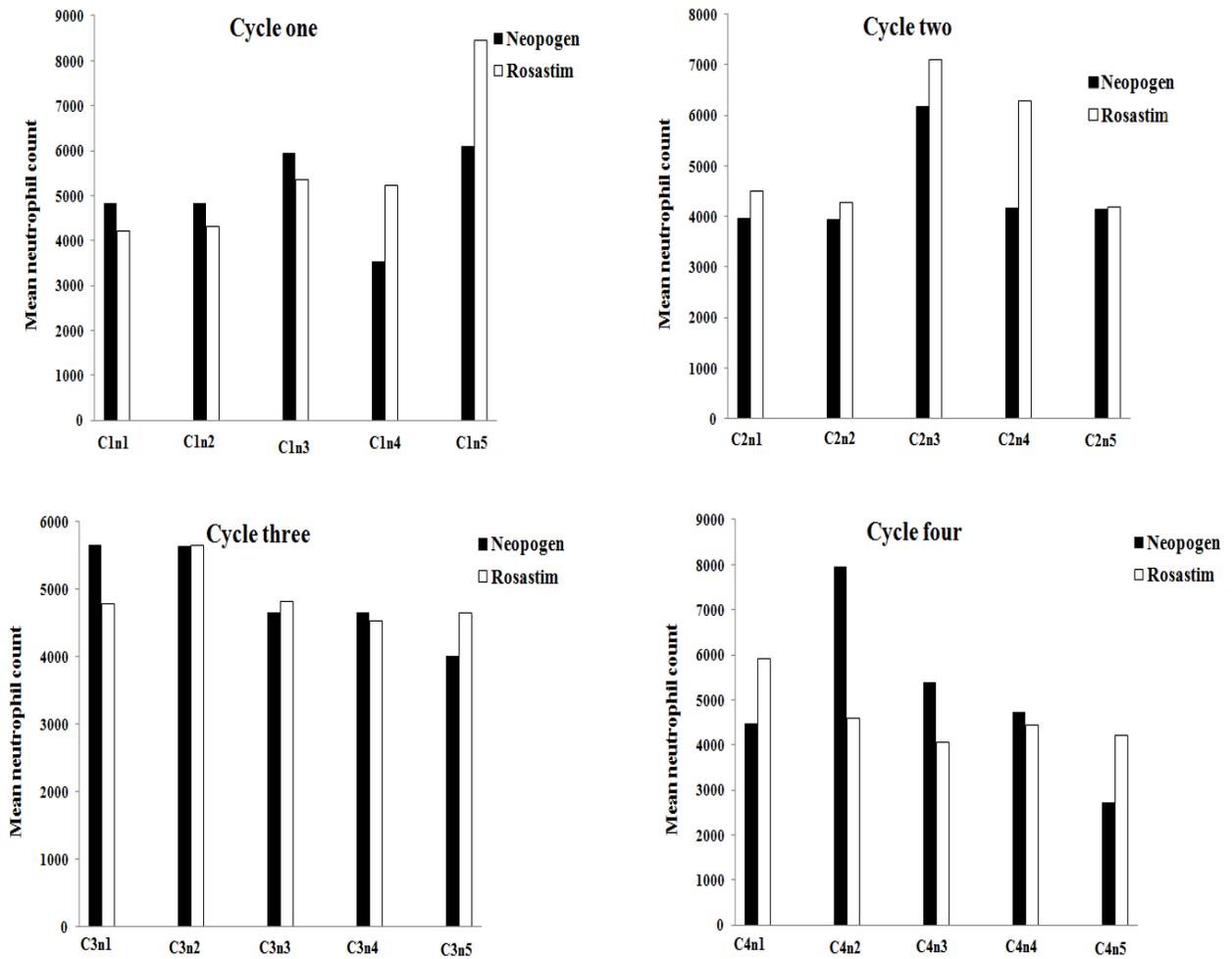
	Value	df	Asymp. Sig. (2-sided)	Exact Sig. (2-sided)	Exact Sig. (1-sided)
Pearson Chi-Square	.200a	1	.655		
Continuity Correction <sup>b</sup>	.022	1	.881		
Likelihood Ratio	.200	1	.655		
Fisher's Exact Test				.769	.440
Linear-by-Linear Association	.197	1	.657		
N of Valid Cases <sup>b</sup>	60				

a.0 cells (.0%) have expected count less than 5. The minimum expected counts is 7.25

b.computed only for 2x2 table

**Table 2.** Independent sample t-test for evaluation of effect of age in two groups

		Levene's Test for Equality of Variances		t-test for Equality of Means						
		F	Sig.	t	df	Sig. (2-tailed)	Mean Difference	Std. Error Difference	95% Confidence Interval of the Difference	
									Lower	Upper
age	Equal variances assumed	.537	.467	-.119	58	.906	-.28587	2.41180	-5.11361	4.54186
	Equal variances not assumed			-.121	48.311	.905	-.28587	2.37068	-5.05165	4.47990



**Figure 1.** Mean neutrophil counts in cycle one, two, three and four

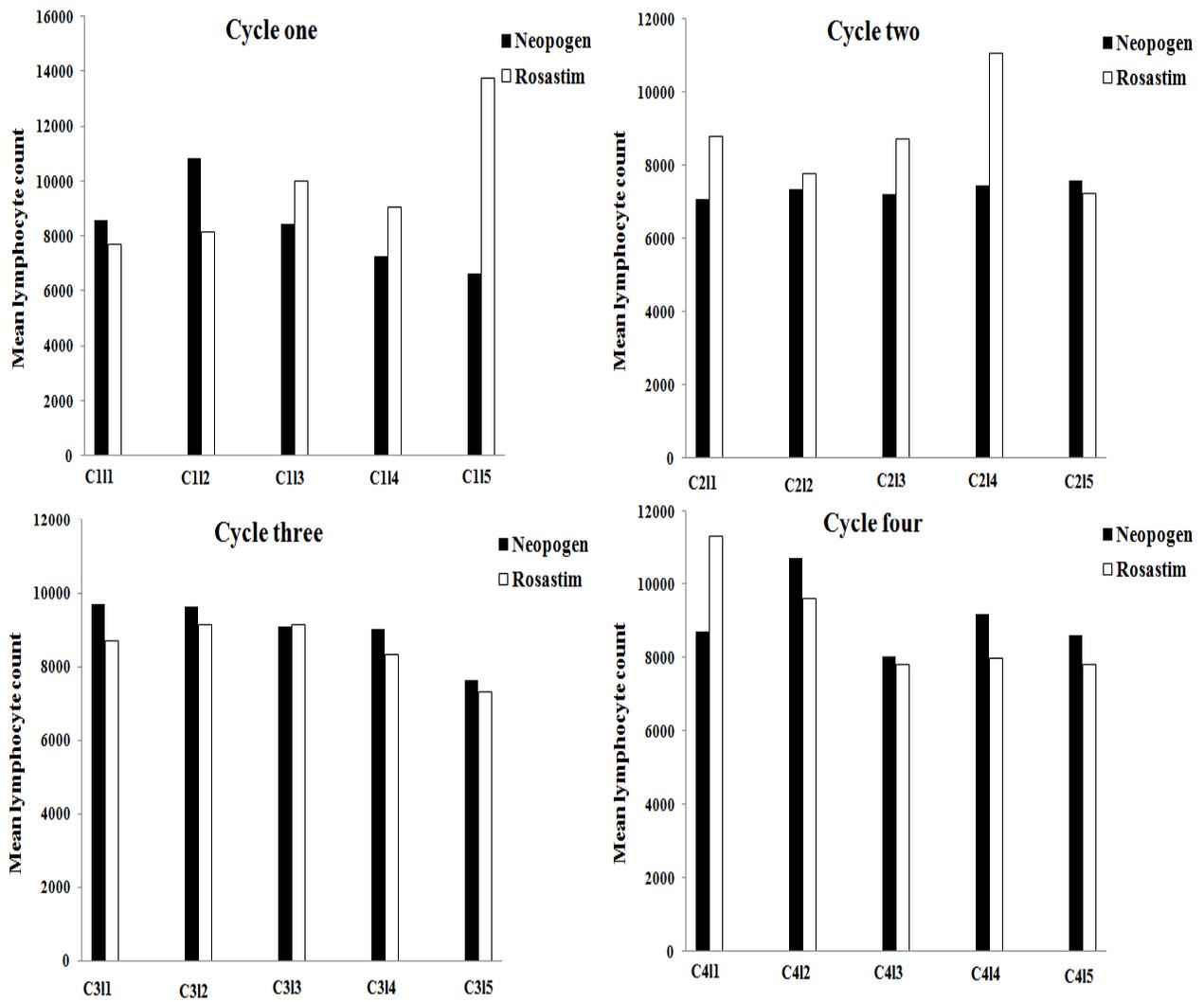
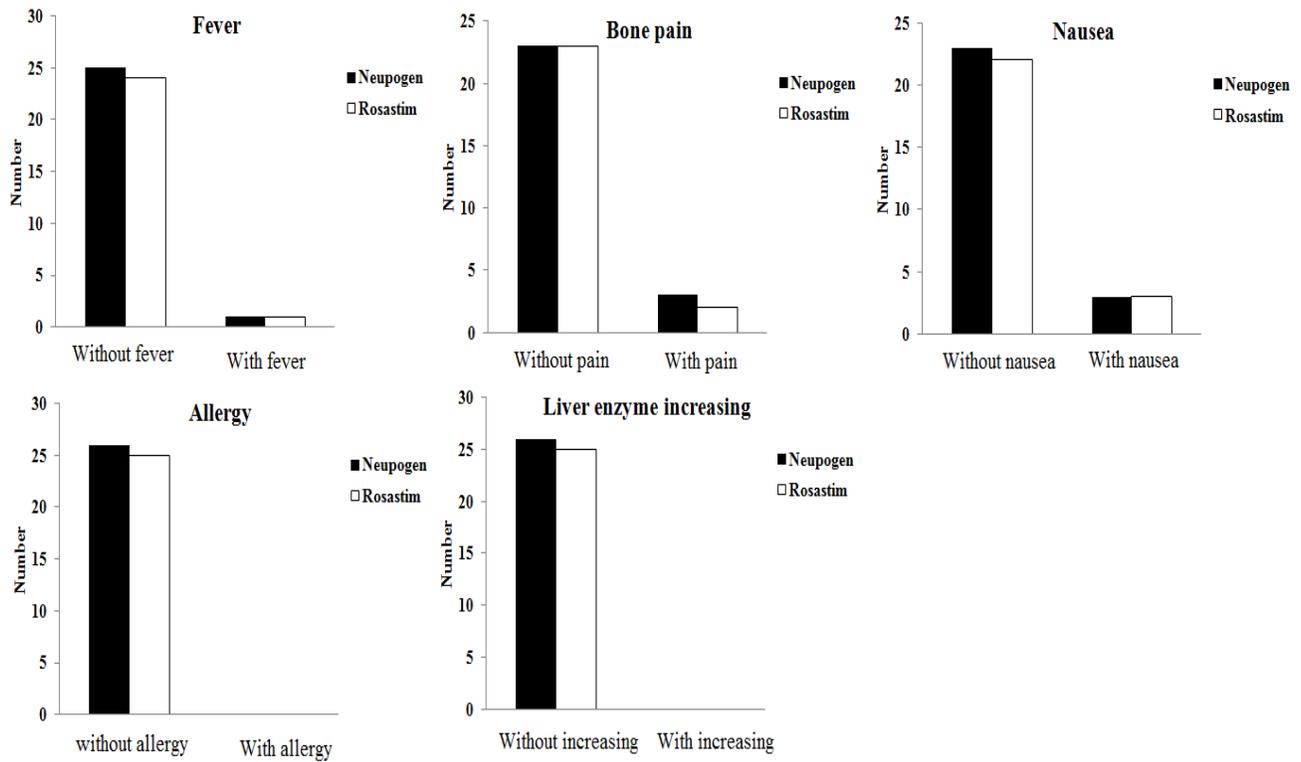


Figure 2. Mean lymphocyte counts in cycle one, two, three and four



**Figure 3.** Frequency of fever, bone pain, nausea allergy and liver enzyme increasing in two groups in cycle 1