Effect and Safety of Granulocyte-Monocyte Adsorption Apheresis for Patients with Ulcerative Colitis Positive for Cytomegalovirus in Comparison with Immunosuppressants

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<u>**Abbreviations used in this paper**</u>: PSL, predonisolone; GMA, granulocyte-monocyte adsorption apheresis; IMT, immunosuppressive therapies.

Key Words: **ulcerative colitis, cytomegalovirus, GMA, ganciclovir**

Abstract

Background: Cytomegalovirus (CMV) infection exacerbates ulcerative colitis (UC) refractory to immunosuppressive therapies. However, the underlying UC remained active in some UC patients, despite the fact that CMV-DNA in colonic mucosa became negative after antiviral therapy. Therefore, new therapeutic strategies for UC patients concomitant with CMV infection in mucosa are required.

Aims: The aim of this study was to evaluate the effect and safety of granulocyte-monocyte adsorption apheresis (GMA) in UC patients positive for CMV infection after antiviral therapy.

Methods: From October 2003 to December 2008, 64 patients with UC refractory to immunosuppressive therapies (IMT), including steroids and immunomodulators, were enrolled in this retrospective, observational, multicenter study, which was reviewed and approved by the Institutional Review Board of Kyoto University. CMV infection was investigated by three methods (histologic examination, CMV antigenemia, and polymerase chain reaction). We investigated clinical outcomes of GMA and IMT after 2weeks of treatment with ganciclovir.

Results: Thirty-one (48.4%) of 64 patients with UC refractory to IMT were positive for CMV. Of 31 patients, 4 (12.9%) underwent colectomy. Twenty-seven patients (87.1%) underwent antiviral therapy. Seven of 27 patients achieved remission following antiviral therapy alone. Of the remaining 20 patients that did not achieve remission despite the disappearance of CMV-DNA, 11 and 9 patients were treated with additional GMA (GMA group) and IMT (IMT group), respectively. Nine of 11 patients (GMA group) achieved remission and 2 underwent colectomy. Four of 9 patients (IMT group) achieved remission and 5 underwent colectomy. CMV-DNA was not detected in 11

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patients after GMA, but it was detected again in all 5 patients of the IMT group, that underwent colectomy. Total colectomy rate in UC patients positive for CMV was 35.5% (11/31). In addition, colectomy-free survival in the CMV relapse (+) group was estimated to be 12.9% at 65 months, while that in the CMV relapse (-) group was estimated to be 100% at 60 months.

Conclusion: The colectomy ratio tends to be high in refractory UC patients with recurrent CMV reactivation or infection. Therefore, GMA might be a safe and effective treatment for UC patients positive for CMV because it does not induce avoiding CMV reactivation.

Introduction

Cytomegalovirus (CMV) infection is considered to be an important exacerbating factor in patients with ulcerative colitis (UC) [1, 2]. Therefore, accurate and rapid diagnosis of CMV infection is critical for treatment for UC patients refractory to immunosuppressive therapies (IMT). We previously reported the usefulness of quantitative real-time polymerase chain reaction using colonic mucosa (mucosal PCR) for accurately diagnosing CMV infection in patients with UC refractory to IMT [3,4]. Based on the results of mucosal PCR for CMV-DNA in inflamed mucosa, we applied either antiviral therapy or modulating IMT to UC patients [3,4]. However, colonic inflammation remained active in some UC patients, even in those whose colonic mucosa became CMV-DNA negative after antiviral therapy. Domènech et al reported that CMV disease in UC patients occurs in the presence of colonic mucosal inflammation and ongoing IMT [5]. In addition, the results of experiments in a mouse model of colitis suggested that the underlying CMV infection alters mucosal immunity, potentially increasing the tendency of CMV-infected hosts to develop colitis [6]. Therefore, to establish anti-inflammatory therapy without reactivating CMV in UC patients with concomitant CMV infection is a very important issue.

It was recently established in Japan that granulocytapheresis (GCAP) using an Adacolumn (Japan Immunoresearch Laboratories, Takasaki, Japan) is safe and effective for active UC [7]. This apheresis column is filled with cellulose diacetate carriers that selectively adsorb granulocytes and monocytes/macrophages. Shimoyama *et al.* reported that a high remission rate was achieved in UC patients following GCAP therapy [5]. GCAP is a promising option for patients with UC refractory to conventional therapy, including prednisolone (PSL).

In general, after primary CMV infection, CMV persists within the host, in whom peripheral and bone marrow monocytes constitute a major site of viral latency. The differentiation of CMV latent monocytes into tissue macrophages due to granulocyte macrophage colony-stimulating factor and proinflammatory cytokines such as tumor necrosis factor (TNF)-α leads to CMV replication and CMV reactivation [8]. A recent in vitro study showed that CMV could force monocytes to acquire an M1 proinflammatory phenotype and differentiate into long-lived macrophages [9]. CMV-infected monocytes with the M1 phenotype induce cytokine expression of interleukin (IL)-6, TNF-α, and IL-1β, which might be involved in colonic inflammation of patients with inflammatory bowel disease (IBD). Considering the mechanism of CMV reactivation, granulocyte-monocyte adsorption apheresis (GMA) may be effective for UC patients concomitant with CMV infection, because it can remove granulocytes and monocytes/macrophages in which CMV infects latently and reactivates. Also, because treatment with steroids and IMT can be avoided, GMA might not affect the immune response to CMV reactivation.

In this study, we first evaluated whether or not flare-ups of UC positive for CMV can improve after antiviral therapy alone. Next, we evaluated the effect and safety of GMA in comparison with IMT as additional therapies in case the underlying UC remained active even after antiviral therapy.

Materials and Methods

Patients: From October 2003 to December 2008, 64 patients with UC refractory to IMT, including steroids and immunomodulators, were enrolled in this retrospective, observational, multicenter study, which was reviewed and approved by the Institutional Review Board of Kyoto University.

The diagnosis of UC was based on clinical, endoscopic, radiologic, and histological parameters. Fecal bacterial culture yielded no specific pathogens in any of the patients. All patients had been treated with IMT, and had active UC defined as moderate to severe using the disease activity index (DAI) criteria, with a score greater than 6 points. We defined patients with refractory UC as follows: (1) patients who had ongoing active disease despite continuous acute-phase treatment with systemic corticosteroids in daily doses of more than 1 mg PSL equivalent per kilogram body weight or administration of tacrolimus with optimal trough level to induce remission for at least 14 days; (2) patients who had chronic active UC for more than 6months despite taking more than 10mg PSL; and (3) patients who had relapsed, irrespective of azathioprine treatment for at least 3 months.

Assessment of endoscopic severity: Endoscopic severity of UC was based on endoscopic findings and scored from 1 to 4 according to Matts grade [10] as follows: normal=1; mild granularity and edema=2; marked granularity and edema, and spontaneous bleeding=3; and severe ulceration=4.

Histopathology: Colonic biopsies were fixed in formalin, embedded in paraffin, and stained with hematoxylin and eosin, and immunohistochemistry was performed using anti-CMV monoclonal antibodies (Dako Cytomation, Kyoto, Japan) [3,4].

CMV antigenemia: The antigenemia assay was performed using a monoclonal antibody (C7HRP or C10C11) against a CMV structural protein of the 65kDa lower-matrix phosphoprotein (pp65).

Quantitative real-time PCR: DNA for real-time PCR assay was extracted from inflamed mucosa obtained from patients at endoscopic examination using a QIAamp DNA Blood Mini Kit (QIAGEN, Tokyo, Japan) according to the manufacturer's instructions. The assay was performed using an ABI Prism 7700 Sequence Detector System (Perkin Elmer Applied Biosystems, Foster City, CA) as described previously. The oligonucleotide primers used for CMV-DNA amplification were constructed to detect the immediate early gene. The upstream primer was 5́-GACTAGTGTGATGCTGGCCAAG-3́ and the downstream primer was 5́-GCTACAATAGCCTCTTCCTCATCTG-3́. The 6-carboxyfluorescein-labeled probe was 5́- AGCCTGAGGTTATCAGTGTAATGAAGCGCC-3́. The PCR conditions were incubation at 95 °C for 10 minutes, 50 cycles of 95 °C for 15 seconds, followed by incubation at 62 °C for 1 minute. Cases in which the CMV-DNA copy number was over 10 copies/μg DNA were defined as positive for CMV [3, 4].

Diagnosis of CMV infection: Cases in which CMV was detected by at least one of these methods (histological examination, CMV antigenemia, and quantitative real- time PCR) were defined as positive for CMV.

Treatment: All patients defined as positive for CMV were hospitalized at the time of initiation of ganciclovir (DENOSINE®, Mitsubishi Tanabe Pharma, Osaka, Japan) . Ganciclovir (5mg/kg body weight) was intravenously administered twice a day for 2 weeks. If patients did not complete the 2weeks of ganciclovir treatment due to the disease activity, surgical treatment was performed. After starting ganciclovir, tacrolimus

and azathioprine were completely withdrawn and corticosteroids were tapered by reducing the dose 5 to 10 mg/week (more slowly in patients with high disease activity) and completely withdrawn, if possible. After patients completed 2weeks treatment with ganciclovir, disease activity and CMV status were evaluated. If a patient who became negative for CMV after 2weeks antiviral therapy did not achieve clinical remission, the patient received additional GMA or IMT to induce remission.

Assessment: As a first evaluation of the therapeutic effect, we investigated whether or not UC patients went into remission following antiviral therapy alone. As a second evaluation of the therapeutic effect, including colectomy-free survival, the effect of additional GMA in patients with UC who did not achieve remission after antiviral therapy was compared with that of additional IMT.

Statistical Analysis: Categorical and continuous data were compared using a two-tailed Fisher exact test and Mann-Whitney U test. A P value of less than 0.05 was considered statistically significant. Colectomy-free survival was assessed using the Kaplan-Meier method.

Results

Demographic and clinical characteristics: From October 2003 to December 2008, we evaluated 64 patients with UC refractory to IMT including corticosteroids and immunomodulators. CMV-DNA was detected in inflamed mucosa of 31 patients (48.4%) based on mucosal PCR. On the other hand, CMV antigenemia and histological examination were positive in only 7 (10.9%) and 3 (4.7%) of the 64 patients with UC refractory to IMT, respectively. Moreover, none of the patients negative for CMV-DNA in the colonic mucosa was positive for either CMV antigenemia or histological examination. The clinical characteristics of these patients were as follows. Mean age of the 31 patients was 43.5±18.7 years (range; 17-80 years) and the mean DAI score was 9.9±1.7. The extent of disease was proctitis (3.2%), left-sided colitis (35.5%), and pancolitis (61.3%). The mean Matts grade was 3.2±0.8. Of the 31 patients, 27 (87.1%) had been treated with corticosteroids, 8 (25.8%) with azathioprine, 8 (25.8%) with tacrolimus, and 4 (12.9%) with cytapheresis when patients were diagnosed as CMV-positive.

Effect of antiviral therapy on UC patients positive for CMV: Of 31 patients positive for CMV, 4 patients (12.9%) underwent colectomy before finishing 2weeks treatment with ganciclovir therapy due to the disease activity. The remaining 27 patients (87.1%) were treated with ganciclovir. CMV-DNA in colonic mucosa became negative in all patients that received antiviral therapy. Seven patients (25.9%) went into remission following antiviral therapy only. We evaluated the difference in age, DAI score, disease extent and Matts grade of endoscopic severity between UC patients who achieved

remission following antiviral therapy alone and those who did not. As shown in Table 1, there was no significant difference in the clinical parameters between UC patients who went into remission after antiviral therapy alone and those who did not. Moreover, the initial treatment did not affect the result of antiviral therapy, because all UC patients positive for CMV-DNA became negative for CMV-DNA after antiviral therapy.

Effect of GMA or IMT on UC patients after antiviral therapy: In 20 (74.1%) of 27 UC patients, the underlying UC remained active, despite the disappearance of CMV-DNA in the colonic mucosa after treatment with antiviral therapy (Figure 1). Clinical parameters of both GMA and IMT groups are shown in Table 1. Eleven (55%) of the 20 patients were treated with additional GMA (GMA group). Six (54.5%) of them achieved remission, while 5 (45.5%) patients did not. Finally, 2 of these 5 patients required colectomy. Interestingly, in all patients treated with GMA, CMV-DNA was not detected in the colonic tissue after GMA treatment. On the other hand, 9 (45.0%) of the 20 patients in whom the underlying UC remained were treated with additional IMT (IMT group). Eight of the 9 patients (88.9%) were treated with tacrolimus, and remaining 1 patient (11.1%) was treated with combination of tacrolimus and infliximab. Four (44.4%) of the 9 patients achieving remission were negative for CMV-DNA, while 5 (55.6%) patients undergoing colectomy were positive for CMV-DNA. In all 5 patients requiring colectomy, CMV-DNA in the colonic tissue was detected again after IMT.

Association between colectomy-free survival and CMV relapse: We evaluated the association between the relapse of CMV infection and colectomy-free survival in UC patients who were treated with GMA or IMT after 2weeks treatment with ganciclovir. Within a median follow up of 21.5 months, 7 patients (25.9%) underwent colectomy. Based on Kaplan-Meier analysis, overall cumulative colectomy-free survival was estimated to be 53.7% at 65 months. Moreover, we evaluated the difference in colectomy-free survival between patients enrolled in this study in whom CMV infection was detected again after antiviral therapy [CMV relapse (+)] and those in whom CMV infection was not detected [CMV relapse (-)]. As shown in Figure 2, colectomy-free survival in the CMV relapse (+) group was estimated to be 12.9% at 65 months, while that of CMV relapse (-) group was estimated to be 100% at 60 months. The test statistics for the equality of survival distribution were *P*=0.0243 (log-rank test), *P*=0.0164 (Breslow test) and *P*=0.0190 (Tarone-Ware test).

Discussion

In this study, we demonstrated that the therapeutic effect of GMA in patients with UC was similar to that of IMT, and GMA did not induce the reactivation of CMV. These data suggest that GMA could be effective and safe for refractory UC patients positive for CMV and help to avoid recurrence of CMV reactivation and infection.

Previously, it was reported that CMV infection exacerbates UC refractory to IMT and the ratio of colectomy and mortality was high in UC patients concomitant with CMV infection [11, 12, 13]. In this study, 48.4% of UC patients refractory to IMT were diagnosed to be positive for CMV. Interestingly, 25.9% of UC patients positive for CMV achieved remission after treatment with antiviral therapy alone, suggesting that CMV could be a significant pathogen in some patients with UC refractory to IMT. Therefore, it is clinically important to identify UC patients positive for CMV whose disease improves with antiviral therapy in future studies.

Despite the disappearance of CMV-DNA in the colonic mucosa after 2 weeks treatment with antiviral therapy, 20 (74.1%) of 27 UC patients did not achieve remission. In these patients, it is difficult to judge whether CMV was only a bystander reactivation or played a role in exacerbating a UC flare-up by the therapeutic effect of antiviral therapy on clinical activity. Our data support the notion that CMV reactivation or infection would be associated not only with the use of immunosuppressive drugs, but also with UC activity itself. In this regard, we consider anti-inflammatory treatments that will not induce CMV reactivation, for UC patients with a history of CMV infection [14]. Therefore, we next evaluated the safety and efficacy of GMA for UC patients who became negative for CMV after antiviral therapy in comparison with IMT.

Eleven and 9 patients were treated with GMA and IMT, respectively. As a result, 54.5%

(6/11) of the GMA group achieved clinical remission, while 44.4% (4/9) of the IMT group achieved remission. Of note, CMV-DNA in the colonic tissue was not detected in all patients of the GMA group, while 5 patients of the IMT group became positive for CMV after treatment with IMT, who eventually required colectomy. As expected, our clinical data suggested that GMA did not affect CMV reactivation because GMA theoretically removes granulocytes and monocytes/macrophages in which CMV infects latently and reactivates. Immunosuppressants are exogenous factors that influence the reactivation of CMV [15]. In this study, four patients who had been treated with IMT went into remission and 5 did not, although there was no significant difference in the clinical features between patients successfully treated with IMT and those not. Of note, all 5 patients who did not achieve remission became positive for CMV despite the disappearance of CMV reactivation in the colonic mucosa by antiviral therapy. As Domènech et al reported, CMV disease in UC patients occurs in the presence of colonic mucosal inflammation and ongoing immunosuppressant therapy [5]. These data suggested that if IMT unsuccessfully regulates colonic inflammation, IMT might facilitate CMV reactivation in UC patients with a history of CMV reactivation or infection and thereby complicate the disease status of UC. Therefore, treatment of UC patients with a history of CMV reactivation or infection by IMT requires careful follow-up, keeping in mind the possible involvement of CMV infection. Taken together, these findings suggest that GMA would be safer for UC patients with a history of CMV infection due to the avoidance of CMV reactivation compared with IMT.

Finally, overall cumulative colectomy-free survival of patients treated with additional GMA or IMT after 2weeks antiviral therapy was estimated to be 53.7% at 65 months. Colectomy-free survival was significantly different between the CMV relapse (+) group

(12.9%) and the CMV relapse (-) group (100%). CMV pathogenicity in IBD patients remains controversial. The findings of our study support that CMV infection in patients with IBD is associated with a poor prognosis because colectomy-free survival in the CMV relapse (+) group was significantly lower than that in the CMV relapse (-) group. In this regard, UC treatment should be based on the ability to avoid CMV reactivation and infection in patients with refractory UC under IMT, although the pathogenic role of CMV in patients with IBD requires further elucidation.

Is there another therapy for patients with UC positive for CMV? Considering that TNF-α plays an important role in reactivation of CMV in both monocytes and dendritic cells, infliximab is a potential candidate for UC patients with concomitant CMV infection [16, 17]. However, the efficacy of infliximab on UC patients with concomitant CMV infection remains controversial because there are few case reports [17, 18].

In conclusion, GMA is safe and effective for UC patients positive for CMV. GMA alone or in combination with antiviral therapy would be a new therapeutic option for UC patients with concomitant CMV infection or reactivation, although further studies with a larger number of patients are necessary.

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Figure legends:

Figure 1. Clinical course of 31 UC patients positive for CMV-DNA in colonic mucosa. Shaded rectangular columns with double lines show final outcomes. As for clinical outcome of refractory cases to tacrolimus enrolled in this study, all patients refractory to tacrolimus positive for CMV-DNA were treated with ganciclovir for 2weeks and tacrolimus was completely withdrawn. After the antiviral therapy, one patient went into remission. Two were treated with additional GMA, and five were treated with additional IMT. As for clinical outcome of refractory cases to cytapheresis, all patients refractory to cytapheresis positive for CMV-DNA were treated with ganciclovir for 2weeks and cytapheresis was stopped. After antiviral therapy, two of four patients went into remission. One was treated with additional GMA Remaining one was treated with additional IMT (tacrolimus).

Figure 2. Colectomy free survival of CMV relapse (+) group and CMV relapse (-) group. Colectomy free survival of UC patients in whom CMV infection was detected again after antiviral therapy (CMV relapse (+)) is depicted by the dash line, and that of UC patients in whom CMV infection was not detected (CMV relapse (-)) is depicted by the solid line.
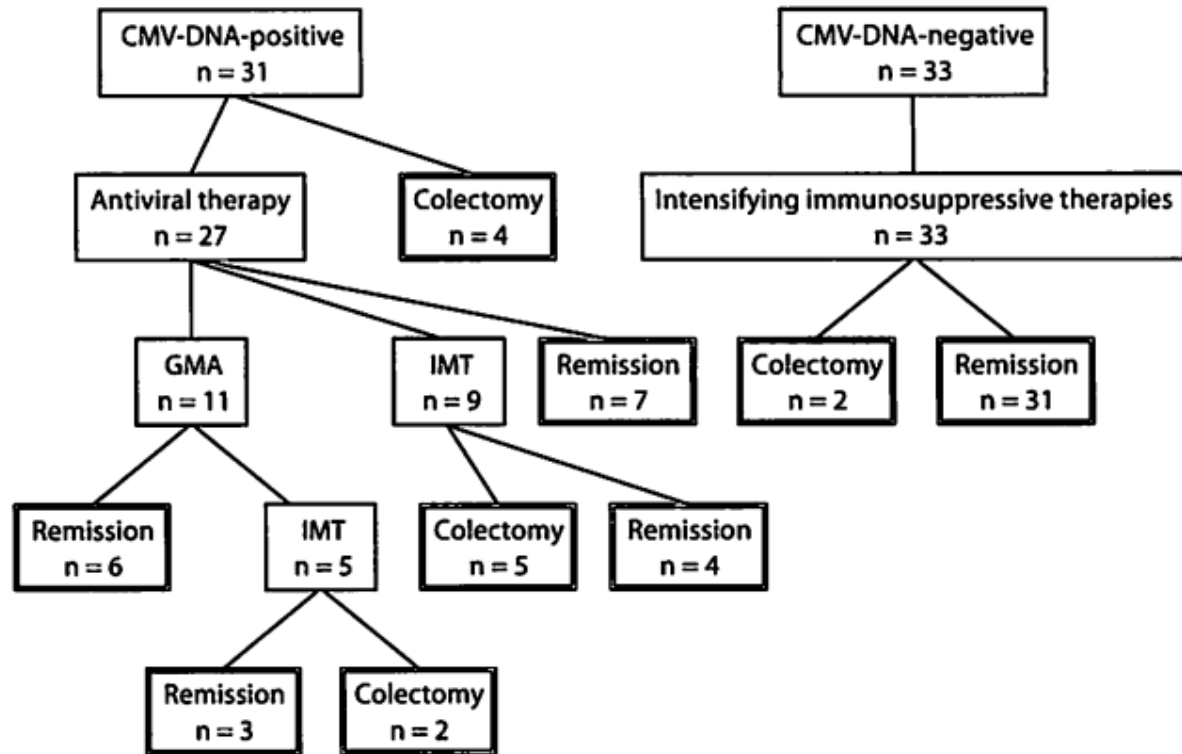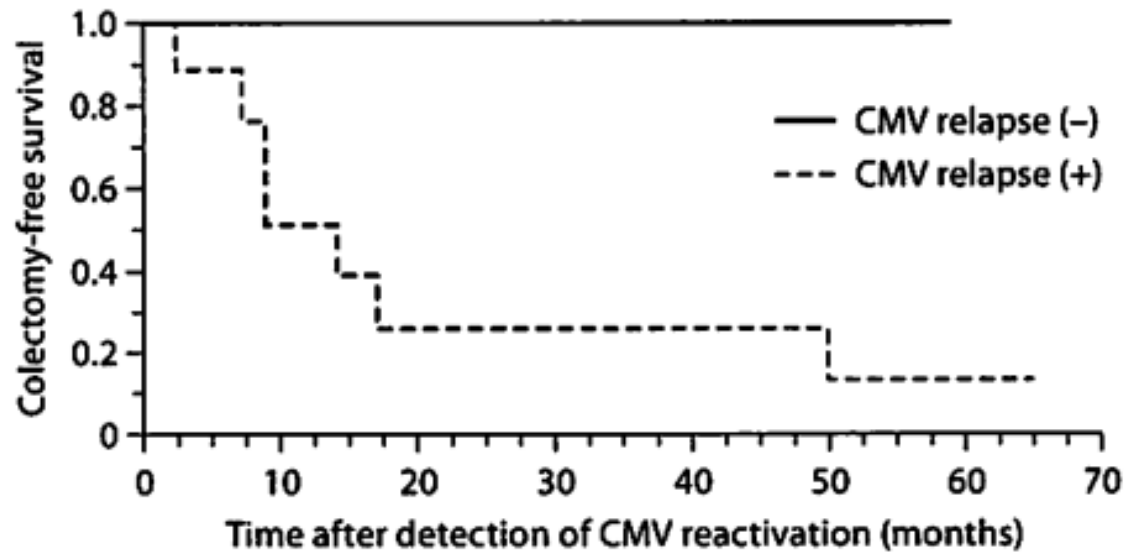
Fig 1

Fig 2

Table 1

	Remission (n = 7)	Not remission (n = 20) (GMA group (n = 11), IMT group (n = 9))	p value
Age	41.3 ± 21.9	48.0 ± 16.2 (46.2 ± 18.1, 46.4 ± 21.9)	0.389 (0.977)
DAI score	9.3 ± 1.9	9.9 ± 1.8 (10.4 ± 1.3, 9.6 ± 2.1)	0.478 (0.307)
Extent of disease			
Left-sided	3	7 (3, 4)	0.933 (0.642)
Pancolitis	4	13 (8, 5)	
Matts grade	3.6 ± 0.5	3.0 ± 0.8 (3.0 ± 0.9, 2.9 ± 0.8)	0.104 (0.774)

Number of patients is shown. Age, DAI score, and Matts grade are presented as mean $\pm$ SD.
The data from the GMA group and IMT group are shown in parentheses.