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## Is there a place for granulocyte colony-stimulating factor in non-neutropenic critically ill patients?

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**Abstract** Immunoparalysis, characterised by impairments in neutrophil and monocyte/macrophage function, is common in critically ill patients. The theoretical ability of granulocyte colony-stimulating factor (G-CSF) to improve the functions of both neutrophils and monocytes/macrophages provides a rationale for G-CSF therapy in non-neutropenic critically ill patients with infection or a high risk of nosocomial infection. The expression of the receptors that mediate G-CSF effects in neutrophils and monocytes/macrophages is regulated by bacterial products, cytokines and endogenous G-CSF levels, accounting for the variable effects of G-CSF on the neutrophil functions of critically ill patients. This variability should be taken into account

when designing studies on the use of G-CSF in ICU-patients. Studies are still needed to identify the subset of patients who may benefit from G-CSF therapy.

**Keywords** Granulocyte colony-stimulating factor (G-CSF) · Intensive care · Sepsis · Safety · Controlled trials · Acute respiratory distress syndrome · Experimental · Randomised controlled trials · Immunomodulation · Neutrophils

### Introduction

Human granulocyte colony-stimulating factor (G-CSF) is the most important regulatory cytokine capable of stimulating the production of neutrophils from committed hematopoietic progenitor cells both *in vitro* and *in vivo* [1]. G-CSF not only increases neutrophil counts, but also enhances and primes many neutrophil functions. It is widely used in cancer patients to hasten recovery from chemotherapy-induced neutropenia and to mobilise peripheral blood progenitor cells for bone marrow transplantation [2, 3, 4, 5]. G-CSF has proved safe and effective when used for longer periods in patients with drug-induced neutropenia, particularly following solid organ transplantation [6]. The prevention or curtailing of cytotoxic drug-induced neutropenia is ascribable to quantita-

tive stimulation of granulopoiesis by G-CSF. In patients with lymphoma and even leukaemia or solid tumours, G-CSF not only shortens the duration of neutropenia, thus providing higher complete remission rates and longer disease-free survival, but also reduces the incidence of infectious episodes (about half reduction of febrile neutropenia) [7, 8]. Interestingly, cost-effectiveness analyses have suggested that the prophylactic therapy of patients with chemotherapy-induced febrile neutropenia with recombinant colony-stimulating factors may be justified if the expected risk of neutropenic fever is greater than 40%.

This review will focus on the potential clinical usefulness of G-CSF in non-neutropenic critically ill patients. After delineating the rationale for G-CSF therapy in these patients, we will describe the effects of G-CSF on

neutrophil functions, the results of G-CSF therapy of sepsis in non-neutropenic acutely ill patients with various comorbidities and the results of studies in critically ill patients given G-CSF as prophylaxis of nosocomial infection.

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### **The biological plausibility of granulocyte colony-stimulating factor treatment**

What are the properties of granulocyte colony-stimulating factor and what is the rationale for granulocyte colony-stimulating factor treatment in critically ill non-neutropenic patients?

Because G-CSF increases both the number and the functions of neutrophils, it is an attractive candidate for biological immunotherapy in non-neutropenic patients with serious infections [9]. In this situation, benefits can be expected to arise from the qualitative effects of G-CSF, which enhance host defences by increasing neutrophil survival, proliferation, maturation, differentiation, adhesion, chemotaxis, respiratory burst, antibody-dependent cellular toxicity and intracellular microbial killing [10]. All these effects have been demonstrated mainly on blood neutrophils from healthy volunteers. G-CSF effects are mediated via specific receptors whose number on the neutrophil membrane is subjected to regulation by microbial products and cytokines [11, 12]. Furthermore, intracellular events induced by G-CSF [13] are modulated by the effects of other mediators. Consequently, G-CSF effects are probably influenced by the local environment (e.g., the pro-inflammatory/anti-inflammatory cytokine balance). Monocytes/macrophages also carry G-CSF receptors [14] and their functions, including cytokine synthesis, are sensitive to G-CSF. Few studies have investigated G-CSF effects on isolated monocytes/macrophages. A pro-inflammatory effect or an anti-inflammatory effect of G-CSF on monocytes has been suggested based on the influence of G-CSF on the cytokine balance [13]. These discrepancies may stem from differences across study populations (e.g., healthy individuals versus critically ill patients).

Granulocyte colony-stimulating factor is a natural component of host defences. Most patients with infection have high plasma levels of endogenous G-CSF, and those who do not seem to have a worse prognosis [15]. The main reason for using G-CSF in non-neutropenic critically ill patients is the acquired impairment of cellular immunity that affects both neutrophils and monocytes/macrophages in these patients [16, 17]. This immunoparalysis may delay the eradication of infection, despite appropriate antibiotic treatment, or increase the risk of subsequent nosocomial infection. Impairments in blood neutrophil functions were described many years ago in critically ill patients with severe infection [18].

They include reduced production of reactive oxygen species, reduced intracellular bacterial killing and impaired migration to infected sites [17, 19]. As expected, these impairments in neutrophil functions increase the risk of nosocomial infection [17]. Monocyte functions are also impaired: deactivation (impaired response to subsequent endotoxin challenge) has been demonstrated, as well as reduced HLA-DR expression responsible for the impaired recognition of foreign antigens [16, 20]. Macrophage functions have been less extensively studied, at least in humans. Experiments carried out using animal models of peritonitis have shown reduced CMH expression, impaired secretion of TNF- $\alpha$  and nitric oxide, and reduced bactericidal properties [21].

Can granulocyte colony-stimulating factor correct neutrophil abnormalities in non-neutropenic critically ill patients?

The biological effects of G-CSF administration on neutrophil functions have been assessed in critically ill patients in vivo, ex vivo and in vitro.

Weiss and colleagues evaluated prophylactic G-CSF treatment in two phase-II studies that included 30 post-operative/post-traumatic patients at risk of sepsis or with sepsis. In the first study, they showed that neutrophil function impairment (decreased oxygen radical production) was reversed by G-CSF treatment in ten patients, as compared to ten controls [22]. In the second phase-II study in 20 postoperative/post-traumatic patients at risk of sepsis or with sepsis, G-CSF improved neutrophil generation and function and appeared to counter-regulate hyperactivation of pro-inflammatory processes [23]. Weiss et al. suggested that administration of G-CSF might correct neutrophil abnormalities, thereby reducing the risk of nosocomial infection. In a phase I/II safety study, Gross-Weege and colleagues reported that low-dose G-CSF (1  $\mu\text{g}/\text{kg}$  per day) was safe in 20 surgical ICU patients (10 with systemic inflammatory response syndrome and 10 with sepsis) [24]. Oxygen radical production by neutrophils increased, whereas IL-6 and IL-8 levels declined. Sepsis did not develop in any of the ten patients with systemic inflammatory response syndrome.

Gerber and co-workers investigated the functional response of neutrophils to G-CSF in 30 non-neutropenic surgical ICU patients with severely impaired wound healing. G-CSF enhanced respiratory burst activity, phagocytic activity and intracellular calcium concentration. These effects were associated with clinical improvement in most patients [25]. Tanaka and colleagues investigated whether G-CSF administration changed leukocyte deformability, thereby causing lung injury in patients with sepsis. They randomly assigned 12 of 25 consecutive patients with sepsis to G-CSF (2  $\mu\text{g}/\text{kg}$  per day) and 13 to a placebo. G-CSF caused leukocyte stiffness but attenuat-

ed the inflammatory response without inducing lung injury [15].

More recently, Weiss and colleagues conducted a randomised double-blind study to evaluate the effects of G-CSF on neutrophil functions of post-surgical patients [26]. In this study, which had a stronger design than did the earlier studies carried out by this group, G-CSF had no detectable stimulatory effect on the release of reactive oxygen species. Moreover, G-CSF effects varied with the initial endogenous G-CSF levels. Interestingly, in a study of in vitro G-CSF effects on blood neutrophils from critically ill patients, Yang and co-workers found variable effects on respiratory burst and bacterial killing, ranging from inhibition to stimulation and depending on concentrations of both endogenous G-CSF and IL-10 [27].

The effects of G-CSF seem to vary, one of the factors being the concentration of endogenous G-CSF. Clearly, G-CSF therapy cannot be expected to benefit all critically ill patients. This should be taken into account when calculating sample sizes for future prospective studies with clinical end points.

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### **Clinical effects of granulocyte colony-stimulating factor treatment**

The in vivo effect of G-CSF therapy on severe infection has been assessed in experimental models and in humans. Some negative results in human trials are probably related to the heterogeneity of the patient populations (see above). For instance, in the post-hoc analysis of one study, G-CSF during pneumonia seemed more effective in patients with cirrhosis [28], a condition known to be associated with neutrophil function impairment [29].

#### Effects of granulocyte colony-stimulating factor in experimental models

Increased production of endogenous G-CSF during the acute-phase response is a key component of normal host defences [30]. Investigations of several animal models of severe bacterial infection without neutropenia found that G-CSF, either alone or in combination with antibiotics, significantly enhanced host defences or improved survival rates [31]. This result was somewhat surprising, since it had been reported that bacterial antigens down-regulated the G-CSF receptors at the surface of granulocytes [12]. During canine bacterial pneumonia, G-CSF decreased plasma endotoxin and serum TNF- $\alpha$  levels, attenuated sepsis-associated myocardial dysfunction and prolonged survival [32]. Similar results have been obtained in a rabbit model of *Pasteurella multocida* pneumonia [33], in a granulocytopenic mouse model of

*Pseudomonas aeruginosa* lung infection [34], in a model of *Klebsiella pneumoniae* [35] and in models of pneumococcal pneumonia [36, 37]. G-CSF also has therapeutic effects in experimental *Candida* pneumonia [38].

Granulocyte colony-stimulating factor therapy initiated at the time of resuscitation improved host defences to shock and polymicrobial sepsis and reduced the consequences of post-trauma sepsis by increasing neutrophil number and function [39]. In a model of haemorrhagic shock, Abraham and Stevens showed that G-CSF increased resistance to *Pseudomonas aeruginosa* pneumonia [40]. Similar results were obtained by Attalah and colleagues in a model of peritonitis complicated by bacterial pneumonia, in which G-CSF enhanced host defences in rats with immunoparalysis [41].

#### Effects of granulocyte colony-stimulating factor in humans

The encouraging results presented above supported the use of G-CSF in non-neutropenic patients with acute sepsis or secondary functional neutrophil impairment. Clinically, the earliest and largest experience with G-CSF in non-neutropenic patients involved administration at the onset of infection. Importantly, most of the conditions in which G-CSF seems effective are associated with pre-existing neutrophil function impairment. We have only detailed the use of G-CSF in patients with community-acquired pneumonia. Other conditions not associated with critical illness are mentioned in Table 1 for completeness.

#### *Patients with community-acquired pneumonia*

In several large trials in patients with complicated community-acquired pneumonia or with pneumonia and sepsis, G-CSF, although safe, was not beneficial overall, in keeping with some preclinical studies [42]. The safety and efficacy of G-CSF therapy in 30 non-neutropenic patients with severe community-acquired pneumonia were evaluated by deBoisblanc and colleagues in an open-label, dose-ranging, clinical trial. There was no evidence of G-CSF-related lung injury or extra-pulmonary toxicity [43]. G-CSF had no dose-response effect on clinical variables related to pneumonia. In a randomised, placebo-controlled, multicentre trial of G-CSF (300  $\mu$ g/day) as an adjunct to antibiotics in patients with severe community-acquired pneumonia, G-CSF did not affect mortality or length of hospitalisation. G-CSF treatment, however, accelerated radiological improvements and appeared to reduce serious complications (e.g., empyema, adult respiratory distress syndrome and disseminated intravascular coagulation). G-CSF was safe and well tolerated in these patients [28].

**Table 1** Indications in which granulocyte colony-stimulating factor at infection onset has been evaluated in non-neutropenic acutely ill patients

| References       | Indication                                 | Results   | Safe |
|------------------|--|---|------|
| [28, 43, 44, 45] | Patients with community-acquired pneumonia | Accelerated radiological improvement<br>Reduction of serious complications (empyema, ARDS and DICV)   | Yes  |
| [61, 62, 63, 64] | HIV patients                               | Reduced incidence of bacterial infections, bacteraemia and the number of consequent days of hospitalisation<br>Prolonged survival<br>Reversal of neutropenia associated with HIV and CMV infections | Yes  |
| [65, 66]         | Neonatal sepsis                            | Reduced incidence of nosocomial infections  | Yes  |
| [67]             | <i>Streptococcus pneumoniae</i> meningitis | All 22 patients recovered<br>Rapid improvement of inflammation indices in the cerebrospinal fluid   | Yes  |
| [68, 69, 70, 71] | Diabetic foot infection                    | Improved clinical outcome of foot infection with lower rate of amputation in three of four studies  | Yes  |
| [29, 72, 73]     | Acute liver failure or cirrhosis           | G-CSF reversed neutrophil function impairments in patients with acute liver failure and enhanced transendothelial migration of neutrophils in cirrhotic patients                                    | Yes  |
| [74, 75]         | Liver transplantation                      | Decreases in sepsis episodes, sepsis-related deaths and rejection in the study by Foster, but not in the study by Winston   | Yes  |

*G-CSF* granulocyte colony-stimulating factor, *ARDS* acute respiratory distress syndrome, *DICV* disseminated intravascular coagulation, *HIV* human immunodeficiency virus, *CMV* cytomegalovirus

Another randomised, controlled trial conducted by the same group assessed the safety and efficacy of G-CSF in hospitalised adults with multilobar community-acquired pneumonia [44]. The two treatment groups were not significantly different regarding the study end points; however, there was a trend toward lower mortality in the patients with pneumococcal bacteraemia. Lastly, in a phase III, double-blind, placebo-controlled trial in patients with community-acquired pneumonia, G-CSF had no effect on mortality, length of hospital stay or time to resolution of morbidity [45]. However, G-CSF accelerated the radiographic resolution of pneumonia and reduced the rate of serious complications, most noticeably in those patients with multilobar pneumonia. Based on these studies, additional trials were performed in patients with multilobar pneumonia or with severe pneumonia and sepsis. Although mortality was not different in the G-CSF-treated patients, further analyses of these studies are ongoing.

#### *Trials of granulocyte colony-stimulating factor in the intensive care unit*

Granulocyte colony-stimulating factor administration has been used in the ICU both to prevent nosocomial infections in mechanically ventilated patients and to prevent the progression of sepsis. Nosocomial infections and

multiple organ dysfunction are the leading causes of mortality in patients admitted to the ICU.

Granulocyte colony-stimulating factor to prevent nosocomial infection in critically ill patients

Three clinical trials have investigated the effects of prophylactic G-CSF in non-neutropenic critically ill patients. In patients with severe head injury or cerebral haemorrhage, prophylactic G-CSF increased circulating neutrophil counts and decreased the incidence of bacteraemia, but had no impact on mortality rates, length of stay or the global incidence of nosocomial infection [46]. In another prospective, randomised, placebo-controlled, double-blind trial, Petilla and colleagues investigated the safety of G-CSF in the prevention of nosocomial infections in consecutive medical/surgical intubated ICU patients, with special attention to possible deleterious effects on acute respiratory distress syndrome and on the development of multiple organ dysfunction. In the interim analysis in the first 59 patients, G-CSF was found to be safe but ineffective in decreasing nosocomial infections, mortality rates and organ dysfunction [47]; the final results are not yet available. Lastly, Wunderink and colleagues evaluated G-CSF in 18 patients with pneumonia and either septic shock or severe sepsis who were receiving mechanical ventilation [48]. There were no differences between the two groups in types or occurrence rates of adverse events, including ARDS, or in outcomes.

The two completed studies lacked statistical power to demonstrate a beneficial effect of G-CSF on the incidence of nosocomial infection. The ongoing study by Petila and colleagues will perhaps clarify the clinical impact of G-CSF. However, the wide inter-individual variations in the biological effects of G-CSF were probably not taken into account in the design of this study.

Granulocyte colony-stimulating factor in critically ill patients at the onset of community- or hospital-acquired infection

Two uncontrolled studies have been reported before the results of a large trial (see below). In a prospective randomised study, Meyanci et al. investigated the role of G-CSF in combination with antibacterial agents for the treatment of ventilator-associated nosocomial pneumonia in patients intubated for acute respiratory failure. G-CSF (5 µg/kg per day) was given to 14 patients after they had been diagnosed with nosocomial pneumonia. As compared to the 14 controls, the G-CSF-treated patients had better outcomes and the difference was largest in the patients with the lowest leukocyte counts [49]. More recently, Stephens and colleagues compared outcomes in 36 patients with community-acquired septic shock treated with G-CSF (300 µg/day) in addition to standard treatment and in a historical cohort of 11 similar patients. They found that G-CSF was safe and decreased the mortality rate [50].

The recently published, multicentre, double-blind, placebo-controlled study of the Pneumonia Study Group gives a clear negative response for the use of G-CSF (filgrastim) in the context of severe pneumonia (699 patients, 80% community- and 20% hospital-acquired pneumonia) [51]. Although safe, G-CSF (5 µg/kg per day for 5 days) did not afford any beneficial effect in terms of mortality, subsequent organ dysfunction, time to discharge from ICU and number of days on mechanical ventilation.

### **Is granulocyte colony-stimulating factor safe in non-neutropenic critically ill patients?**

The neutrophil has been strongly implicated in the pathogenesis of inflammatory lung injury [52, 53] and there has been theoretical concern that G-CSF-induced neutrophil activation may exacerbate lung injury. Preclinical experience with prophylactic G-CSF showed that the stimulatory effects on immune responses were po-

tentially harmful, with worse lung injury and outcomes in some types of bacterial pneumonia [54, 55, 56, 57, 58]. G-CSF has been reported to exacerbate the pulmonary toxicity of cancer chemotherapy or acute lung injury related to an infectious process, during or after neutropenia recovery [58, 59, 60]. These facts raise concern regarding the use of G-CSF in mechanically ventilated ICU patients. However, in non-neutropenic patients given G-CSF, either at infection onset to prevent multiple organ dysfunction or later in the ICU stay to prevent nosocomial infection, no serious adverse pulmonary effects have been reported [22, 23, 24, 25, 28, 43, 44, 45, 46, 47, 49, 50] (Table 1). It should be borne in mind that the total number of non-neutropenic critically ill patients who have been treated with G-CSF is very small, as compared to the number of neutropenic patients. However, exacerbation of acute lung injury, which was the main concern based on theoretical grounds, has not been demonstrated and a thought-provoking finding is that G-CSF is the only therapeutic agent that has been associated with prevention of ARDS in human trials [28].

### **Conclusion**

At present, G-CSF has been proven effective and safe in reducing the incidence of infection and sepsis in selected subgroups of immunocompromised patients after cancer chemotherapy, as well as in neutropenic patients. In experimental models, G-CSF has benefited the resolution of infection, especially pneumonia, and of subsequent sepsis, multi-organ dysfunction and septic shock. These effects result directly from improved infection control and indirectly from attenuation of the overwhelming, damaging, pro-inflammatory response.

Conversely, in non-neutropenic critically ill patients, G-CSF has no proven clinical benefit in terms of morbidity (prevention of hospital-acquired infection, resolution of severe community-acquired infection) and mortality. The explanation probably lies in the wide variability of the biological response in ICU patients, which modulates G-CSF effects. Further studies in patients at high risk of hospital-acquired infection should assess whether a biological marker of G-CSF efficiency could be identified (i.e., endogenous G-CSF levels). Also, treatment schedules and dosages that are likely to be effective should be determined.

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