

# Granulocyte Colony Stimulating Factor in Burn Patients with Neutropenia

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## ABSTRACT

**Objective:** To assess the role of granulocyte-colony stimulating factor (G-CSF) for improving neutropenia in burns patients with neutropenia.

**Study Design:** Experimental study.

**Place and Duration of Study:** Jinnah Burn and Reconstructive Surgery Centre, Lahore, from May to October 2017.

**Methodology:** Patients with burn injury, having absolute neutrophil count (ANC) <500 /  $\mu$ L or where it was expected to decrease to <500/ $\mu$ L within the next 48 hours, were recruited in the study. A detailed demographic profile of patients was taken, burn site was evaluated, and sample collection by phlebotomy was done in the complete blood count (CBC) vial. Samples were run in a CBC analyser and verification of neutrophil count on the Neubauer chamber was done. ANC was taken for 3 days for each patient. Injection Filgrastim was given 300  $\mu$ g subcutaneous (S/C) or intravenous (I/V) once daily until the neutropenia improved. Improvement was categorised as good, moderate and poor, depending on the number of days for improvement in ANC. The response was further stratified on the basis of age, gender and percentage of burn.

**Results:** A total of 39 patients with mean age of  $32.1 \pm 14.4$  years included 84.6% (n=33) males and 15.4% (n=6) females. Mean percentage of burn was  $40.5 \pm 15.7\%$ . In 12-40 years of age, there were 30/39 (76.9%) patients. Among them, 11/30 (36.6%) were good, 13/30 (43.3%) were moderate, and 6/30 (20%) were poor responders. In 41-70 years of age, there were 9/39 (23.1%) patients. Among them, 2/9 (22.2%) were good, 4/9 (44.44%) were moderate, and 3/9 (33.3%) were poor responders (p = 0.616).

**Conclusion:** The addition of G-CSF injections to the standard treatment of burn injury markedly improve the neutrophil counts in burn patients with neutropenia.

**Key Words:** Granulocyte colony stimulating factor, Burn, Neutropenia.

## INTRODUCTION

Human granulocyte-colony stimulating factor (G-CSF) is regulatory cytokine which helps neutrophils production. Both *in vitro* and *in vivo* studies have shown the increase neutrophil production when it stimulates hematopoietic progenitor cells.<sup>1-8</sup> G-CSF has shown improvement in neutrophil functions as well. This improvement suggests its role in host defence in neutropenic as well as non-neutropenic immunocompromised patients. Treatment with G-CSF, in combination with antibiotics, is tried in critically ill patients like burns, complicated diabetes mellitus, brain injury, community acquired pneumonia and neonatal bacterial sepsis with non-neutropenic patients.<sup>9-14</sup>

Medical treatment of patients with neutropenia is based on the cause of illness, duration and severity and is mostly supportive. Specific treatment in neutropenia is

required in fever and infection to treat the disease. G-CSF use as treatment in chronic neutropenia is also effective as it specifically stimulates the functionally active neutrophils. Filgrastim and pegfilgrastim are examples of G-CSF.

The availability of G-CSF has improved the management of agranulocytosis. Its administration has shown to improve the recovery time and duration of infection, if used before the establishment of sepsis. The G-CSF is agent of choice in severe congenital neutropenia (SCN) and cyclic neutropenia (CN) with associated serious infections and now increasingly used in neutropenia of burn patients.

Severe burn is a form of injury with resultant impairment of immune response of the body leading to multi-organ failure. In major burns, this immunosuppression and loss of skin barrier leads to increase risk of infection and sepsis. This alteration of immune response also leads to reduce number and function of neutrophils.

Myeloid growth factors, specifically G-CSF, may shorten the duration of neutropenia in patients with different total body surface area (TBSA). The main purpose of this study was to assess the role of G-CSF to improve neutropenia in patients with burn injuries. There is lack of literature evidence from our part of the world. The study was designed to produce an evidence of G-CSF use and its role in improvement of neutrophil count in our population.

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The objective of this study was to assess the role of G-CSF for improving neutropenia in patients with burn injuries.

## METHODOLOGY

After approval from ethical committee, this study was conducted at Jinnah Burn and Reconstructive Surgery Centre (JB & RSC), Lahore, from May to October 2017. A total of 39 burn patients with neutropenia were recruited through a non-probability / purposive sampling. Sample size was calculated with 95% confidence interval and 10% margin of error, assuming 92% patient neutropenia will be improved. All the burn patients having the absolute neutrophil count  $<500/\mu\text{L}$  were included. Neutropenia was defined as an absolute neutrophil count (ANC) of  $<500/\mu\text{L}$  or an ANC that is expected to decrease to  $<500/\mu\text{L}$  within the next 48 hours. Profound neutropenia was defined as an ANC  $<100/\mu\text{L}$ . Patients with ANC  $>500/\mu\text{L}$  were excluded from the study.

A detailed demographic profile of subjects was taken. Burn site was evaluated for the percentage involved and the type of burn. A sample collection by phlebotomy in the complete blood count vial was done, samples were run in a CBC analyser; and verification of neutrophil count on the Neubauer chamber was done. ANC was taken for 3 days for each patient. All the information was entered in the structured questionnaire. Filgrastim is a sterile, clear, colourless, preservative free liquid containing at a specific activity of  $1.0 \pm 6 \times 10^8$  unit per mg (As measured by a cell mitogen assay). Injection filgrastim was given  $300\mu\text{g}$  s/c or I/V once daily until the neutropenia improved. Data was entered and analysed in SPSS (version 21.0). Mean and standard deviation was calculated for numerical variables like age, length of stay, the baseline and the end neutrophil count. Frequencies and percentages were calculated for qualitative variables like gender and type of burn. Paired t-test was used to evaluate statistical significance with  $p < .05$  as statistical significance. The response of

G-CSF injection was categorised in 3 groups: good responders were those whose neutrophil count improved within one day of G-CSF injection; moderate responders were those whose neutrophil count improved within two to three days of G-CSF injection; poor responders were those whose neutrophil count did not improve after G-CSF injection.<sup>11</sup> For quality assurance, data was collected by a trained co-investigator and data entry mistakes were checked by all investigators.

## RESULTS

The mean age of patients was  $32.10 \pm 14.35$ , 84.6% (n=33) males and 15.4% (n=6) females. Mean percentage of burn was  $40.56 \pm 15.73$ . Mean baseline neutrophil count was  $3.0641 \pm 0.79785 \times 10^9/\text{L}$  and final count was  $5.3564 \pm 3.61086 \times 10^9/\text{L}$ , ( $t = -4.299$ ,  $p < 0.001$ ). Mean baseline ANC count was  $1.8769 \pm 0.62554\%$  and final count was  $4.0590 \pm 3.51371\%$  ( $t = -3.722$ ,  $p < 0.001$ , Table I).

Response of therapy was categorised as good, moderate, and poor; and was stratified for age and gender. In 12-40 years of age, there were 30/39 (76.9%) patients. Among them, 11/30 (36.6%) were good responders, 13/30 (43.3%) were moderate responders, and 6/30 (20%) were poor responders. In 1-70 years of age, there were 9/39 (23.1%) patients. Among them, 2/9 (22.2%) were good responders, 4/9 (44.44%) were moderate responders, and 3/9 (33.3%) were poor responders. The differences were not significant ( $\chi^2 = 0.968$ ,  $p = 0.616$ ).

The response of G-CSF injection compared among genders showed that among 33/39 (84.6%) males, 12/33 (36.4%) were good responders, 13/33 (39.4%) were moderate responders, and 8/33 (24.2%) were poor responders. Among 6/39 (15.3%) females, 1/6 (16.7%) was good responder, 4/6 (66.7%) were moderate responders, and 1/6 (16.7%) was poor responder. Chi-Square test was applied which was not significant ( $\chi^2 = 1.583^a$ ,  $p = 0.453$ , Table II).

The response of G-CSF injection compared to different percentage of burns showed that in patients with less than 30% burn, 6/12 (50.0%) were good responders, 6/12 (50.0%) were moderate responders, and 0/12 (0.0%) was poor responder. In 31-50% burns, there were 17 patients (43.5%). Among them, none (41.2%) were good responders, 5/17 (29.4%) were moderate

**Table I:** Comparison of neutrophil and ANC count before and after therapy.

Count n=39	Mean	SD	t-test and p-value
Baseline neutrophil count ( $\times 10^9/\text{L}$ )	3.0641	0.79785	$t = -4.299$
Final neutrophil count ( $\times 10^9/\text{L}$ )	5.3564	3.61086	$p < 0.001$
Baseline ANC (%)	1.8769	0.62554	$t = -3.72$
Final ANC (%)	4.0590	3.51371	$p < 0.001$

**Table II:** Therapy response, age and gender cross tabulation.

Variables	Response			Total	Chi-square p-value
	Poor responder	Moderate responder	Good responder		
Age					
12 - 40 years	6 (20.0%)	13 (43.4%)	11 (36.6%)	30 (100.0%)	$\chi^2 = 0.968$ $p = 0.616$
41 - 70 years	3 (33.3%)	4 (44.4%)	2 (22.2%)	9 (100.0%)	
Gender					
Male	8 (24.2%)	13 (39.4%)	12 (36.4%)	33 (100.0%)	$\chi^2 = 1.583$ $p = 0.453$
Female	1 (16.7%)	4 (66.6%)	1 (16.7%)	6 (100.0%)	
Total	9 (23.1%)	17 (43.6%)	13 (33.3%)	39 (100.0%)	

responders, and 5/17 (29.4%) were poor responders. In 51-70% burns, there were 10 patients (25.6%). Among them, 0/10 (0.0%) was good responder, 6/10 (60.0%) were moderate responders, and 4/10 (40.0%) were poor responders ( $\chi^2=10.468^a$ ,  $p=0.033$ ).

## DISCUSSION

Major burn may be a cause of decrease neutrophils count and function and was proved by different animal studies, resulting in overall immunosuppression. In different clinical trials on humans, it has been proved that burn causes decrease immunoglobulins and neutrophil count and neutrophil function.<sup>11</sup> The ability to improve the functional capacity of neutrophils can contribute to minimise morbidity and mortality from sepsis following thermal injury. With the use of G-CSF, a rising level of femoral marrow granulocyte progenitor cells and circulating neutrophils as well as the survival rate after burn wound infection.<sup>15</sup> Treatment with G-CSF injections significantly affect the neutrophil count in burn patients with neutropenia. Administration of G-CSF has shown decreased mortality in different studies.<sup>16-18</sup> Analysis of posttraumatic gene expression patterns in humans reveals that they are also consistent with a role for G-CSF as a switch that activates innate immune responses and suppresses adaptive immune responses. This specific study findings suggest that the G-CSF STAT3 axis constitutes a key protective mechanism induced by thermal injury to reduce the risk of post-traumatic infection.<sup>19</sup> However, prospective, randomised, multicentre, double-blind placebo-controlled studies failed to confirm these benefits.<sup>20-23</sup> There are some limitations in available studies, including a delay in G-CSF administration,<sup>21</sup> differences in duration of G-CSF therapy (5 days *versus* 10 days),<sup>16-18,20-22</sup> difference in type of agents used (lenograstim filgrastim),<sup>16,18,20-22</sup> lack of power to detect a difference,<sup>20</sup> use of traditional treatments,<sup>8,10,24</sup> inability and differences in baseline patient characteristics.<sup>17</sup>

Treatment with G-CSF reduces the duration of antibiotic treatment; but this needs further studies to confirm, as it will reduce antibiotic associated side effects, cost and most importantly the development of resistance. Moreover, there is a need to do gene study on humans to prove the role of G-CSF to activate innate and to suppress the adaptive immune responses.<sup>19</sup>

This study, based on the use of G-CSF injections in different age groups, genders and different percentage of burn, showed better outcomes in the younger age group with less burn TBSA percentage of burn as compared to older age groups with more burn TBSA.

## CONCLUSION

Adding G-CSF injections to the standard treatment significantly affect the neutrophil count in burn patients

with neutropenia; but the clinical significance and the profound effect of G-CSF on the patient's morbidity and survival is yet to be established. Moreover, G-CSF may be considered as an adjunct to other appropriate care of burn patients with neutropenia; but further studies are needed to support this evidence for better outcome and survival of burn patients.

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