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## *Letters to the editor*

# *Pneumocystis carinii* pneumonia in patients with ulcerative colitis

*Pneumocystis carinii* pneumonia (PCP) causes pneumonia in immunocompromised patients suffering from diseases such as leukemia, malignant lymphoma, and other cancers, and those treated with corticosteroids and/or immunosuppressive agents. However, it has rarely been reported in patients with ulcerative colitis (UC). We report three patients with UC who developed PCP during corticosteroid and/or azathioprine (AZA) administration.

Case 1 was a 26-year-old woman with an 8-month history of ulcerative colitis. She had hematochezia, and colonoscopy showed severe pancolitis. She had a blood transfusion and was treated with prednisolone, at a dose of 60 mg/day. Her disease was unresponsive to therapy with prednisolone. AZA at 100 mg/day was added. Her symptoms improved gradually, but AZA was stopped on the day 34 after the beginning of administration because of leukocytopenia. The following day she had a high-grade fever. Laboratory data were as follows: white blood cell count (WBC), 2500/mm$^3$; lymphocyte count (Ly), 650/mm$^3$; hemoglobin (Hb), 10.3 g/dl; and platelet count (Plt), 24.1 × 10$^4$/mm$^3$. Arterial blood gases in room air were: pH 7.44, $PCO_2$, 36.0 mmHg; and $PO_2$, 56.0 mmHg. The CD4/CD8 ratio in peripheral blood was decreased, at 0.5. X-ray and computed tomography (CT) of the chest revealed bilateral "ground-glass" shadows (Fig. 1).

Case 2 was a 68-year-old woman with an 11-year history of left-sided ulcerative colitis. She presented with acute colitis. We started pulse steroid therapy. The response was excellent and she entered the remission stage on clinical tests. She developed a high fever 2 days after the start of maintenance therapy with prednisolone at a dose of 7.5 mg/day. Laboratory data were as follows: WBC, 6200/mm$^3$; Ly, 990/mm$^3$; Hb, 10.5 g/dl; and Plt, 35.9 × 10$^4$/mm$^3$. Arterial blood gases in room air were: pH 7.47, $PCO_2$, 32.7 mmHg; and $PO_2$, 70.0 mmHg. The CD4/CD8 ratio in peripheral blood was normal (1.1). Chest X-ray and CT showed bilateral "ground-glass" shadows.

Case 3 was a 29-year-old man with a 4-month history of ulcerative colitis. He presented with acute colitis exacerbation. Colonoscopy showed total colitis. We changed the corticosteroid from prednisolone, 40 mg/day, to betamethasone, 4 mg/day. AZA, 100 mg/day, was added and the response was satisfactory. Then we began to reduce betamethasone gradually, to 1.5 mg/day. His clinical course was excellent and he was discharged. Four months later, he was readmitted with a high fever. Laboratory data were as follows: WBC, 3400/mm$^3$; Ly, 880/mm$^3$; Hb, 11.0 g/dl; and Plt, 15.6 × 10$^4$/mm$^3$. Arterial blood gases in room air were: pH, 7.39; $PCO_2$, 41.0 mmHg; and $PO_2$, 91.0 mmHg. Chest X-ray was normal, but chest CT revealed faint "ground-glass" shadow in the right lung (Fig. 2).

In all three patients, polymerase chain reaction (PCR) for detection of *P. carinii* in sputa was positive, and we diagnosed PCP. Cytomegalovirus antigen in peripheral blood was negative in all the patients. After the diagnosis of PCP, they were treated

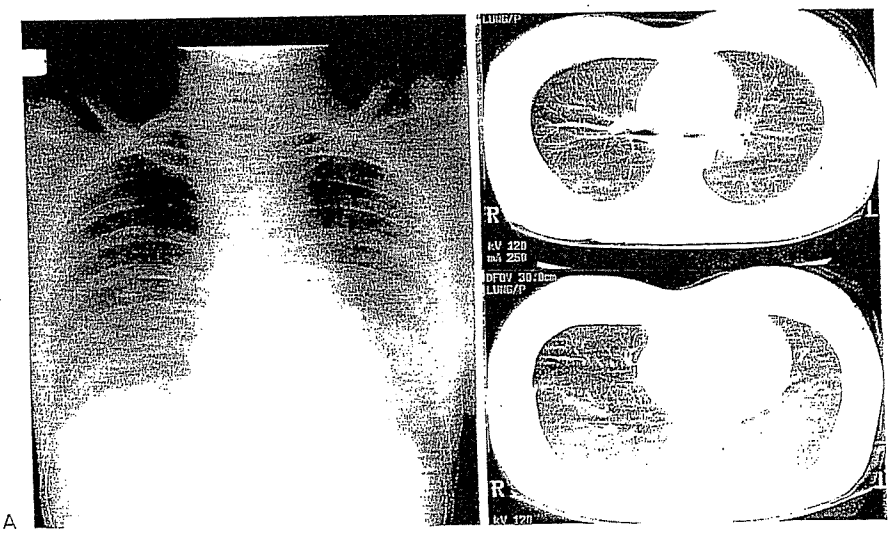
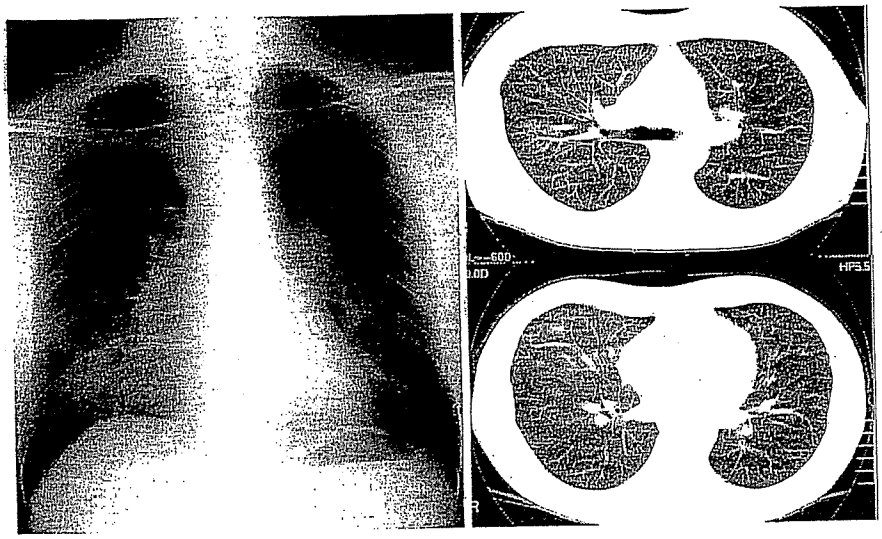



Fig. 1. A Chest X-ray shows interstitial shadows in bilateral middle and lower lung fields. B Chest computed tomography (CT) reveals diffuse "ground-glass" opacity in bilateral lung fields, with a small amount of pleural effusion. Both images are from case 1, taken on the same day



A B

Fig. 2. A Chest X-ray shows almost normal findings. B Chest CT shows faint "ground-glass" opacity in the right lung. Both images are from case 3, taken on the same day

with intravenous trimethoprim-sulfamethoxazole (TMP-SMX) and improved quickly.

The symptoms of PCP worsen without appropriate therapy. As chest X-rays are often normal, as shown in our case 3, or show a faint ground-glass shadow, chest CT is very useful for detecting abnormalities at the early stage of PCP.[1] This modality usually reveals bilateral diffuse ground-glass opacity. Diagnostic identification of *P. carinii* is based on the microscopic detection of the organism in pulmonary materials. However, it is difficult to do such an invasive examination as bronchoscopy when the patient's condition is worsening. Noninvasive diagnosis of *P. carinii* infection using PCR of patient's sputa is of great benefit.

For the prevension of PCP in patients with UC who are receiving immunosuppressive therapy, prophylactic treatment with TMP-SMX should be considered.[2–4] Administration of prednisolone, at more than 16 mg/day over a 2-months period,[5] or at 20 mg/day over a 4-weeks period,[6] is suggested as the indication for preventive therapy. In the present case report, we have described three patients with UC who developed PCP during immunosuppressive therapy; one patient received pulsed steroid therapy, and two received AZA together with corticosteroids. Thus, we believe that prophylaxis has to be carried out in UC patients receiving immunosuppressive therapy with high doses of corticosteroid, as well as in those receiving combination therapy with corticosteroids and immunosuppressants such as AZA, considering the benefit of such prophylaxis and the high mortality of PCP.

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# A pregnant patient with fulminant hepatic failure was found to carry a novel missense mutation in the argininosuccinate synthetase gene

Classical citrullinemia (CTLN1; OMIM no. 215700) caused by mutations of the argininosuccinate synthetase (*ASS*; EC6.3.4.5) gene on chromosome 9q34 differs from adult-onset type II citrullinemia (CTLN2; OMIM no. 603471), which is caused by mutations of citrin encoded by *SLC25A13* on chromosome 7q21.3. Plasma citrulline/arginine levels enable the distinction of CTLN1 (2500 ± 1040/58 ± 31 nmol/ml) from CTLN2 with liver-specific *ASS* deficiency (521 ± 290/232 ± 167 nmol/ml). To date, 50 *ASS* mutations have been identified in CTLN1 patients, and differences among races have been noted.[1] Most CTLN1 patients are neonatal- or infantile-onset, but some adult-

# Increased serum concentrations and surface expression on peripheral white blood cells of decay-accelerating factor (CD55) in patients with active ulcerative colitis

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**Inflammatory stimuli induce expression and release of decay-accelerating factor (DAF), a complement-regulatory protein present on peripheral-blood cells. Therefore, in ulcerative colitis (UC), an inflammatory colonic disease in which activated leukocytes are involved, DAF may be released from leukocytes into the circulation. In this study we compared serum DAF concentrations and surface DAF expression on peripheral-blood cells in patients with UC with disease activity. Peripheral-blood samples were obtained from 60 patients with UC (30 with active and 30 with inactive disease) and 19 healthy volunteers. Serum DAF concentrations were determined by means of immunoassay, and surface DAF expression on blood cells was examined with the use of flow cytometry. Serum DAF concentrations in patients with active disease (mean 48.6 ng/mL) were significantly higher than those in patients whose disease was in remission (33.3 ng/mL; $P = .0003$) and those in healthy controls (32.3 ng/mL; $P = .0007$). Surface DAF expression on neutrophils, CD14+ monocytes, and subsets of lymphocytes in patients with active UC was significantly increased compared with that in patients with UC in remission and in healthy controls. The increased serum DAF concentrations and surface DAF expression on leukocyte fractions in patients with active disease fell to significantly lower levels when the disease had gone into remission after medical therapy. Serum DAF concentrations are increased in UC patients in relation to disease activity. The likely source of increased DAF concentrations is peripheral-blood leukocytes that have been activated as part of the UC disease process. (J Lab Clin Med 2004;143:152-8)**

**Abbreviations:** DAF = decay-accelerating factor; FITC = fluorescein isothiocyanate; HRP = horseradish peroxidase; IL = interleukin; SCR = short consensus repeat; TNF = tumor necrosis factor; UC = ulcerative colitis; WBC = white blood cell

Altered regulation of cell- and humoral factor–mediated immune responses against intestinal constituents may play a role in the development of UC.[1] The complement system is a major effector pathway in humoral factor–mediated immunity, and we have previously shown the activation and degradation of complement in mucosal lesions of active UC.[2] Autologous complement activation is regulated by complement-regulatory proteins to protect host cells. One of these proteins, DAF (CD55), is a glycosylphosphatidyl inositol–anchored protein that inhibits

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**Table I.** Characteristics of UC patients and controls

| Characteristics | UC | | | Healthy controls ($n$ = 19) |
|---|---|---|---|---|
| | Total ($n$ = 60) | Active ($n$ = 30) | Remission ($n$ = 30) | |
| Age (yr)* | 39.0 ± 2.0 (14-74) | 38.2 ± 2.5 (14-62) | 39.8 ± 3.1 (14-74) | 35.8 ± 1.8 (27-59) |
| Sex (M/F) | 32/28 | 14/16 | 18/12 | 11/8 |
| Age at diagnosis (yr)* | 31.8 ± 1.8 (9-71) | 31.3 ± 2.2 (13-59) | 32.2 ± 3.0 (9-71) | |
| Duration of disease (yr)* | 7.2 ± 0.9 (0-30) | 6.7 ± 1.3 (0-30) | 7.6 ± 1.3 (0-26) | |
| Location of disease | | | | |
| Proctitis | 8 (13%) | 3 (10%) | 5 (17%) | |
| Left-sided colitis† | 21 (35%) | 13 (43%) | 8 (27%) | |
| Pancolitis | 31 (52%) | 14 (47%) | 17 (56%) | |

*Data expressed as mean ± SEM (range).
†Inflammation up to the splenic flexure.

the formation and promotes the catabolism of C3 and C5 convertases.[3] We have found that DAF expression is markedly increased in the inflamed mucosa of UC patients[4] and that DAF is released into the stools of patients with active UC.[5] In the regulation of DAF expression, we have shown that inflammatory cytokines IL-4 and IL-1β markedly enhance the expression and release of DAF from cultured HT29 intestinal epithelial cells.[6] These inflammatory cytokines also activate polymorphonuclear leukocytes (neutrophils), and activated neutrophils express increased amounts of DAF molecules on their surfaces.[7] In addition, both resting and stimulated neutrophils have released DAF into culture supernatants.[8] Therefore DAF may be released from activated leukocyte into the circulation in patients with active UC. In this study, we examined serum DAF concentrations and cell-surface expression of DAF on peripheral-blood cells in patients with UC in relation to the severity of disease activity.

## METHODS

**Patients and study design.** Peripheral-blood samples were obtained from 60 patients with UC (28 women, 32 men; mean age 39 years, age range 14-74 years) and 19 healthy volunteers (8 women, 11 men; mean age 36 years, range 27-59 years). The diagnosis of UC was based on history, clinical symptoms, and endoscopic and histologic findings. Thirty-one patients had pancolitis, 21 had left-sided colitis, and eight had proctitis. Disease activity was graded on the basis of clinical features and laboratory data in accordance with the criteria of Truelove and Witts[9,10] by 2 gastroenterologists (MM and HO) who had no knowledge of the DAF levels in serum and on the surfaces of peripheral-blood cells of the patients under evaluation. Remission was defined as a mild degree or better of clinical activity, without hematochezia; or better, WBC counts were not used in the evaluation. Thirty patients had active disease (19 moderate, 11 severe), and 30 had colitis in remission when blood samples were obtained. The patients' characteristics are presented in Table I. Serum samples were kept frozen at −80°C until use. We also obtained heparinized blood samples from 14 patients with active UC (11 moderate, 3 severe), 13 patients with inactive UC, and 14 healthy volunteers for the analysis of surface expression of DAF on blood cells by means of flow cytometry. We obtained blood samples from 7 patients with active UC when their disease went into remission after medical treatment. Two of the 7 were women and 5 were men (mean age 31 years, range 14-44 years); 6 had pancolitis and 1 had left-sided colitis; the colitis was severe in 2 and moderate in 5.

The study was conducted in accordance with the guidelines of the Declaration of Helsinki. Our local ethics committee approved the study protocol. The objective of the study was explained to each patient before the study, and written informed consent was obtained from each patient.

**Determination of serum DAF protein.** Serum samples were diluted with phosphate-buffered saline solution containing 1% bovine serum albumin (Sigma-Aldrich, St Louis, Mo), 0.05% Tween 20, and 1 mmol/L phenylmethylsulfonylfluoride with increased NaCl concentration (0.4 mol/L) to reduce nonspecific reactions as described.[11] Details of the immunoassay for the measurement of DAF have been described.[5,11-14] In brief, human DAF was purified from pooled human erythrocyte stroma, and mouse monoclonal antibodies to DAF were prepared.[15] Two of the mouse monoclonal antibodies ($IgG_1$), clones 1C6 and 4F11, were used. The 1C6 antibody is directed to the active site on the DAF molecule (ie, SCR 3), and the 4F11 antibody recognizes SCR4.[16] The 1C6 monoclonal antibody was labeled with HRP as described.[17] The wells of microtiter plates were coated with 4F11 monoclonal anti-DAF, and serially diluted serum samples were added to the wells. After washing, HRP-labeled 1C6 anti-DAF was added. After further washing, bound 1C6 antibody was detected with 2,2'-azino-di-3-ethylbenzo-thiazoline-6-sulfonic acid as substrate. Optical densities at 415 nm were measured with an automated ELISA plate reader. A calibration curve was obtained from several dilutions of known quantities of purified DAF, and the concentrations of serum DAF were calculated. Samples were analyzed in duplicate.

**Flow-cytometric analysis of DAF expression on peripheral-blood cells.** Heparinized blood cells were immediately suspended in RPMI culture medium (Gibco-BRL, Gaithersburg, Md) with 10% fetal calf serum at 37°C and separated into mononuclear-cell, neutrophil, and red blood cell fractions. Mononuclear cells were obtained by means of centrifugation with Ficoll-paque (Amersham Bioscience, Piscataway, NJ). After being washed with phosphate-buffered saline solution containing 1% bovine serum albumin, cells in each fraction were incubated with 1C6 mouse anti-DAF monoclonal antibody on ice for 30 minutes, then labeled with FITC-conjugated $F(ab')_2$ fragments of rabbit anti-mouse IgG (DAKO, Glostrup, Denmark).

The mononuclear-cell fraction was separated into subclasses on the basis of CD markers: CD3+ pan–T-cells, CD4+ helper/inducer T-cells, CD8+ suppressor/cytotoxic T-cells, CD14+ monocytes, and CD19+ B-cells. Cell-surface DAF expression in each subclass was analyzed with the use of 2-color flow cytometry. After performing FITC labeling with anti-DAF and blocking with normal mouse serum, we labeled mononuclear cells with a mouse monoclonal antibody conjugated with phycoerythrin — anti-CD3, anti-CD4, anti-CD8, anti-CD14, or anti-CD19 (DAKO) — on ice for 30 minutes. Mouse monoclonal antibody of the $IgG_1$ subclass specific for an irrelevant antigen was used as a negative control. After washing, $1.0 \times 10^4$ cells were analyzed in a FACScan apparatus (Becton Dickinson, Franklin Lakes, NJ). Data were analyzed with the use of Cellquest software (Becton Dickinson) in accordance with the manufacturer's instructions, and cell-surface DAF expression was presented as mean fluorescence intensity of DAF staining.

**Statistical analysis.** Data are expressed as mean ± SEM. Data sets were examined with Scheffé's multiple-comparison test and Wilcoxon's signed-rank test.

## RESULTS

**Serum DAF concentrations in UC patients and controls.** The distribution of serum DAF concentrations in each patient group is shown in Fig 1. Concentrations of serum DAF in patients with active disease (48.6 ± 3.7 ng/mL) were significantly higher than those in patients whose disease was inactive (33.3 ± 1.3 ng/mL; $P$ = .0003) or those in healthy controls (32.3 ± 1.6 ng/mL; $P$ = .0007).

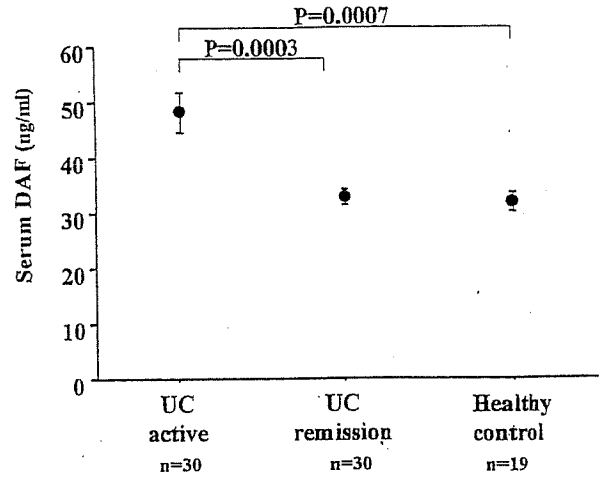


Fig 1. Serum DAF concentrations in patients with active and inactive UC and healthy controls. We measured serum DAF concentrations with the use of an immunoassay. Concentrations of serum DAF in patients with active UC were significantly higher than those in patients with inactive UC or healthy controls. Comparisons were made with the use of Scheffé's multiple-comparison test.

**Surface DAF expression on peripheral-blood cells.** To investigate the origin of increased serum DAF concentrations in patients with active UC, we examined surface DAF expression on peripheral-blood cells. Numbers of total WBCs and neutrophils in blood of patients with active UC were significantly higher than those in patients whose disease was inactive (total WBCs, $P$ = .016; neutrophils, $P$ = .01) and those in healthy controls (total WBCs, $P$ = .002; neutrophils, $P$ = .0004; Table II). We noted no significant difference in lymphocyte and monocyte counts among the groups. Cell-surface DAF expression on each blood-cell fraction is shown in Fig 2. Surface DAF expression on neutrophils ($P$ = .041), CD14+ monocytes ($P$ = .0002), CD19+ lymphocytes ($P$ = .01), CD4+ lymphocytes ($P$ =

**Table II.** Blood-cell counts in patients with active and inactive UC and healthy controls

| | UC | | | *P* (vs active UC)* | |
|---|---|---|---|---|---|
| Blood cell | Active ($n$ = 30) | Remission ($n$ = 30) | Healthy controls ($n$ = 19) | Remission | Control |
| WBC | 9.8 ± 1.1 | 6.8 ± 0.4 | 5.5 ± 0.3 | .016 | .002 |
| Neutrophil | 6.8 ± 0.8 | 4.3 ± 0.4 | 3.0 ± 0.2 | .01 | .0004 |
| Lymphocyte | 2.0 ± 0.2 | 1.9 ± 0.1 | 1.9 ± 0.1 | NS | NS |
| Monocyte | 0.62 ± 0.10 | 0.45 ± 0.03 | 0.38 ± 0.02 | NS | NS |

*NS*, not significant; *WBC*, white blood cell.
Data expressed as mean ± SEM ($\times 10^3/\mu L$).
*Scheffé's multiple-comparison test.

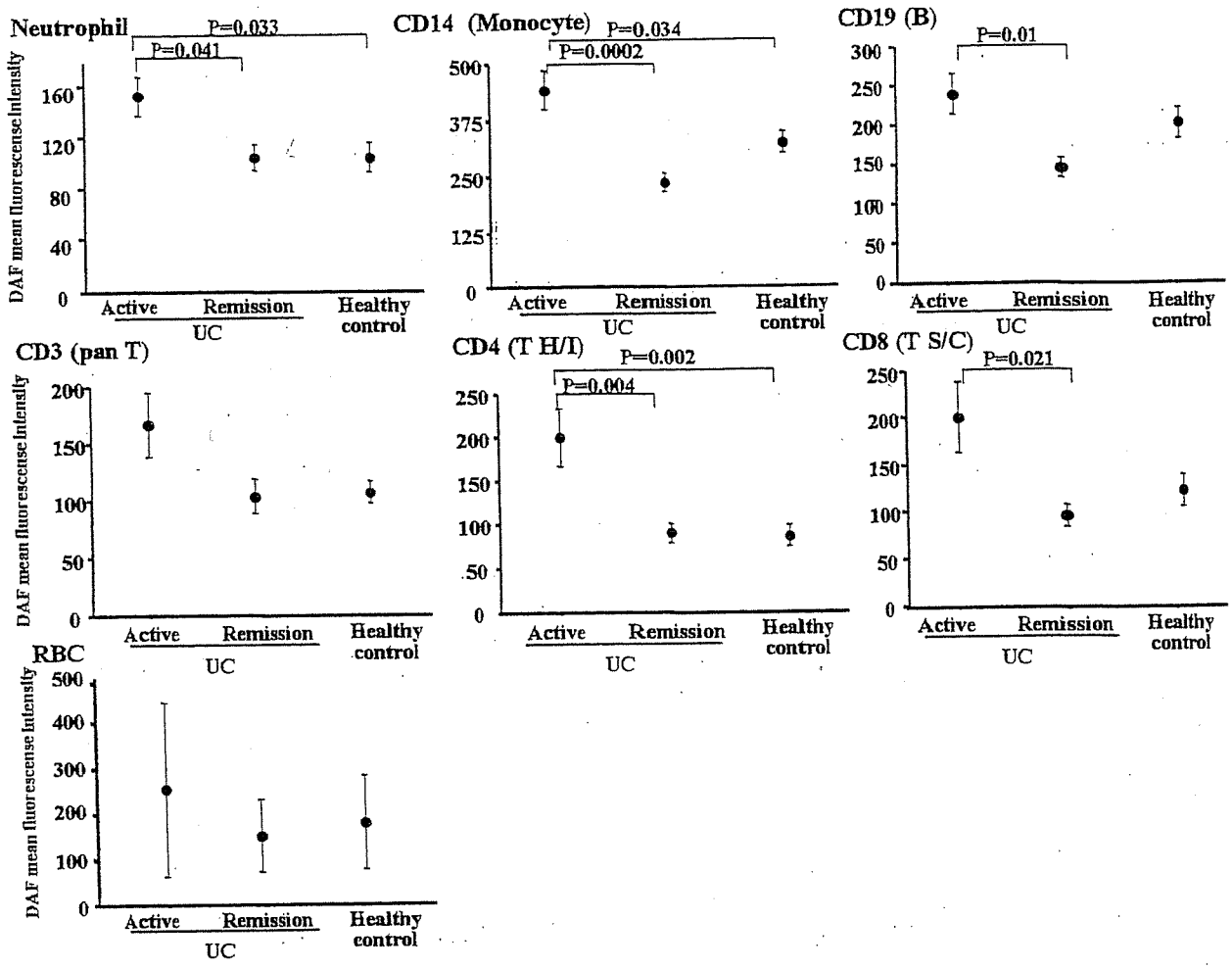


Fig 2. Flow-cytometric analysis of surface DAF expression on peripheral-blood cells in patients with active or inactive UC and healthy controls. DAF expression on the surfaces of peripheral-blood cells was analyzed with the use of flow cytometry; cell-surface DAF expression is presented as mean fluorescence intensity. Surface DAF expression on neutrophils, CD14+ monocytes, CD19+ B lymphocytes, CD4+ helper/inducer T (*T H/I*)-lymphocytes, and CD8+ suppressor/cytotoxic T (*T S/C*) lymphocytes in patients with active UC ($n$ = 14) was significantly increased compared with that in patients whose UC was in remission ($n$ =13). Surface DAF expression on neutrophils, CD14+ monocytes, and CD4+ lymphocytes was also significantly increased compared with that in healthy controls ($n$ = 14). Comparisons were made with the use of Scheffé's multiple-comparison test. *RBC*, red blood cell.

.004), and CD8+ lymphocytes ($P$ = .021) in patients with active UC was significantly increased compared with that in patients whose UC was in remission. Surface DAF expression on neutrophils ($P$ = .033), CD14+ monocytes ($P$ = .034), and CD4+ lymphocytes ($P$ = .002) was also significantly increased compared with that in healthy controls. We noted no apparent difference in surface DAF expression on erythrocytes among these groups.

**Effects of medical treatment on serum DAF concentrations and surface DAF expression on peripheral-blood cells.** We evaluated the effects of medical treatment on serum DAF concentrations and surface DAF expression on peripheral-blood cells. A pair of blood samples was obtained from each of 7 UC patients. The first samples were taken when disease was active, the second when the disease was in remission after medical therapy. The increased serum DAF concentrations (37.3 ± 5.1 ng/mL) in patients with active disease decreased to significantly lower levels in sera obtained when the disease had gone into remission (25.4 ± 2.1 ng/mL; $P$ = .018; Fig 3). The enhanced surface DAF expression on 5 of the 6 WBC fractions examined (neutrophils, CD14+ monocytes, CD19+ lymphocytes, CD3+ lymphocytes and CD8+ lymphocytes) also declined significantly after the patients' disease had gone into remission.

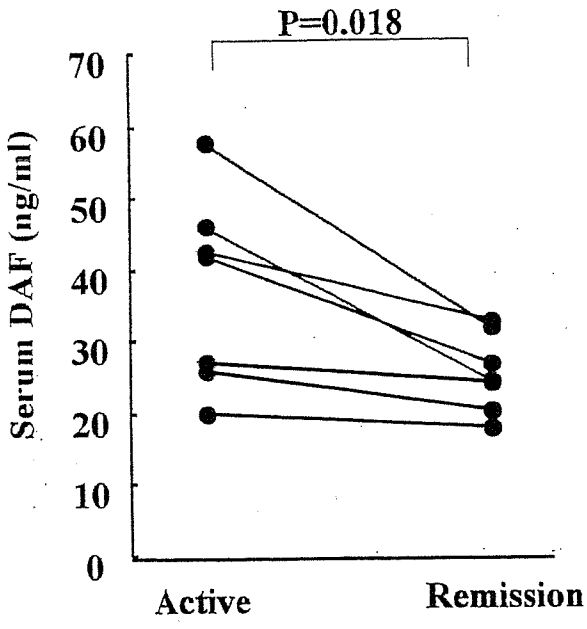


Fig 3. Serum DAF concentrations in patients with active UC before and after medical treatment. A pair of sera was obtained from each of 7 UC patients. The first sample was taken when disease was active, the second when disease was in remission after medical therapy. The increased serum DAF concentrations in patients with active disease were noted to have fallen to significantly lower levels in sera obtained when the disease was remission (Wilcoxon's signed-rank test).

There was no apparent change in DAF expression on erythrocyte surfaces after medical treatment (Fig 4).

## DISCUSSION

In this study, we first found significantly increased serum DAF concentrations in patients with active UC. These concentrations declined significantly after the disease had gone into remission induced by medical treatment. Correspondingly, DAF expression on neutrophils, monocytes, and subsets of lymphocyte was increased when the disease was active and fell after medical therapy. These parallel observations suggest that the increased amounts of serum DAF associated with active UC are likely derived from peripheral-blood cells. DAF was isolated first from human erythrocyte membrane as a molecule that protects erythrocytes from hemolysis by regulating the autologous complement activation.[3] DAF is also present on other blood cells: neutrophils, monocytes, lymphocytes, and platelets,[18,19] with the greatest amounts on neutrophils and monocytes. Surface DAF expression on neutrophils was doubled when the cells were activated.[7] In active UC, large numbers of neutrophils and monocytes are activated and extravasate into the colonic mucosa, where they are believed to cause mucosal injury. Our finding of increased DAF expression on circulating leukocytes in active UC indicates that these circulating cells are likely in an activated state. However, up-regulation of surface DAF on activation of neutrophils paralleled the up-regulation of complement receptor types 1 and 3.[7] The surface expression of other leukocyte molecules such as CD26 (dipeptidyl peptidase IV) was reportedly increased in peripheral-blood lymphocytes from patients with UC.[20] The increase in the DAF expression may therefore be a manifestation of generalized leukocyte activation in active UC.

Inflammatory cytokines and mediators such as TNF-$\alpha$, IL-1$\beta$, and leukotoriene B4, concentrations of which are increased in the inflamed mucosa of UC patients,[21-24] facilitate the activation and infiltration of leukocytes. TNF-$\alpha$ and IL-1$\beta$ reportedly increase DAF expression in various types of cells.[6,25-28] It therefore seems reasonable to assume that these inflammatory cytokines are responsible for the enhanced expression of DAF on leukocyte surfaces in patients with active UC.

Soluble variants of DAF are present in various extracellular fluids (eg, tears, saliva, urine, blood plasma, serum).[29-31] Although there have been a few reports of DAF concentrations in plasma, serum concentration have not been reported until recently, and then only in healthy subjects.[31] The reported serum DAF concentration (29.6 $\pm$ 5.4 ng/mL) is similar to that in our healthy control subjects (32.3 $\pm$ 7.1 ng/mL). Our observation that serum DAF concentrations declined in patients whose disease had gone into remission with treatment is also consistent with the proposition that the serum DAF concentration was derived from activated WBCs. It is known that DAF can be released into culture medium from various types of cells (eg, neutrophils, umbilical-vein endothelial cells, HT-29 human intestinal epithelial cells).[6,8,32] Therefore the increase in serum DAF concentration associated with active UC conceivably originate from intestinal epithelial cells, leukocytes, and/or vascular endothelial cells, but our observations support leukocytes as the most likely source.

Because we used 2 monoclonal antibodies recognizing different DAF epitopes, including the complement-regulatory domain SCR3[16] in our immunoassay, the DAF we detected in serum likely contains a significant portion of its complete structure. Consistent with this opinion is the observation that DAF spontaneously shed from cultured cells transfected with human DAF complementary DNA inhibited both the classical and alternative pathways of complement activation.[33] DAF in serum likely retains its function as a complement regulator. Activation and degradation of complement are observed in mucosal lesions of active UC.[2,34,35]

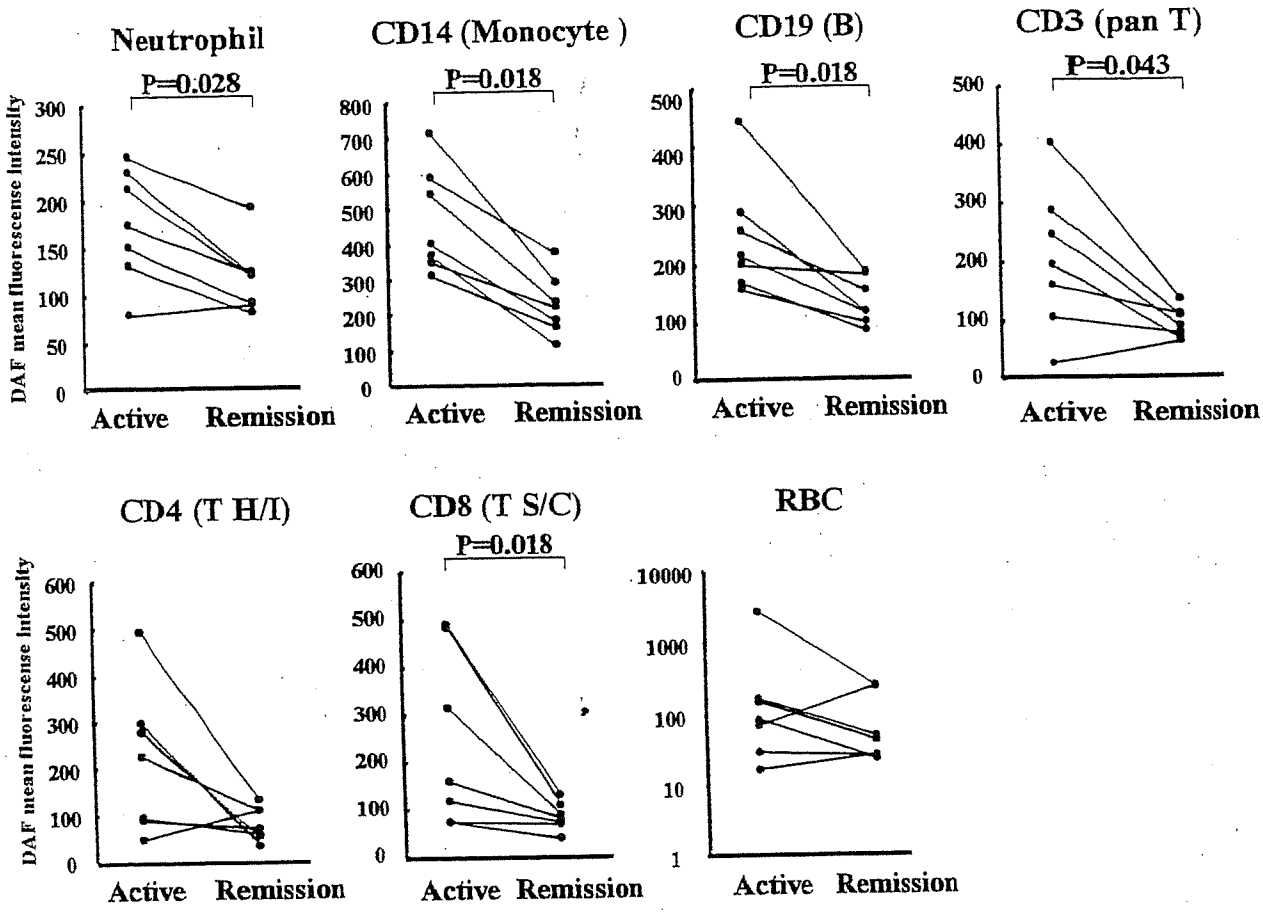


Fig 4. Flow-cytometric analysis of surface DAF expression on peripheral-blood cells from 7 patients with active UC before and after medical therapy. The enhanced surface DAF expression on 5 of the 6 WBC fractions examined was noted to have fallen significantly when the disease went into remission. The decreases on neutrophils, CD14+ monocytes, CD19+ B-lymphocytes, CD3+ pan–T-lymphocytes, and CD8+ suppressor/cytotoxic T (*T S/C*)-lymphocytes were statistically significant (Wilcoxon's signed-rank test). *RBC*, red blood cell.

Whether serum DAF plays a role in these immune responses in UC awaits clarification.

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# Difference in *Ulex europaeus* agglutinin I-binding activity of decay-accelerating factor detected in the stools of patients with colorectal cancer and ulcerative colitis

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**Expression of decay-accelerating factor (DAF, CD55), a complement-regulatory glycoprotein, is enhanced in colorectal-cancer (CC) cells and colonic epithelium in ulcerative colitis (UC), and stools from these patients contain increased amounts of DAF. Carbohydrate chains of glycoproteins are often altered during malignant transformation or inflammation. In this study, we investigated whether DAF molecules in patients with CC and those with UC differ with respect to oligosaccharide side chains. We analyzed DAF in stools and homogenates of colonic-tissue specimens obtained from patients with CC or UC using solid-phase enzyme-linked assay and Western blotting for reactivity with the lectins *Ulex europaeus* agglutinin I (UEA-I), wheat-germ agglutinin, peanut agglutinin, and concanavalin A. UEA-I bound to DAF in stools from patients with UC but not in that from the stools of CC patients, as demonstrated on the solid-phase enzyme-linked assay ($P < .05$, Mann-Whitney U test) and Western blotting. Binding of UEA-I was specifically inhibited by the addition of fucose. The difference in UEA-I reactivity with DAF was observed also in colonic-tissue homogenates from patients with UC and those with CC. DAF expressed in the mucosa and excreted into the stools of UC patients is different from that expressed in CC with regard to UEA-I reactivity. Future studies should be directed toward determining whether a qualitatively unique isoform of DAF is present, of which sugar chains are specific to CC in UC patients. (J Lab Clin Med 2004;143:169-74)**

**Abbreviations:** CC = colorectal cancer; ConA = concanavalin A; DAF = decay-accelerating factor; EDTA = ethylenediaminetetraacetate; HRP = horseradish peroxidase; OD = optical density; PMSF = phenylmethylsulfonylfluoride; PNA = peanut agglutinin; UEA-I = *Ulex europaeus* agglutinin I; UC = ulcerative colitis; WGA = wheat-germ agglutinin

Decay-accelerating factor (CD55) is a membrane glycoprotein that regulates complement activation by inhibiting the formation of C3/C5 convertases.[1] We have reported that the expression of DAF is enhanced in CC cells[2] and the colonic epithelium of UC in relation to the degree of mucosal inflammation.[3]

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Drs Mizuno, Fujita, and Tsuji have patented the method of detecting stool decay-accelerating factor described in this article.

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We have also found that stools from patients with CC and those with UC contain increased amounts of DAF.[4,5] These findings suggest that measurement of stool DAF would be useful in CC screening and the monitoring of disease activity in UC.

DAF has both *N*- and *O*-linked glycosylation sites.[6] Carbohydrate portions of glycoproteins are often altered during malignant transformation[7] and in various inflammatory conditions,[8,9] and DAF detected in UC patients may be different from that expressed CC in terms of the structure of oligosaccharide side chains. Lectins are proteins that can bind to certain carbohydrate chains; changes in reactivity with various lectins (eg, PNA, UEA-I) have been reported in the colonic mucosa of malignant[10-13] and inflammatory conditions.[8,14] In this study, we sought to determine whether differential binding of various lectins to DAF oligosaccharide side chains could be used to distinguish between the DAF in the stools and colonic tissues of patients with CC and those with UC.

## METHODS

**Patients.** Spontaneously passed stool specimens and samples of colonic tissue were obtained from 10 patients with CC (4 women, 6 men; mean age 61 years, range 44-78) and 13 patients with UC (7 women, 6 men; mean age 32 years; range 15-68). Histologically, the colorectal tumors were well-differentiated adenocarcinoma ($n$ = 5), moderately differentiated adenocarcinoma ($n$ = 4), and mucinous adenocarcinoma ($n$ = 1). The tumors were located in the ascending colon ($n$ = 2), sigmoid colon ($n$ = 5), and rectum ($n$ = 3). TNM stages[15] included I ($n$ = 4), II ($n$ = 4), and III ($n$ = 2). The diagnosis of UC was based on history, clinical symptoms, and endoscopic and histologic findings. Ten patients had total colits; 3 had left-sided colitis. Disease activity, graded on the basis of clinical features and laboratory data in accordance with the criteria of Truelove and Witts,[16,17] was severe ($n$ = 8), moderate ($n$ = 3), or mild ($n$ = 2) at the time when specimens were obtained. When stool samples were obtained, 11 patients with UC received prednisolone (mean dose 40 mg/day), 4 received sulfasalazine (mean dose 4.5 g/day), and 7 received mesalazine (mean dose 2.1 g/day). Stool samples were obtained also from 10 control subjects (5 women, 5 men; mean age 51 years, range 30-67) who underwent total colonoscopic examination because of abdominal symptoms or screening for CC but were found to have no colorectal disease.

Specimens of tumor tissue or colonic mucosa (in UC) were obtained from each patient at the time of endoscopic examination or surgical resection. Stool and tissue specimens were quickly frozen and kept at −80°C until being used. White blood cells and red blood cells were obtained from the patients' peripheral blood by means of centrifugation with Ficoll (Amersham Bioscience, Piscataway, NJ). The study was conducted in accordance with the guidelines of the Declaration of Helsinki. The study protocol was approved by the local ethics committee, and informed consent was obtained from each patient.

**Lectin-binding assay on microtiter plates.** Stools were weighed; suspended in an equal volume of phosphate-buffered saline solution containing 1% bovine serum albumin, 0.05% Tween 20, and 1 mmol/L PMSF with increased NaCl concentration (0.4 mol/L)[18]; and centrifuged at 20,000$g$ for 15 minutes, after which the supernatants were collected. Tissue specimens were homogenized in an equal volume of cold lysis buffer (phosphate-buffered saline solution containing 1% Nonidet P-40, 10 mmol/L EDTA, and 1 mmol/L PMSF). The homogenate was centrifuged at 20,000$g$ for 15 minutes at 4°C, after which the supernatant was collected. The amount of total protein in each sample was estimated with BCA protein assay reagent (Pierce, Rockford, Ill). The amount of DAF in each sample was measured with the use of an enzyme-linked immunosorbent assay as described.[4,5,18,19]

We examined the reactivity of DAF in stool and tissue specimens with various lectins using the following HRP-labeled lectins (HONEN Corp, Tokyo, Japan); PNA, which reacts with galactose β1-3*N*-acetyl-D-galactosamine residues[20]; WGA, which reacts with β-D-*N*-acetyl-glucosamine residues;[21] UEA-I, which reacts with terminal α-L-fucose residues;[22,23] and ConA, which reacts with α-D-mannose.[24] Stool supernatants adjusted to DAF concentrations of 5 ng/mL were added to wells of microtiter plates coated with 4F11 mouse monoclonal anti-DAF antibody[25] and incubated at 4°C overnight. After washing, HRP-labeled PNA, WGA, UEA-I, and ConA lectin were added to different wells and incubated at room temperature for 2 hours. After further washing, 2,2'-azino-di-3-ethylbenzo-thiazoline-6-sulfonic acid was added as substrate and ODs at 415 nm were measured. Samples were analyzed in duplicate.

**Western-blot analysis.** We analyzed the reactivity of DAF in stool specimens with UEA-I through the use of Western blotting. Stool extracts and crude extracts of human erythrocyte stroma, a positive control for DAF, were first immunoprecipitated with Sepharose 4B beads (Amersham Bioscience) labeled with 1C6 mouse anti-DAF monoclonal antibody.[25,26] In brief, samples were preabsorbed with Sepharose CL-4B beads, after which the 1C6 antibody–labeled Sepharose beads were mixed and incubated with the samples overnight at 4°C with continuous rotation. After washing, immunoprecipitates were subjected to 7.5% sodium dodecyl sulfate–polyacrylamide gel electrophoresis under nonreducing conditions and transferred to a polyvinylidene difluoride membrane (Immobilon; Millipore Corp, Bedford, Mass). As a positive control for UEA-I binding, we also subjected α2-macroglobulin[27] to SDS-PAGE and blotting. The membrane was incubated either with HRP-labeled UEA-I lectin in the absence or presence of 200 mmol/L fucose or HRP-labeled 1C6 mouse anti-DAF monoclonal antibody, prepared as described.[25,28] After washing, bound reactivity was detected with the use of a chemiluminescence-based detection kit (Hyperfilm-ECL and ECL detection reagent; Amersham Bioscience) in accordance with the manufacturer's protocols.

**Statistical analysis.** We used the Mann-Whitney U test for statistical analysis.

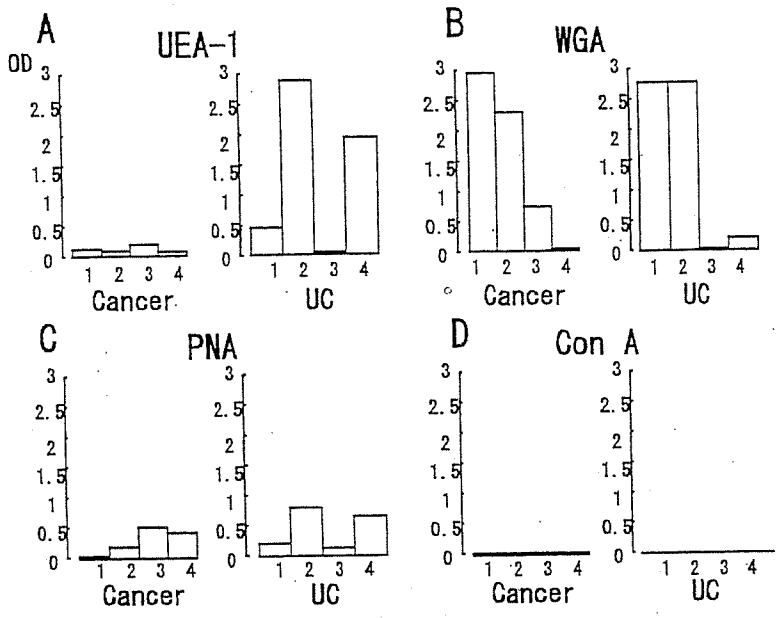


Fig 1. Reactivity of various lectins with stool DAF. Stool supernatants from 4 patients with CC and 4 with UC were added to the wells of microtiter plates coated with anti-DAF antibody and then reacted with HRP-labeled UEA-I (A), WGA (B), PNA (C), or ConA (D) lectins. A difference in reactivity with UEA-I, but not with the other 3 lectins, between samples from CC and UC patients is evident.

## RESULTS

**Lectin-binding assay on microtiter plates.** Medians (range) of stool DAF concentrations in the UC patients, the patients with CC, and the control subjects were 467 ng/g stool (119–1465 ng/g), 41 ng (30–283 ng/g), and 0.4 ng/g (0.4–0.7 ng/g), respectively. Because the amount of DAF in stool in the control subjects was negligible, we performed the following lectin-binding experiments with stools from the UC patients and from the patients with CC.

We first analyzed the reactivity of DAF in stool specimens from 4 CC patients and 4 UC patients with the lectins PNA, WGA, UEA-I, and ConA (Fig 1). DAF in the stool specimens from 3 of 4 UC patients, but none of the CC patients, bound UEA-I. DAF from most of the CC patients, as well as that from the UC patients, bound WGA and PNA lectin, but the binding to PNA was weaker than that to WGA. We found no apparent reactivity of stool DAF and ConA lectin in patients with CC or those with UC.

On the basis of the results of this pilot experiment, we examined the reactivity of UEA-I and WGA with DAF in stool specimens from 10 patients with CC and 13 with UC (Fig 2). Reactivity of UEA-I with stool DAF from UC patients was significantly higher than that with DAF from the stool of CC patients ($P = .04$, Mann-Whitney U test). We detected no difference in WGA binding with stool DAF from the 2 patient populations. With regard to the effects of medications for UC, 2 patients with UC received only corticosteroid, and reactivities of UEA-I (ODs) with stool DAF in the 2 patients were 1.99 and 0.03. Another 2 patients received sulfasalazine but not corticosteroids, and ODs of their stool DAF in UEA-I binding assay were 2.95 and 0.02.

Next we examined UEA-I reactivity with DAF in colonic-tissue homogenates (Fig 3). Reactivity of UEA-I with DAF in inflamed colonic tissues from UC patients was significantly higher than that with DAF from CC patients ($P = .02$, Mann-Whitney U test). UEA-I binding to DAF in peripheral red blood cells and leukocytes obtained from UC patients was negligible (data not shown).

To document the specificity of the reactivity of UEA-I with stool DAF from patients with UC, we tested inhibition of lectin binding by adding monosaccharides. As illustrated in Fig 4, the addition of fucose specifically inhibited the binding of UEA-I in a dose-dependent manner, whereas the nonrelevant monosaccharides galactose and *N*-acetyl-glucosamine did not inhibit binding.

**Specificity of UEA-I binding to stool DAF on Western-blot analysis.** Next we analyzed the reactivity of DAF in stool specimens with UEA-I by means of Western blotting (Fig 5). Stool DAF proteins in UC and CC were present as a broad band with a molecular weight of around 70 kD. UEA-I bound to stool DAF from UC

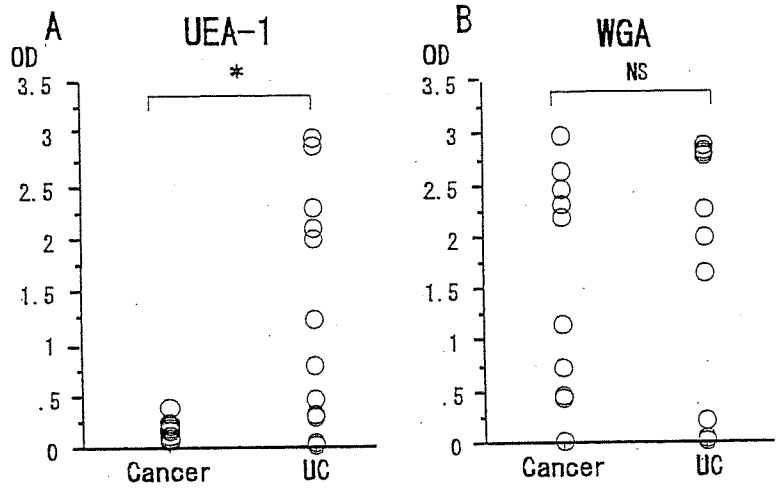


Fig 2. Reactivity of UEA-I and WGA lectins with stool DAF. Stool supernatants from 10 patients with CC and 13 with UC were added to the wells of microtiter plates coated with anti-DAF antibody and then reacted with HRP-labeled UEA-I (A) or WGA (B). Reactivity of UEA-I with stool DAF from UC patients was significantly higher than that with DAF from CC patients.
*$P$ = .04, Mann-Whitney U test. *NS*, not significant.

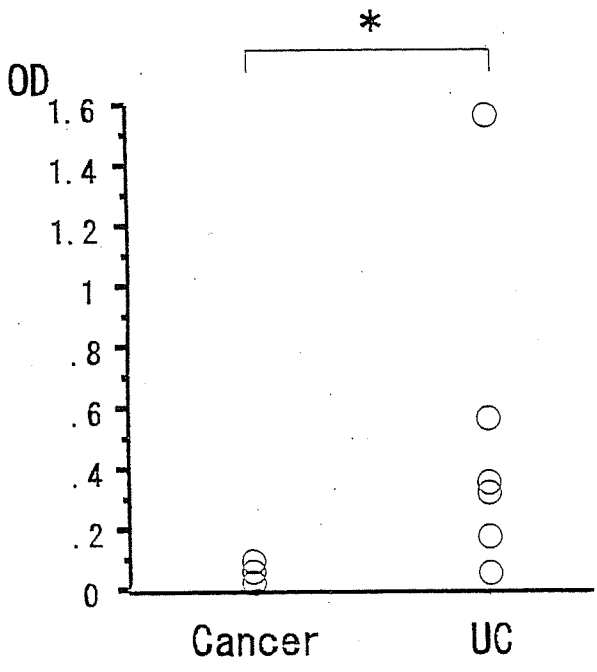


Fig 3. Reactivity of UEA-I lectin with colonic-tissue DAF. Tissue homogenates from 5 patients with CC and 7 with UC were added to the wells of microtiter plates coated with anti-DAF antibody and then reacted with HRP-labeled UEA-I. Reactivity of UEA-I with tissue DAF from UC patients was significantly higher than that with DAF from CC patients.
*$P$ = .02, Mann-Whitney U test.

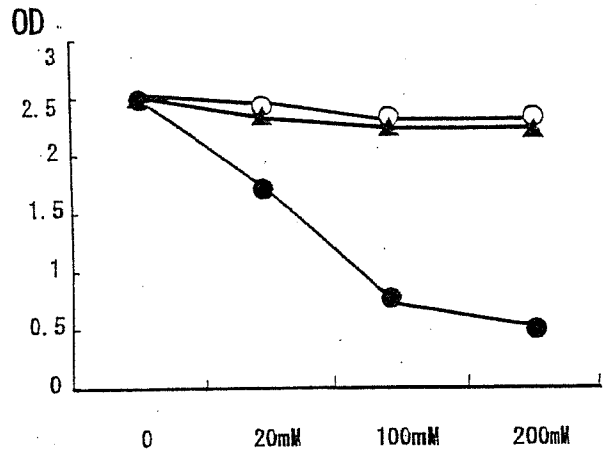


Fig 4. Inhibition of UEA-I binding to stool DAF from UC patients by monosaccharides. Stool supernatants from UC patients were added to wells coated with anti-DAF antibody. Next, serially diluted fucose (*black circles*), galactose (*open circles*), or *N*-acetyl-glucosamine (*black triangles*) was added to the wells, together with HRP-labeled UEA-I. The binding of UEA-I lectin was specifically inhibited by the addition of fucose in a dose-dependent manner. Data represent the mean of 3 experiments.

patients with a molecular weight comparable to that of the DAF band, and UEA-I binding was inhibited by the addition of 200 mmol/L fucose. In contrast, UEA-I did not bind to DAF in stool specimens from CC patients.

## DISCUSSION

In this study we examined the reactivity of various lectins with stool DAF from patients with UC or CC. The major finding was that UEA-I bound to stool DAF in the UC patients but not in the CC patients. This

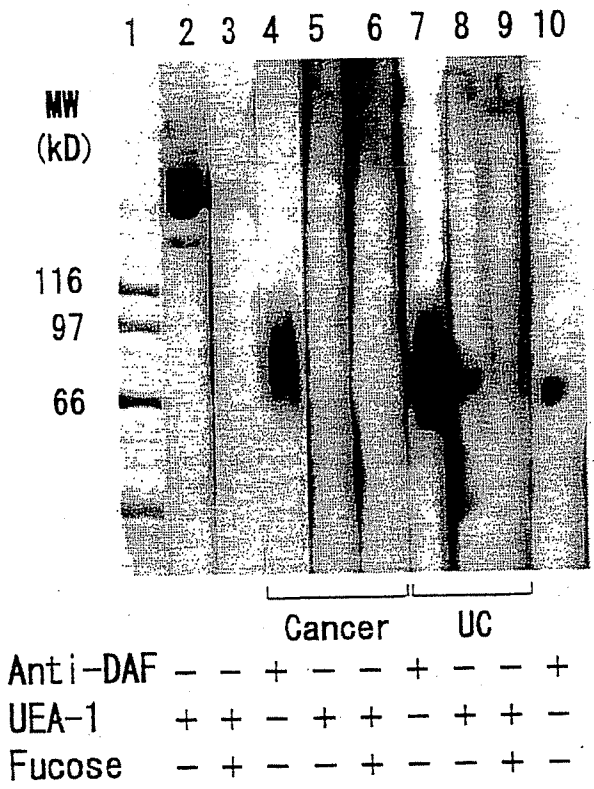


Fig 5. Western-blot analysis of UEA-I binding to stool DAF. Stool supernatants (*lanes 4–9*) and crude extracts of human erythrocyte stroma (*lane 10*), as a positive control for DAF, were immunoprecipitated with anti-DAF antibody-conjugated beads. The immunoprecipitated DAF and α2-macroglobulin (*lanes 2 and 3*), a positive control for UEA-I, were subjected to 7.5% SDS-PAGE and transferred to a polyvinylidene difluoride membrane. The membrane was incubated with HRP-labeled UEA-I lectin, either in the absence (*lanes 2, 5, and 8*) or presence (*lanes 3, 6, and 9*) of fucose or HRP-labeled anti-DAF monoclonal antibody (*lanes 4, 7, and 10*). Stool DAF proteins in CC (*lane 4*) and UC patients (*lane 7*) are visible as a broad band with a molecular weight of around 70 kD. Binding of UEA-I is visible with stool DAF from UC patients, with a molecular weight of around 70 kD, comparable to that of the DAF band (*lane 8*). UEA-I binding was inhibited by the addition of 200 mmol/L fucose (*lane 9*). UEA-I reactivity was not detected in stool DAF from CC patients (*lanes 5 and 6*). All these lanes were from the same experiment, but the exposure was shorter for the film used for the anti-DAF antibody probe (*lanes 4, 7, and 10*) than for UEA-I probe (*lanes 2, 3, 5, 6, 8, and 9*) because the DAF band detected by anti-DAF antibody developed rapidly. *Lane 1* contains molecular markers.

difference was also observed between tissue homogenates of inflamed colonic mucosa from UC patients and the tissues of CC patients. Binding of UEA-I to DAF was not observed in the peripheral-blood cells of UC patients, suggesting that the UEA-I reactivity with DAF in UC patients' stools was not due to UEA-I binding to red blood cells or leukocytes passed in the stool. Rather, DAF expressed in UC mucosa is evidently different from DAF expressed in CC cells in terms of UEA-I reactivity. Moreover, this difference in glycosylation of DAF is also expressed in the DAF passed in these patients' stools.

UEA-I is a lectin that binds specifically to terminal α-linked fucose residues of glycoproteins and glycolipids.[22,23] Malignant transformation of cells is frequently accompanied by alteration in surface oligosaccharides, such as the expression of carbohydrate determinants containing sialylated or fucosylated structures. Binding of UEA-I to CC cells has been reported,[11,12] whereas UEA-I binding has been observed infrequently in the inflamed mucosa of UC patients.[8,14] We had therefore expected to find UEA-I binding to stool DAF from CC patients but not to the DAF from UC patients, but we found the opposite. We have no ready explanation for this disparity.

In altered glycosylation processes, terminal fucose residues to which UEA-I binds might be expressed in either *N*- or *O*-linked sugar chains by either the addition of fucose residues or the loss of other terminal sugars. The matured membrane form of DAF contains a single *N*-linked complex-type oligosaccharide chain and multiple sialylated *O*-linked oligosaccharide side chains.[6] We found that ConA lectin, which has a high affinity for mannose residues of *N*-linked sugar chains, did not bind to stool DAF from either UC or CC patients, supporting the *O*-linked sugar chain–rich nature of the DAF. It therefore seems likely that alterations in the *O*-linked sugar chains of DAF account for the UEA-I binding we observed in UC patients' stools and tissues, but this possibility merits verification.

With regard to the effects of the medications used to treat UC, corticosteroids are shown to influence the glycosylation and fucosylation processes of glycoproteins in rat small intestine.[29] Although stool DAF from 2 patients not receiving sulfasalazine or corticosteroid showed high affinity for UEA-I, the sample size was small and appropriate control samples (eg, stools from patients with CC receiving these drugs) were not available. We could therefore not rule out the possibility that the UEA-I binding observed in stool DAF in UC patients was an effect of the medication given to these patients.

Patients with chronic UC, particularly extensive disease of more than 8 to 10 years' duration, are at increased risk for CC.[30-32] If a difference in the DAF present in CC and UC can be identified, it might be helpful in the construction of strategies for detecting CC in UC patients. In this study, we demonstrated that DAF induced by colonic inflammation is different from DAF induced by malignant transformation in terms of the structure of oligosaccharide side chains as revealed by UEA-I reactivity. Future studies should be directed

toward determining whether a qualitatively unique isoform of DAF is present of which sugar chains are specific to CC in patients with long-standing UC.

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*Original Article*

# Family Experience with Palliative Sedation Therapy for Terminally Ill Cancer Patients

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*Abstract*

*Symptomatic sedation is often required in terminally ill cancer patients, and could cause significant distress to their family. The aims of this study were to clarify the family experience during palliative sedation therapy, including their satisfaction and distress levels, and the determinants of family dissatisfaction and high-level distress. A multicenter questionnaire survey assessed 280 bereaved families of cancer patients who received sedation in 7 palliative care units in Japan. A total of 185 responses were analyzed (response rate, 73%). The families reported that 69% of the patients were considerably or very distressed before sedation. Fifty-five percent of the patients expressed an explicit wish for sedation, and 89% of families were clearly informed. Overall, 78% of the families were satisfied with the treatment, whereas 25% expressed a high level of emotional distress. The independent determinants of low levels of family satisfaction were: poor symptom palliation after sedation, insufficient information-giving, concerns that sedation might shorten the patient's life, and feelings that there might be other ways to achieve symptom relief. The independent determinants of high levels of family distress were: poor symptom palliation after sedation, feeling the burden of responsibility for the decision, feeling unprepared for changes in the patient's condition, feeling that the physicians and nurses were not sufficiently compassionate, and shorter interval to patient death. Palliative sedation therapy was principally performed to relieve severe suffering based on family and patient consent. Although the majority of families were comfortable with this practice,*

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*clinicians should minimize family distress by regular monitoring of patient distress and timely modification of sedation protocols, providing sufficient information, sharing the responsibility of the decision, facilitating grief, and providing emotional support.* J Pain Symptom Manage 2004;28:557–565. 

*Key Words*
*Palliative sedation therapy, family, palliative care, neoplasms*

## *Introduction*

Palliative sedation therapy has been the focus of a strong debate in the recent medical literature.[1–3] Empirical studies have reported that 10–50% of terminally ill cancer patients require sedation for acceptable symptomatic relief.[4,5]

Palliative care specialists stress the importance of extensive care for the family members of sedated patients, because it may cause profound family distress, and one of the chief aims of palliative care is to relieve family distress.[1–3] Understanding family experiences with palliative sedation therapy is of value to develop effective care strategies for family members, but no empirical studies have systemically investigated them.

In intensive care settings, several studies have revealed how the family felt about the withdrawal of treatments and the behavior of physicians and nurses that was helpful or harmful.[6–10] A qualitative study by Tilden et al. has identified that timely communication, clarification of family roles, facilitating family consensus, accommodating family grief, and avoiding placing the full burden of decision-making on one person were regarded as helpful behavior by medical professionals.[10] These clinical observations have been integrated into recommendations about how medical professionals should care for family members in such complex situations.[11–13]

This survey was performed with the primary aims to 1) clarify the actual experience of family members in palliative sedation therapy, 2) clarify the overall family evaluation about sedation, and 3) identify the factors influencing the family evaluation.

## *Methods*

This was a cross-sectional, anonymous multicenter survey of the bereaved families of cancer patients who received palliative sedation at specialized palliative care units in Japan. We mailed questionnaires to bereaved families in October 2002, and again in November 2002 to non-responding families.

We initially identified all 23 palliative care units as potential participating institutions that met the following criteria: 1) formally approved by the Japanese Association of Hospice and Palliative Care Units, 2) having 15 or more beds, and 3) belonging to a hospital with more than 350 beds. We then approached 10 palliative care units conveniently selected from them, and finally 7 palliative care units agreed to participate in this survey.

We identified the potential participants consecutively through a chart review by primary physicians. The inclusion criteria were: 1) bereaved family members of an adult cancer patient who died January to November 2000 after receiving sedation (one family member for one patient), 2) aged 20 or more, 3) capable of replying to a self-reported questionnaire, and 4) no serious psychological distress recognized by the primary physicians. The last criterion was adopted on the assumption that primary physicians could identify families who might suffer serious psychological burden by this survey. Physicians cared for the families closely in inpatient care settings, with a mean admission period of 47 days (unpublished data). We adopted 2 years as the minimal time interval between patient death and this survey despite the possibility of recall bias, because the authors agreed that shorter time intervals might cause significant emotional burden on family members.

The responsible physicians recorded the patients' backgrounds: age, sex, types of sedation, all target symptoms, medications used for sedation, and the duration of continuous-deep sedation. Delirium was diagnosed following the

*Diagnostic and Statistical Manual of Mental Disorders* (4th edition).[14]

Palliative sedation therapies investigated in this survey were intermittent-deep sedation and continuous-deep sedation.[15,16] The former was defined as the intermittent use of sedative medications to relieve intolerable and refractory distress by achieving almost or complete unconsciousness, and the latter was defined as the continuous use of sedative medications to relieve intolerable and refractory distress by achieving almost or complete unconsciousness until death.[15,16] The researchers in each institution agreed with these definitions prior to the survey. The indications and medical practice of sedation was determined on the basis of standard clinical practice of each institution.

The ethical and scientific validity was confirmed by the institutional review boards of each hospital.

*Questionnaire*

The questionnaire (available from the authors) was developed by the authors based on a literature review, in-depth interviews with 3 bereaved family members, and a preliminary questionnaire survey of 100 bereaved family at an single inpatient hospice.[1–13,17–21] The term palliative sedation therapy was paraphrased into the "treatment to alleviate discomfort by inducing sleep" throughout the survey, with short descriptions.

The level of family satisfaction with palliative sedation therapy was rated on an 8-point scale from "1 - very dissatisfied" to "8 - completely satisfied," and the level of the family-perceived distress was rated on a 5-point scale from "1 - not distressed at all" to "5 - very distressed." In addition, the family-perceived appropriateness of when sedation was started was rated on a 5-point scale of "too early," "maybe too early," "appropriate," "maybe too late," and "too late."

The respondents provided information about age, sex, relationship to the patient, interval from patient death, health status during the admission periods (good, fair, poor, and very poor), and the presence or absence social support (someone with whom the respondents could consult). They were requested to report on 5 variables related to their actual experience during palliative sedation: 1) the level of patient distress before sedation (very distressed, considerably distressed, not so distressed, and difficult to determine); 2) the level of patient distress after sedation (completely resolved, mostly resolved, sometimes distressed, often distressed, and constantly distressed); 3) the change in the frequency of physicians' visits to the patient after sedation (increased, same, slightly decreased, or decreased); 4) the change in the nurses' attitude toward the patient (more attentive, same, slightly less attentive, or less attentive); and 5) whether the physicians or nurses who performed sedation did or did not know the patient well.

The respondents were requested to describe 9 variables related to the decision-making process: 1) the presence or absence of prior discussion about the preferred end-of-life treatment between patients and family; 2) the presence or absence of prior discussion about sedation between the family and medical staff; 3) the presence or absence of the patient's explicit wish for sedation; 4) the family-perceived adequacy of the frequency of information giving about sedation (sufficient, slightly insufficient, insufficient); 5) the person who explained about sedation to the family members; 6) the time interval from the first discussion to the actual initiation of sedation; and 7) the presence or absence of a conflict in the opinions about sedation among the family members, between the patient and family, and between the family and medical staff.

Finally, the respondents were requested to rate their degree of agreement with 13 statements concerning the concerns the families might have about palliative sedation therapy on a 5-point Likert-type scale of "1 - disagree" to "5 - strongly agree."

*Analyses*

To explore the determinants of family satisfaction and distress levels related to sedation, we initially screened 10 background variables (Table 1), 5 variables related to experience in palliative sedation, 9 variables related to the decision-making process, and 13 family-reported concerns about sedation by univariate analyses. Univariate analyses were performed using the Mann-Whitney U-test and the chi square test (Fisher's exact methods), where appropriate. To assess the chance results in 37 comparisons, the $P$ value necessary for statistical significance was defined as 0.001 ($<0.0013 = 0.05/37$) using the Bonferroni correction.

*Table 1*
**Backgrounds of Patients and the Bereaved Families**

| | |
|---|---|
| Patients | |
| Age (years) | 63 ± 13 |
| Sex, % (*n*) | |
| Male | 56 (104) |
| Female | 44 (81) |
| Sedation types, % (*n*) | |
| Intermittent alone | 22 (41) |
| Continuous | 78 (144) |
| Target symptoms,[a] % (*n*) | |
| Agitated delirium | 68 (127) |
| Dyspnea | 32 (60) |
| Pain | 14 (26) |
| Myoclonus/convulsion | 3.2 (6) |
| Others | 2.1 (4) |
| Bereaved families | |
| Age (years) | 57 ± 12 |
| Sex, % (*n*) | |
| Male | 35 (64) |
| Female | 64 (119) |
| Relationship to the deceased, % (*n*) | |
| Spouse | 55 (101) |
| Child | 25 (47) |
| Sibling | 9.2 (17) |
| Son-/daughter-in-law | 3.8 (7) |
| Parent | 2.7 (5) |
| Others | 2.7 (5) |
| Mean interval from patient death (months) | 28 ± 3.9 |
| Health status, % (*n*) | |
| Good, fair | 81 (149) |
| Poor, very poor | 19 (35) |
| Social support, % (*n*) | |
| Presence | 86 (159) |
| Absence | 14 (25) |

Percentages do not add up to 100% due to missing values.
[a]Duplicated answers.

For the comparisons, the respondents were classified into two groups: families who rated their satisfaction level as "very satisfied" or "completely satisfied" (defined as high-level satisfaction) and the others (low-level satisfaction); and families who rated their distress levels as "distressed" or "very distressed" (high-level distress) and the others (low-level distress). This classification was determined on the basis of the actual data distribution, and empirical findings that satisfaction scores usually have a highly skewed distribution toward satisfaction.[22]

Multiple linear regression analyses were then performed using the satisfaction and distress levels as dependent variables, and all the potentially significant predictors ($P < 0.01$) identified by univariate analyses were entered into these models as independent variables in a forward elimination fashion.

We calculated the percentages based on the whole numbers of data; and the numbers of missing values were additionally described, if more than 5%. We reported only the results from all families, because the subgroup analyses of the families of patients who received continuous-deep sedation achieved essentially the same results.

All analyses were performed using the Statistical Package for the Social Sciences (version 9.0).

## Results

Of 764 patients who died at the participating institutions during the study periods, 310 patients (41%) received sedation (intermittent sedation alone, 7.9%, $n = 60$; continuous-deep sedation with or without intermittent sedation, 33%, $n = 250$). As 30 cases were excluded due to serious psychological distress of families ($n = 24$) and no competent family available ($n = 6$), questionnaires were sent to a total of 280 family members. Of these, 16 were mailed back due to a wrong address and 197 were returned. As 12 responses were excluded due to missing values or late arrival, 185 responses were analyzed (effective response rate, 73%, 185/252).

Table 1 summarizes the backgrounds of the patients and family. The medications used for sedation were benzodiazepines (86%, $n = 160$), barbiturates (38%, $n = 71$), ketamine (2.7%, $n = 5$), and phenothiazines (1.1%, $n = 2$). The median sedation period for continuous-deep sedation was 2 days (<7 days in 97%, 140/144).

### *Family Experience in Palliative Sedation and the Decision-Making Process*

The families perceived that 69% of the patients were considerably or very distressed before sedation, whereas 14% reported that the patients were not so distressed (Table 2). After sedation, the symptom frequency reduced to sometimes or less in 88%, and the patients were still often or consistently distressed in 11% (Table 2). Also, 94% of the families ($n = 173$) reported that the physicians visited the patient as frequently as before or more frequently, and 95% ($n = 176$) reported the nurses cared for the patients as attentively as before or more attentively. In addition, 96% ($n = 177$) reported that physicians or nurses who knew the patient well performed the sedation.

*Table 2*
**Patient Distress Before and After Palliative Sedation Therapy**

| Before | % (*n*) | After | % (*n*) |
|---|---|---|---|
| Very distressed | 37 (69) | Constantly distressed | 3.2 (6) |
| Considerably distressed | 32 (60) | Often distressed | 8.1 (15) |
| Not so distressed | 14 (26) | Sometimes distressed | 28 (51) |
| Difficult to determine the degree of distress | 15 (28) | Mostly resolved | 47 (87) |
| | | Completely resolved | 13 (24) |

Percentages do not add up to 100% due to missing values.

The families reported that 55% of the patients ($n = 101$) expressed an explicit wish for sedation, whereas the others could not express their wishes. Eighty-nine percent (89%) of the family members ($n = 165$) received a clear explanation about sedation from physicians (68%, $n = 112$), nurses (6.7%, $n = 11$), or both (24%, $n = 39$), and 8.1% ($n = 15$) reported they had no clear information. The percentages of the families who were informed about the treatment goal (symptom palliation), the degree of achievable communication after sedation, the predicted physical changes after sedation, and the predicted physical status and prognosis if sedation was not induced were: 86% ($n = 160$), 67% ($n = 124$), 68% ($n = 125$), and 60% ($n = 111$), respectively. Although 75% of the families ($n = 139$) regarded the frequency of information giving as sufficient, 22% ($n = 40$) evaluated it as slightly insufficient and 2.2% ($n = 4$) as insufficient.

Prior discussion about preferred end-of-life treatment before actual deterioration of patient conditions was held between the patient and the family in 79% ($n = 146$), and between the family and the medical staff in 75% ($n = 139$; missing, 5.9%). The time interval from the first discussion to the actual initiation of sedation was: less than 1 day (22%, $n = 41$), 1 day to 1 week (32%, $n = 59$), 1 week to 1 month (22%, $n = 41$), and more than 1 month (9.7%, $n = 18$; missing, 5.9%). Conflicts in the opinions were observed among the family members in 15% ($n = 27$; missing, 6.5%), between the patient and the family in 7.6% ($n = 14$; missing, 12%), and between the family and the medical staff in 9.7% ($n = 18$; missing, 7.0%).

### *Family's Satisfaction and Distress Levels*

Of 185 bereaved family members, 144 (78%) expressed some level of satisfaction with sedation therapy: completely satisfied (8.1%, $n = 15$), very satisfied (17%, $n = 31$), satisfied (39%, $n = 72$), slightly satisfied (14%, $n = 26$), not sure (16%, $n = 30$), slightly dissatisfied (2.2%, $n = 4$), dissatisfied (1.6%, $n = 3$), and very dissatisfied (1.1%, $n = 2$). Also, 143 families (77%) evaluated the time when sedation was started as appropriate, although the others evaluated it as too early (1.6%, $n = 3$), maybe too early (7.6%, $n = 14$), maybe too late (7.0%, $n = 13$), and too late (2.7%, $n = 5$). On the other hand, 47 families (25%) expressed high levels of emotional distress about sedation: very distressed (10%, $n = 19$), distressed (15%, $n = 28$), slightly distressed (35%, $n = 64$), not so distressed (26%, $n = 48$), and not distressed at all (14%, $n = 25$).

### *Family-Reported Concerns About Palliative Sedation Therapy*

Table 3 summarizes the family concerns about sedation. Half of the families reported that they were distressed they could not communicate with the patient. About one-third of the families reported taking responsibility for the decision as a burden, and were concerned that sedation might shorten the patient's life. On the other hand, more than 85% of the families disagreed that the patient's status of sleeping was not dignified, and that they found no meaning in being with the patients.

### *Determinants of Family Satisfaction and Distress*

Compared with the highly satisfied family members, families with low-level satisfaction were significantly more likely to report higher levels of patient distress after sedation, evaluate the frequency of information-giving as insufficient, have concerns that sedation might shorten the patient's life, feel unprepared for changes of patient conditions, and think the physicians and nurses were not sufficiently compassionate; they also were less likely to have a prior discussion with the patients (Table 4).