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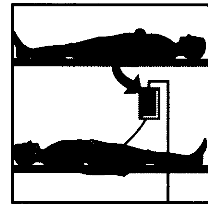

# Elevated endogenous serum macrophage colony-stimulating factor in the early stage of fungemia following bone marrow transplantation

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## Abstract

Murine studies have reported elevated serum macrophage colony-stimulating factor (M-CSF) concentrations in animals inoculated with fungus; however, the human cytokine response to fungemia has not been described. Endogenous M-CSF serum concentrations were measured in 18 autologous bone marrow transplant patients with positive blood fungal cultures. Seventeen of the 18 patients received the same high-dose chemotherapy regimen with autologous hematopoietic support. M-CSF concentrations were determined in serum samples obtained 1 week before and within 2 days of the first positive blood culture. Serum M-CSF rose more than three-fold in a majority of patients at the time of positive culture in contrast to concentrations obtained in the previous week (medians 11.1 and 2.8 ng/mL, respectively; $p$=0.001). Median values at the time of positive blood culture were also significantly higher than those obtained in a matched control group of patients without positive blood cultures (n=18; median 2.60 ng/mL; $p$=0.001). These data demonstrate that endogenous serum M-CSF is elevated in the early stages of human systemic fungal infection and thus may have important diagnostic and therapeutic implications.

**Key words:** M-CSF—Fungemia—Transplantation

## Introduction

M-CSF is an important component in monocyte/macrophage maturation and functional activation. Early and prolonged increases in endogenous serum M-CSF have been described in mice chronically infected with a fungus such as *Candida albicans* [1]. Initial human investigation evaluating M-CSF as an antifungal agent used in vitro exposure of human mononuclear phagocytes with the cytokine. This procedure resulted in enhanced killing of the fungal organisms [2]. Additional human evaluation of M-CSF has included administration of a recombinant form of the protein which yielded in vivo amplification of monocyte-mediated antifungal activity [3].

Fungal infections are a major cause of morbidity and mortality in patients receiving myelosuppressive chemotherapy despite the aggressive use of antifungal drugs. Thus, a phase I trial of recombinant M-CSF in cancer patients with invasive fungal infections was conducted early in the recombinant cytokine's development. Results of the trial suggested a beneficial role of administering recombinant M-CSF in this setting and have led to an ongoing randomized study [4]; however, no data are available regarding the endogenous levels of M-CSF in the serum of cancer patients with fungemia.

The purpose of this study was to evaluate the endogenous serum concentration of M-CSF in patients with fungemia following high-dose, combination alkylating agent chemotherapy and autologous bone marrow transplantation.

## Materials and methods

### *Patient set*

Patients were identified from the Duke Bone Marrow Transplant Program database who had at least one positive, posttransplant blood culture for fungus. All these patients received high-dose chemotherapy with autologous bone marrow support for the treatment of stage II–IV breast cancer or metastatic melanoma. Normal laboratory indicators of renal function (serum creatine <1.5 mg/dL; creatinine clearance >60 mL/min) and liver function (serum total bilirubin <2 mg/dL; serum aspartate aminotransferase <2.5 times normal) were required in all patients before receiving this chemotherapy regimen. Seventeen patients received the same ablative treatment consisting of cyclophosphamide (5625 mg/m$^2$), cisplatin (165 mg/m$^2$), and carmustine (600 mg/m$^2$) administered over the course of 4 days (days –6 to –3), as previously described [5]. One patient (UPN 125) received a similar regimen in which melphalan (40 mg/m$^2$) was substituted for carmustine. Chemotherapy was initiated between 8 a.m. and noon. Some patients received daily recombinant CSFs (granulocyte [G-CSF], granulocyte-macrophage [GM-CSF], interleukin-3 [IL-3], or IL-1) (Table 1) typically starting 3 hours after the first autologous cell reinfusion and continuing for 14 to 21 days as previously described [6–8].

A group of control patients who did not experience fungemia were identified from our previously published study of endogenous cytokines in autologous bone marrow transplantation [9]. These controls were matched to the infected patients using day of therapy, autologous cell support employed, and CSF administered.

### *Sample collection*

Serum samples for the evaluation of endogenous M-CSF concentrations were obtained between 4 and 6 a.m. on day –6 and approximately every third day following marrow reinfusion. All samples were stored at –70°C until analysis. For the purpose of this study, we chose to evaluate M-CSF concentra-

**Table 1.** Patient demographics and outcome

| Unique patient number | Diagnosis | CSF[d] | Day of positive culture(s) | Culture identification | Fungal site | Ampho[a] (mg/kg) | 5-FC[b] (days)[e] | Flu[c] (days)[e] | Outcome |
|---|---|---|---|---|---|---|---|---|---|
| 125 | BrIV[f] | None | +5 | No identification | Blood | 12.7 | — | — | Clinical resolution |
| 156 | BrIV | GM-CSF | +18,+21 | *Aspergillus flavus* ×2 | Blood | 4.3 | — | — | Clinical resolution |
| 158 | BrIV | None | +1 | *Aspergillus flavus* | Blood | 7.3 | — | — | Clinical resolution |
| 167 | Melanoma | GM-CSF | +5 | *Candida tropicalis* | Blood | 26.8 | — | — | D+48[g] MOFS[h] (A)[i] F-[j] |
| 225 | BrIV | None | +5 | *Candida albicans* | Blood | 14.3 | — | — | Clinical resolution |
| 323 | BrIV | None | +4 | *Candida albicans* | Blood | 17.7 | 20 | — | D+30 MOFS |
| 341 | BrIV | IL-1 | +6,7,9,11 | *Candida albicans* ×4 | Blood | 20.4 | — | — | D+33 MOFS Hemorrhage |
| 354 | BrIV | G-CSF | +6 | *Candida tropicalis* | Blood | 12.9 | — | — | D+31 MOFS |
| 367 | BrIV | None | +10 | *Candida parapsilosis* | Blood | 18.8 | — | — | D+36 MOFS Hemorrhage (A)F– |
| 439 | BrIV | None | +27 | *Torulopsis glabrata* | Blood | 16.0 | — | — | D+29 Respiratory failure (A)F+[k] |
| 528 | BrII[l] | GM-CSF | +8 | *Candida krusei* | Blood | 20.1 | — | 10 | Clinical resolution |
| 530 | BrII | GM-CSF | +6,7,10 | *Candida tropicalis* ×3 | Blood | 13.5 | — | — | D+35 MOFS (A)CMV+F+ |
| 649 | BrIV | GM-CSF | +9<br>+22 | No identification<br>*Cunninghamella sp.* | Blood<br>Lung | 3.0 | — | 11 | D+22 Respiratory failure (A)F+ |
| 654 | BrIV | G-CSF | +3,6 | *Candida albicans* ×2 | Blood | 10.5 | 5 | — | D+24 MOFS Hemorrhage (A)F- |
| 659 | BrIV | G-CSF | +6 | *Torulopsis glabrata* | Blood | 12.2 | 12 | — | D+47 MOFS (A)F+ |
| 661 | BrIV | GM-CSF | +4,6 | *Candida tropicalis* ×2 | Blood | 16.2 | 2 | — | Residual renal F+ |
| 671 | BrIV | G-CSF + GM-CSF | +8,9,10 | *Candida albicans* ×3 | Blood | 23.2 | 20 | 5 | D+49 MOFS (A)F- |
| 679 | BrIV | IL-3 + GM-CSF | +7 | *Candida tropicalis* | Blood | 17.0 | 31 | — | Clinical resolution |

[a]Amphotericin (total dose in mg/kg).
[b]5-flucytosine.
[c]Fluconazole.
[d]Recombinant colony-stimulating factor administered.
[e]Duration of treatment in days.
[f]Stage IV breast cancer.
[g]Day of therapy following bone marrow reinfusion.
[h]Multi-organ failure syndrome.
[i]Autopsy.
[j]Fungal culture negative.
[k]Fungal culture positive.
[l]Stage II breast cancer.

tions in samples collected within 2 days of the first positive fungal blood culture and those obtained approximately 7 days before the positive culture.

#### *M-CSF assay*

Immunoreactive M-CSF was measured using a double monoclonal antibody sandwich ELISA specific for M-CSF. The murine anti-M-CSF monoclonal antibody HM7/2.4.4 was used as the capture antibody. After blocking and incubation with serum samples, bound M-CSF was detected with biotinylated murine anti-M-CSF monoclonal antibody HM7/5.3.9.13 (Genetics Institute, Cambridge, MA). Avidin-horseradish peroxidase conjugate (Zymed, San Francisco, CA) was added, and color was developed using ABTS substrate (Kirkegaard & Perry, Gaithersburg, MD). The optical density (OD) was recorded at a wavelength of 405 nm. The lower limit of quantitation of this assay in our laboratory was 0.4 ng/mL. A new standard curve was run with each assay. The intra-assay variability using spiked controls was less than 10 percent.

#### *Statistical methods*

Prior population analyses of cytokine concentrations have noted data distributions that were not normal [9]. Thus we have chosen to analyze the data in this manuscript with non-parametric methods. Statistical comparisons were performed using the Wilcoxon test for paired analysis and the Mann-Whitney test for non-paired group comparison. The criterion for statistical significance was defined as $p<0.05$.

## Results

Eighteen patients were identified who had serum samples available for analysis both surrounding and 1 week before positive fungal blood cultures. The average age of the patients was 42 years (27–56) and all but one were female. A variety of fungal organisms were identified, often on multiple days (Table 1). All patients were treated with antifungal therapy primarily consisting of amphotericin B as outlined in Table 1. Autopsy data were available from 73 percent of the 11 patients who died during the transplant admission. Five of these patients

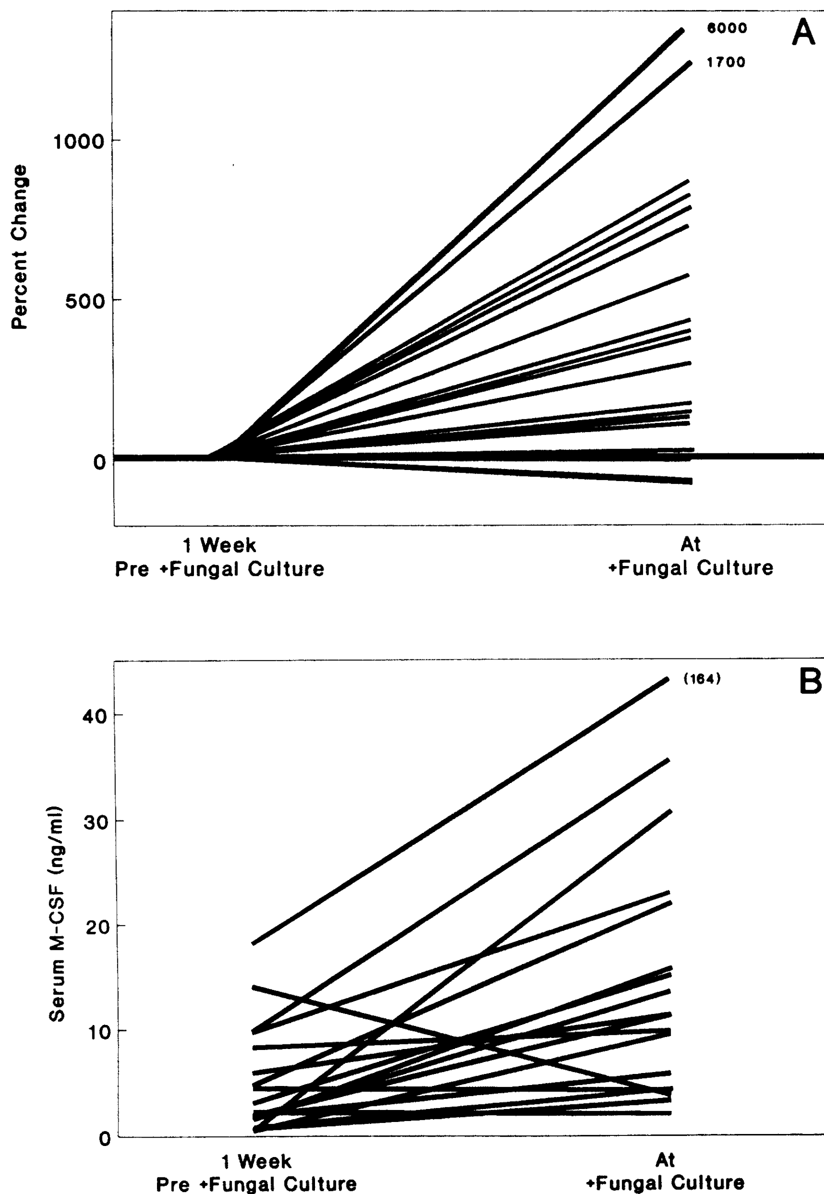


**Fig. 1. A.** Percent change in endogenous serum M-CSF concentrations in 18 patients, collected 1 week before the first positive blood culture for fungus compared to those samples obtained within 2 days of the positive culture ($p$=0.001, Wilcoxon test). **B.** Absolute amounts of M-CSF in serum collected at the time points mentioned above ($p$=0.001, Wilcoxon test).

had residual fungal infection with the same organism that was identified during therapy. One of the surviving patients has a persistent fungal infection of the kidney which is responding to fluconazole treatment administered on an outpatient basis.

Measurable concentrations of M-CSF were found in all patient samples. The median serum concentration at the time of positive fungal culture was 11.1 ng/mL (inter-quartile range 4.3–22.0). These data were significantly higher than those obtained in the same patients 1 week prior to the positive culture (median 2.8 ng/mL, inter-quartile range 1.5–8.6; $p$=0.001) (Fig. 1). The median increase in serum M-CSF was over 300 percent during the 1-week period (Fig. 1).

M-CSF serum concentrations at the time of fungemia were significantly higher in the patients who died before being discharged from the hospital compared to those who survived. The median concentration in the former group was 15.2 ng/mL compared to 4.31 ng/mL in the survivors ($p$=0.01) (Fig. 2).

Eighteen control patients were obtained from our database who matched the infected patients for day of therapy, autologous cell support employed, and CSF administered. The median M-CSF concentration of 2.6 ng/mL (2.0–4.5) in this control group was significantly lower ($p$=0.001) than that of the patients with positive fungal cultures (Fig. 2).

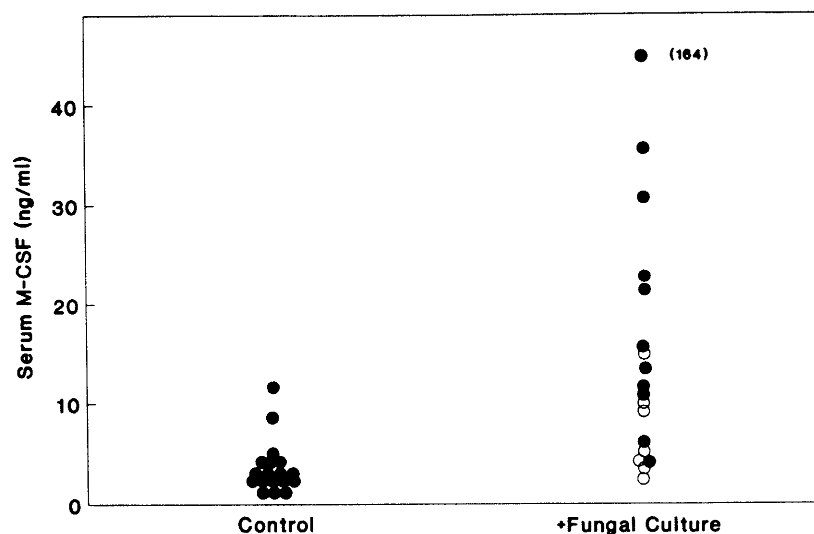


**Fig. 2.** Scatter plot of serum M-CSF in 18 control patients and 18 patients sampled within 2 days of the first positive fungal culture ($p$=0.001, Wilcoxon test). ○ = patients with fungemia who survived and had significantly lower M-CSF concentrations; ● = patients with fungemia who died within 50 days of the transplant ($p$=0.01, Mann-Whitney test).

## Discussion

Endogenous M-CSF production occurs in a variety of cells and is apparently influenced by numerous other cytokines [10]. Elevations in serum M-CSF have been described in association with neutropenia [11]; in patients with ovarian neoplasms [12,13], endometrial cancer [14], or sepsis post–thermal injury [15]; and in mice following bacterial or fungal infection [1]. In addition, we have previously demonstrated differences in endogenous M-CSF depending on the type of hematopoietic support and CSF administered following high-dose chemotherapy [9]. Maximal serum concentrations of M-CSF were found between days 8 and 14 following transplantation with substantially higher concentrations found in patients receiving GM-CSF compared to G-CSF posttransplant. The latter study also noted significantly higher serum M-CSF in patients with renal or hepatic toxicity following autologous bone marrow transplantation [9].

This report is the first to evaluate endogenous serum M-CSF during a period of fungemia in cancer patients. M-CSF concentrations rose considerably over approximately a 1-week period preceding the detection of fungemia. In addition, values obtained at the time of fungemia were significantly higher than those found in control patients. The reason for this phenomenon is not entirely evident. A logical explanation would include the activation of macrophage-mediated M-CSF production as a part of the initial response to the fungus; however, one cannot rule out a reduction in the clearance mechanisms for M-CSF. Receptor attachment and subsequent internalization are thought to be the primary components of M-CSF systemic clearance. Thus, changes in receptor expression or turnover rate surrounding the period of fungemia may significantly influence endogenous concentrations. We have attempted to match the control patients used in this study for as many factors that are known to alter endogenous M-CSF as possible; however, other unstudied factors such as the relationship between neutropenic fever or antibiotic therapy and M-CSF should be evaluated in future trials.

The physiologic role of circulating M-CSF in the neutropenic patient with fungemia has not been determined. Animal and preliminary human data suggest that supplementation of endogenous M-CSF by administration of the recombinant protein may improve antifungal therapy; however, it is also known that M-CSF can stimulate monocytes to produce other cytokines such as tumor necrosis factor (TNF), IL-1, GM-CSF, and interferons [16]. Some of these secondary cytokines may also have antifungal properties.

Heterogeneity in the type and dose of CSF prescribed for patients described in this manuscript precludes a subset analysis to discern if there was any relation between treatment with a particular CSF and the rate of fungemia. It is interesting, however, that we noted a relationship between the degree of M-CSF serum concentration elevation and outcome. The eleven patients who died following detection of fungemia had significantly higher M-CSF concentrations than those who survived. One interpretation of such data is that the fungal inoculum was higher in the group of patients who died, thus producing a greater stimulus for endogenous M-CSF production and worse prognosis. Alternatively, one could speculate that these patients had an insufficient number of cells with M-CSF receptors. This scenario would be consistent with both increased systemic concentrations of M-CSF (secondary to lower overall receptor-mediated clearance) and reduced natural antifungal defenses. These data support the potential prognostic significance of this test in patients with fungemia regardless of the mechanisms involved.

Speculation on the relevance of stimulating endogenous M-CSF secretion by administration of another cytokine such as GM-CSF is intriguing; however, the significance of such induction in the treatment or prevention of fungal infections is unknown. Of equal importance is investigation of the potential alteration of endogenous M-CSF disposition by ancillary drugs commonly used in patients with cancer. Examples of such modulation of other cytokines include the

suppressive effects of pentoxifylline [17], ciprofloxacin [18], or dexamethasone [19] on TNF production.

Future evaluations should investigate if interpatient differences in the amount of endogenous M-CSF correlate to the incidence, severity, or outcome of specific fungal infections. A more frequent sampling scheme could determine the potential for prospectively using serum M-CSF as an early marker for impending fungemia in patients with myelosuppression.

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