(−30.0, −60.0, 15.0), t=4.47} demonstrated a significant negative correlation with age (Fig. 3). Even if the analysis was done on voxels with FA values higher than 0.35, to examine more anisotropic WM areas, the results were essentially unchanged (data not shown).

3.1.3. Correlational analysis between FA values and clinical factors in schizophrenics

There was a significant negative correlation between FA values and duration of illness in widespread WM areas (Fig. 4), while there was no significant correlation of FA values with age of onset, duration of hospitalization or daily dose of antipsychotic drugs (data not shown).

3.2. ROI analyses

3.2.1. ROI-based correlational analysis in both schizophrenics and controls

First, we constituted a General Linear Model putting diagnosis as a fixed factor and age, IQ and relative WM volume as covariates. F values (significance probabilities) were as follows; diagnosis: 10.8 (P=0.001), age: 26.1 (P<0.001), IQ: 0.029 (P=0.865) and relative WM volume: 16.6 (P<0.001). Then, we added diagnosis-by-age interaction into the model. F values (significance probabilities) changed as follows; diagnosis: 2.34 (P=0.130), age: 27.8 (P<0.001), IQ: 0.059 (P=0.809), relative WM volume: 14.1(P<0.001) and diagnosis-by-age interaction: 7.08 (P=0.009). Effect of IQ was not significant in both models. There was a significant diagnosis-by-age interaction effect.

3.2.2. ROI-based correlational analysis in controls

Pearson's correlation coefficients (significance probabilities of the test of significance of the correlation: two-tailed) of mean WM FA value with age, IQ and relative WM volume in controls were as follows; FA vs. age: −0.287 (P=0.065), FA vs. IQ: −0.108 (P=0.496) and FA vs. mean WM volume: 0.481 (P=0.001). Only positive correlation between mean WM FA value and relative WM volume was statistically significant.

3.2.3. ROI-based correlational analysis in schizophrenics

Pearson's correlation coefficients (significance probabilities of the test of significance of the correlation: two-tailed) of mean WM FA value with clinical factors in schizophrenics were as follows; FA vs. age: −0.702 (P< 0.001), FA vs. duration of illness: −0.603 (P<0.001), FA vs. age of onset: −0.305 (P=0.049), FA vs. total daily dose of antipsychotics: 0.110 (P=0.489), FA vs. duration of hospitalization: −0.172 (P=0.277), FA vs. IQ: −0.064 (P=0.686), FA vs. relative WM volume: 0.421 (P=0.006). Significant positive correlation was observed between mean WM FA value and relative WM volume. Fig. 5 shows a scatter plot between age and mean WM FA value in controls and schizophrenics. Fig. 6 shows a scatter plot between duration of illness and mean WM FA value in schizophrenics. Significant negative correlations were observed between mean WM FA value and age (or duration of illness).

4. Discussion

In this study, we obtained three main findings; 1) lower FA values in schizophrenic patients compared with controls in WM areas including frontal and temporal WM, bilateral uncinate fasciculi (external capsules) and cingulum bundles and genu and splenium of corpus callosum, 2) age-related reductions of FA value in the widespread WM were more prominent in schizophrenics than in controls, and 3) a negative correlation between FA value in the widespread WM and duration of illness in schizophrenics.

Recent studies demonstrated age-related FA decline in normal individuals occurred in the prefrontal WM, while temporal WM were relatively preserved (Pfefferbaum et al., 2005; Salat et al., 2005). However, in this study, negative age-dependent effects were observed only in the lenient statistical threshold in the FA values of restricted areas of the WM in controls. This could be explained by the fact that all our subjects were under the age of 60, relatively less old compared to the participants of normal aging studies.

We replicated the results of the most of the previous studies, decreased FA values in the WM of schizophrenics. In the earlier studies concerning FA values in WM of patients with schizophrenia, an inherent abnormality in WM was expected to be detected since the decrease of FA values in the WM of the schizophrenic brain was assumed to occur as neurodevelopmental impairments before onset of the illness. Several studies demonstrated that schizophrenics had reduced FA value in the prefrontal WM (Buchsbaum et al., 1998), prefrontal and parieto-occipital WM (Lim et al., 1999), splenium of the corpus callosum (Agartz et al., 2001) and adjacent occipital WM (forceps major) (Agartz et al., 2001), left uncinate fasciculus and bilateral arcuate fasciculus (Burns et al., 2003), bilateral cingulum bundles (Kubicki et al., 2003). Some of them indicated that the reduction of FA values in schizophrenics might occur independently of reduction of the white matter volume. Although some studies reported no significant FA changes in schizophrenics (Steel et al., 2001; Foong et al., 2002), most studies with chronic

schizophrenia demonstrated lower FA values in schizophrenia (Kanaan et al., 2005). A few DTI studies have examined first episode patients (Price et al., 2005; Szeszko et al., 2005). Szeszko et al. found FA decrease in the left internal capsule and left-hemisphere WM of the middle frontal gyrus and posterior superior temporal gyrus of first-episode schizophrenics and schizoaffective disorder patients, however, the decrease was less pronounced compared with results of the majority of the studies in chronic schizophrenics. On the other hand, Price et al. reported that there was no FA decrease in the corpus callosum of patients with first-episode schizophrenia. They suggested that FA reduction in schizophrenia might reflect neuropathological abnormalities, which may occur after the onset of the disease and could be progressive. Our results, 1) age related FA reduction was more prominent in schizophrenics than controls, and 2) duration of illness was related to FA reduction in schizophrenics, suggest that changes of FA value in schizophrenia are attributable, at least in part, to progressive neuropathological changes after onset of the illness.

Contrary to our results, a previous DTI study demonstrated 'positive' correlation between age and FA in schizophrenics (Jones et al., 2006). They measured FA values of WM tracts captured from tractography, and they set seedpoints of the tracts manually from one slice of FA images. Such methods might overlook general decline of FA values in the WM. Their mean FA values (average of 8 WM tracts in each subjects) were around 0.4, which was relatively higher than those of our study {our mean FA value of entire WM was 0.35+0.01 (mean+S.D.)}. To simulate the analysis of the previous study, we additionally performed an analysis setting masking threshold for FA values of 0.35. As a result, the significant negative correlation remained to be present even in more anisotropic WM areas.

Previous pathological studies demonstrated microscopic abnormalities of the WM in schizophrenia such as decreased expression of myelin and oligodendrocyte-related genes, the decrease in density of oligodendrocytes (Hof et al., 2002), damage of myelin sheath lamellae (Uranova et al., 2001) and maldistribution of interstitial neurons (Akbarian et al., 1996) in prefrontal WM of the brains of schizophrenic patients. Further, a previous longitudinal MR study demonstrated progressive atrophy of the white matter in schizophrenics (Ho et al., 2003). Given these previous findings and ours, it seems likely that age-dependent FA decrease, but not increase, occurs in schizophrenic brains.

As well as a negative correlation with age, FA values of schizophrenics showed negative correlation with duration of illness but not with age of onset or daily dose of antipsychotics. The facts seem to support the hypothesis that FA reduction in schizophrenia might be associated with neuropathological abnormalities which may emerge, at least in part, after the onset of the disease and could be progressive. Further, the spatial distribution of age-related FA reduction in schizophrenics was different from those of normal individuals in previous studies that demonstrated preserved temporal white matter (Pfefferbaum et al., 2005; Salat et al., 2005). Such different distributions suggest that FA changes in schizophrenics might be associated with disease progression rather than merely exaggerated aging effects. However, it is difficult for neuroimaging studies, even for longitudinal studies, to discriminate disease progression from aging effects. The correlational study between DTI findings and pathological findings should be conducted to clarify whether reduction of FA values in schizophrenics reflect pure disease progression or merely exaggerated aging effects.

Several limitations should be considered in our study. First, our study is a cross-sectional study. To confirm progressive pathological process in the WM of the patients of schizophrenia, longitudinal studies should be conducted. Second, IQ score was not matched between groups, i.e., mean IQ score was significantly lower in schizophrenics in our samples. O'Sullivan et al. (2004) reported DTI measures were correlated strongly with cognitive decline in elderly. Thus, it could be problematic whether age-related FA decrease in our study was reflected by cognitive decline. However, no significant correlation was observed between mean WM FA values and IQ in our sample. Also, regarding schizophrenia, it has been hypothesized that most cognitive change takes place early in their psychotic episodes and it remains relatively stable through long term in the illness (Hoff et al., 2005). Hence, at least from our data, we cannot attribute age-related FA decline in schizophrenia to IQ changes. Third, the issue of partial volume effect should be addressed. In schizophrenia, progressive WM atrophy has been reported in the previous studies (Ho et al., 2003). Due to the atrophy, it is possible that the voxels located in the border of the WM and other tissues in schizophrenics were estimated as having lower FA values. However, we minimized the problem by using the high dimensional warping algorithm, threshold masking for FA values and adopting relative WM volume as a nuisance variable. Another issue is the possible effect of long-term medication with antipsychotics. Although daily dose of antipsychotics was not correlated with FA values in schizophrenics, we could not estimate accurate cumulative doses of antipsychotics

throughout the duration of illness. Several morphological MR studies and animal studies suggested that the administration of antipsychotics could affect brain morphology (Wang et al., 2004; Lieberman et al., 2005). It is possible that long-term medication with antipsychotics also affects microstructure of the WM in schizophrenics. The longitudinal animal studies may clarify this issue.

In conclusion, we confirmed decreased FA in schizophrenics, compared to controls in the widespread WM areas in a Japanese sample. We found that age-dependent FA decline was more pronounced in chronic schizophrenics compared to controls, and that such FA decline was significantly correlated with duration of illness in patients. These observations suggest that decreased FA values in schizophrenia might be attributable, at least in part, to progressive changes in the WM after the onset of the illness.

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Regular Article

Effects of switching from conventional antipsychotics to risperidone in Japanese patients with chronic schizophrenia

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Abstract Atypical antipsychotics are rapidly evolving to become the standard pharmacotherapy in schizophrenia; however, the trend of switching to such drugs is not necessarily progressing quickly in East Asia. This might be due to the scarcity of evidence for the efficacy of switching from conventional to atypical antipsychotics, which prompted the authors to examine effects of switching from conventional antipsychotics to an atypical drug, risperidone, in Japanese patients. Fifty patients with chronic schizophrenia completed the study in which combination therapy with other antipsychotics was allowed if monotherapy with risperidone was not tolerated. Symptoms were assessed with the brief psychiatric rating scale (BPRS). Switching to monotherapy was achieved in 34 patients (68%). The number of antipsychotics prescribed to each patient was reduced (from 2.1 to 1.4 drugs; $P < 0.001$) and the use of antiparkinsonian drugs decreased ($P < 0.001$). The mean BPRS score was also reduced 6 months after initiation of the switch ($P < 0.001$). Failure in switching to monotherapy was associated with higher dosage of antipsychotics at baseline. Switching from conventional antipsychotics to risperidone reduced schizophrenia symptoms, antiparkinsonian medication, and polypharmacy. However, a portion of patients, particularly those who receive an excessive dosage of antipsychotics, might not tolerate such switching.

Key words atypical antipsychotics, polypharmacy, risperidone, schizophrenia, switching.

INTRODUCTION

Recent meta-analytic studies have demonstrated the superiority of second-generation antipsychotics to conventional drugs in several clinical aspects in the management of schizophrenia. Atypical antipsychotics have been found to be superior to conventional drugs in the treatment of global schizophrenic symptoms,[1–4] particularly for negative symptoms,[4] and cognitive functions.[5] Furthermore, atypical drugs were associated with higher rates of acceptance, lower rates of movement disorders and less frequent use of antiparkinsonian drugs.[3,5] However, contradictive results have also been reported. Geddes *et al.* reported that atypical antipsychotics have a similar effect on symptoms to that of conventional antipsychotics at an average dose of 12 mg haloperidol.[6] They also concluded that although atypical antipsychotics cause fewer extrapyramidal side-effects (EPS), overall tolerability is similar to that of conventional drugs. Wahlbeck *et al.* failed to find evidence of superiority in acceptability of novel atypical drugs.[7] Leucht *et al.* reported that optimum doses of low-potency conventional antipsychotics

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might not cause more EPS than new-generation drugs.[8] A recent multicenter study found that a typical antipsychotic, perphenazine, was similar or superior to some atypical antipsychotics in terms of the rate of discontinuation.[9]

If atypical drugs are superior to their conventional counterparts, then switching from the latter to the former would result in better clinical outcomes. With respect to risperidone, in particular, switching seems to improve overall symptom severity, psychotic symptoms, disorganization, negative symptoms, depressive symptoms,[10–16] memory functions,[17] subjective sleep quality,[18] and quality of life (QOL),[16] and to reduce EPS or antiparkinsonian medication.[10–12,15,16] However, there are some drawbacks: switching from conventional antipsychotics to risperidone had no effect on QOL,[14] level of employment, standard of living,[13] or antiparkinsonian medication use.[19]

Nonetheless, the majority of studies have provided evidence supporting the superiority of new atypical drugs to conventional antipsychotics; furthermore, atypical antipsychotics, such as risperidone, are rapidly evolving to become the standard in the pharmacotherapy of schizophrenia. At the same time, however, the trend of switching to such drugs is not necessarily progressing quickly. In East Asia, in particular, second-generation drugs comprised less than 30% of all prescribed antipsychotics, and 46% of antipsychotic prescriptions were in the context of polypharmacy until recently.[20] Among East Asian countries, Japan was found to have a relatively low rate of prescribing second-generation drugs (21.5%) and a very high rate of polypharmacy (79%). One reason for the slow progress of switching might be the scarcity of evidence. Indeed, there have been only a few reports from Japan assessing effects of switching from conventional to atypical drugs.[17,21]

The purpose of the present study was to examine changes in symptoms and antiparkinsonian medication after switching from conventional drugs to risperidone in Japanese patients with schizophrenia. In addition, we tried to elucidate clinical factors associated with successful switching to monotherapy. We chose risperidone because it was the only second-generation antipsychotic available whose efficacy and safety had been well demonstrated in Japan before we initiated the present study in early 2001.

METHODS

Subjects

The subjects consisted of 54 patients (25 male, 29 female; age range 25–72 years; mean age 45 ± 13 years; 28 inpatients and 26 outpatients) with chronic schizophrenia who were treated with conventional antipsychotics at two psychiatric hospitals in Japan during the period June 2001–February 2003. Diagnosis of schizophrenia was made according to the *Diagnostic and Statistical Manual of Mental Disorders*, 4th edition (DSM-IV)[22] by a single psychiatrist (S.N.) who was in charge of all the subjects, based on information from detailed interviews and medical records.

Nine subjects were diagnosed with schizophrenia of the paranoid type, and the remaining 45 with schizophrenia of the residual type. Duration of illness ranged between 4 and 42 years with a mean of 21 ± 10 years. All subjects had been clinically stable and compliant for at least 3 months prior to participation in the study. Switching to risperidone was proposed to patients with an expectation of possible reduction of symptoms and side-effects. Patients who had previously been treated with atypical antipsychotics were not enrolled in the study. After a thorough explanation of the study, informed consent was obtained from all subjects.

Switching procedure and assessments

Psychiatric symptoms were assessed with the Brief Psychiatric Rating Scale (BPRS)[23] before switching (baseline) and at 6 months after initiation of switching. BPRS scores were all rated by a psychiatrist (S.N.) who completed a training course on BPRS scoring. Switching was carried out by any of the following procedures: introduction of risperidone at the same time as cessation of the previous medication; gradual reduction of previous medication, following introduction of risperidone; gradual introduction of risperidone in correspondence with the reduction of previous medication. The dose of risperidone was adjusted between 1.0 and 12.0 mg/day depending on clinical responses. Combination therapy with other antipsychotics was allowed if monotherapy with risperidone was not tolerated. Change in medication of benzodiazepines (minor tranquillizers and hypnotics) or mood stabilizers was avoided during the study period. Switching was completed within 8 weeks for each patient.

Information on medications with antipsychotics and antiparkinsonian drugs was obtained at baseline and at 6 months after the initiation of switching. For antipsychotics, dose equivalent to chlorpromazine was determined according to Inagaki *et al.*[24] For antiparkinsonian drugs, dose equivalent to biperiden was similarly determined.

Statistical analysis

Comparisons in proportions were made using the χ^2 test for independence or the McNemar test. Symptom

ratings and number of drugs were compared before and after switching using the Wilcoxon signed-ranks test. Comparisons in mean dose of medication were done with the *t*-test or paired *t*-test when appropriate. Clinical variables associated with successful switching to monotherapy were investigated using logistic regression analysis. All statistical analysis was performed with SPSS version 11 (SPSS Japan, Tokyo, Japan).

RESULTS

Summary of outcomes of all participants are illustrated in Fig. 1. Of the total 54 participants, two patients stopped treatment at the study hospital for unknown reasons. Two additional patients discontinued participation during the switching period because they preferred their original neuroleptics to risperidone. Thus, the overall dropout rate (4/54) was very low (7%). Among the remaining 50 patients who completed the study, switching to antipsychotic monotherapy with risperidone was successfully accomplished in 34 patients (68%; group I). Of the remaining 16 patients (32%; group II), a partial switch to risperidone was carried out, that is, they were maintained on combination therapy (risperidone with other antipsychotics) at follow up (6 months after initiation of switching) because they could not tolerate monotherapy.

Change in medication after switching

Medications (antipsychotics and antiparkinsonian drugs) before and after switching for subjects who completed the study are summarized in Table 1. Conventional antipsychotics that had been prescribed before switching were haloperidol (33 patients), thioridazine ($n = 27$), bromperidol ($n = 9$), mosapramine ($n = 6$), levomepromazine ($n = 6$), zotepine ($n = 6$) and other drugs. Drugs coprescribed with risperidone in group II after switching included olanzapine ($n = 7$), thioridazine ($n = 5$), zotepine ($n = 5$) and others. The mean dose of antipsychotics equivalent to chlorpromazine did not change significantly after switching ($P = 0.31$), but the number of antipsychotic drugs prescribed to each patient was significantly reduced at follow up (from 2.1 to 1.4 drugs on average; $P < 0.001$). The mean (±SD) doses of risperidone at follow up were 5.3 ± 2.8, 4.8 ± 2.5, and 6.4 ± 3.1 mg/day in the total group, group I, and group II, respectively. In group I, 26 patients were managed without antiparkinsonian drugs, while the remaining eight patients needed antiparkinsonian drugs (trihexyphenidyl or biperiden). The average dose of risperidone prescribed in the patients who needed antiparkinsonian drugs was 7.3 ± 3.0 mg/day, while that for those who did not was 4.0 ± 1.9 mg/day ($P = 0.001$, $t = 3.7$, d.f. = 32). In group II, only one patient was medicated with antiparkinsonian drugs at follow up. In the total group, the proportion of patients who were medicated with antiparkinsonian drugs significantly decreased after switching ($P < 0.001$). The mean antiparkinsonian drugs equivalent to biperiden also reduced after switching ($P < 0.001$).

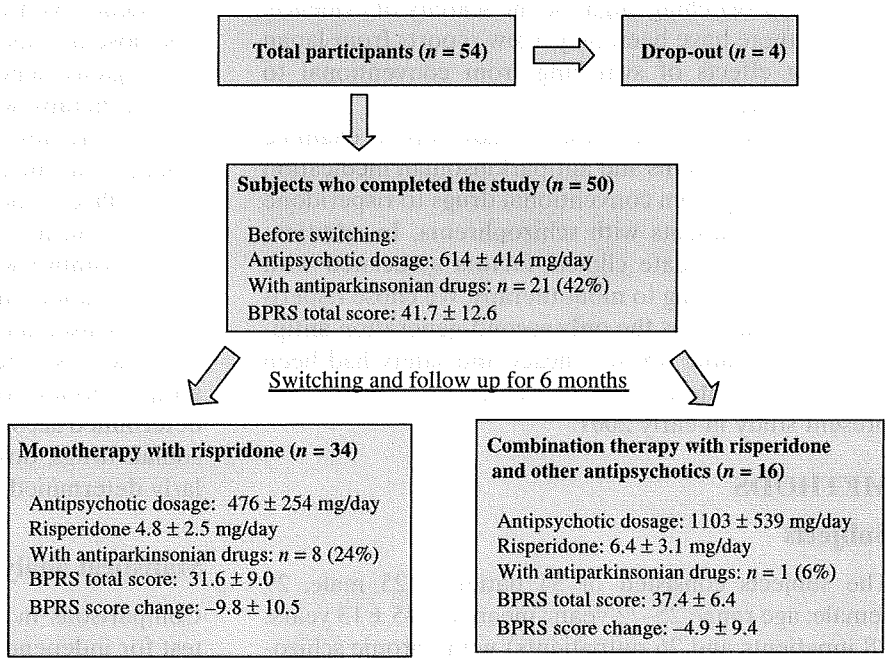

Figure 1. Outcomes flowchart. Antipsychotic dosage is shown as chlorpromazine equivalent dosage. BPRS, Brief Psychiatric Rating Scale.

Table 1. Medications before and after switching for subjects who completed the study ($n = 50$)

	Before switching n (%)	After switching n (%)	P
Antipsychotics			
Monotherapy	7 (14)	34 (68)	<0.001[§]
Combination of 2 drugs	30 (60)	11 (22)	
Combination of 3 drugs	13 (26)	5 (10)	
Total dose[†] (mean ± SD)	614 ± 414	677 ± 469	0.31[¶]
Antiparkinsonian drugs			
With antiparkinsonian drugs	21 (42)	9 (18)	<0.001[††]
Total dose[‡] (mean ± SD)	1.7 ± 2.0	0.5 ± 1.1	<0.001[‡‡]

[†] Equivalent to chlorpromazine (mg/day).
[‡] Equivalent to biperiden (mg/day).
[§] Wilcoxon signed ranks test ($Z = -5.9$).
[¶] Paired t-test ($t = -1.0$, d.f. = 49).
[††] McNemar test.
[‡‡] Paired t-test ($t = 4.3$, d.f. = 49).

Table 2. Changes in schizophrenia symptoms after switching

	BPRS score (mean ± SD)								
	Total ($n = 50$)			Group I ($n = 34$)			Group II ($n = 16$)		
	Baseline	After switching	P	Baseline	After switching	P	Baseline	After switching	P
Total	41.7 ± 12.6	33.5 ± 8.6	<0.001	41.4 ± 14.6	31.6 ± 9.0	<0.001	42.3 ± 7.2	37.4 ± 6.4	0.022
Anxiety-depression	8.4 ± 3.5	7.0 ± 2.6	0.002	8.7 ± 3.8	6.9 ± 2.8	0.003	7.6 ± 2.9	7.2 ± 2.3	0.36
Anergia	13.5 ± 4.4	10.5 ± 3.7	<0.001	13.2 ± 4.6	9.9 ± 4.0	0.001	14.4 ± 4.1	11.7 ± 2.9	0.007
Thought disturbance	8.0 ± 3.9	7.4 ± 2.8	0.059	8.0 ± 4.5	6.9 ± 2.5	0.016	8.1 ± 2.2	8.5 ± 3.2	0.89
Activation	5.6 ± 2.5	4.5 ± 2.1	<0.001	5.5 ± 2.5	4.1 ± 1.9	0.001	6.0 ± 2.5	5.4 ± 2.3	0.24
Hostile-suspiciousness	6.1 ± 4.0	4.0 ± 1.8	<0.001	6.1 ± 4.3	3.7 ± 1.1	0.001	6.2 ± 3.4	4.6 ± 2.5	0.074

Anxiety-depression: total score of somatic concern (item 1), anxiety (2), guilt (5), and depression (9); Anergia: emotional withdrawal (3), motor retardation (13), blunted affect (16), and disorientation (18); Thought disturbance: conceptual disorganization (4), grandiosity (8), hallucinations (12), and unusual thought content (15); Activation: tension (6), mannerisms and posturing (7), and excitement (17); Hostile-suspiciousness: hostility (10), suspiciousness (11), and uncooperativeness (14).
Statistical significance was tested using the Wilcoxon signed-ranks test.

Change in symptom ratings

Table 2 shows changes in BPRS total score and its subscale scores after switching to risperidone. For the total group ($n = 50$), the mean BPRS total score was significantly reduced after switching to risperidone. All the subscales except for thought disturbance showed a significant reduction in score after switching. There was only one subject who had apparent exacerbation at follow-up. Two other subjects had a slight increase in BPRS total score. The remaining 47 subjects had a reduction in total BPRS score or continued to be the same after switching.

When groups I and II were examined separately, both had a significant reduction in BPRS total score after switching. However, there were differences in symptom reduction between the two groups. In group I, all subscales of BPRS showed a significant reduction in symptom rating after switching. For group II, in contrast, only the anergia subscale showed a significant symptom reduction. There were no significant differences at baseline in BPRS total score or any of the subscale scores between the two groups (P not shown), whereas total score ($P = 0.003$), thought disturbance ($P = 0.037$), and activity ($P = 0.024$) scores became significantly lower after switching in group I compared to group II.

Factors associated with successful switching to monotherapy

We then explored clinical factors associated with successful switching to monotherapy with risperidone. We performed a logistic regression analysis, in which either monotherapy or combination therapy was a dependent variable, while age, sex, outpatient or inpatient status, dosage of antipsychotics equivalent to chlorpromazine at baseline, total BPRS score at baseline, and reduction in total BPRS after switching were independent variables. The stepwise variable selection (backward-elimination) procedure of the logistic regression analysis showed that dosage of antipsychotic medication was the only variable that was significantly associated with successful switching to monotherapy ($B = 0.002$, $P = 0.01$). When standard antipsychotic dosage was defined as <1000 mg/day chlorpromazine equivalent, 79% (30/38) and 33% (4/12) of patients who received standard and non-standard dosage (i.e. ≥1000 mg/day), respectively, were successfully switched to monotherapy at follow up ($\chi^2 = 8.7$, d.f. = 1, $P = 0.003$).

DISCUSSION

We found that the majority (approximately two-thirds) of patients successfully completed switching to monotherapy with risperidone. As a result, the proportion of patients on multiple antipsychotics (i.e. polypharmacy) decreased substantially. However, the remaining one-third of patients was unable to be switched to monotherapy. Failure in such switching was found to be related to antipsychotic dosage before switching. We obtained evidence that overall switching procedure resulted in substantial reduction in symptom severity and antiparkinsonian medication, suggesting that switching conventional drugs to risperidone is an effective strategy in the management of schizophrenia.

Global schizophrenia symptoms were substantially reduced (from 42 to 34 in BPRS total score) in our procedure of switching to risperidone. When subscales of BPRS were examined, reduction in score was significant for all subscales except thought disturbance (Table 2). Symptom reduction in thought disturbance only just failed to reach statistical significance, suggesting that switching from conventional drugs to risperidone may be more effective in reducing negative symptoms rather than positive symptoms in the present sample. These findings are generally consistent with previous studies of switching from conventional drugs to risperidone.[10–16] Because the dosage of antipsychotics did not change significantly after switching (Table 1), this symptom reduction cannot be ascribed to change in dosage.

In our procedure, the number of patients on antiparkinsonian drugs and thus the mean dosage of antiparkinsonian drugs significantly reduced, which is consistent with the majority of previous studies,[10–12,15,16] but not with a recent report.[19] In the present study, the average dose of risperidone for patients who needed antiparkinsonian drugs was 7.3 mg, while that for those who did not was 4.0 mg, indicating that use of antiparkinsonian drugs or the development of EPS depends on risperidone dosage.

In the present study approximately two-thirds of patients successfully completed switching to monotherapy with risperidone; however, the remaining patients did not. It has been noted that many clinicians begin the process of switching antipsychotics with the intention of discontinuing the original drug, but ultimately continue with multiple drugs.[25] One reason why a portion of patients were unsuccessful in switching to monotherapy with risperidone might be that they were resistant, at least in part, to risperidone. This possibility is supported by the result that reduction in schizophrenia symptoms was substantially greater in group I than in group II. Furthermore, a principal problem in changing antipsychotics is the potential for withdrawal symptoms resulting from the discontinuation of the existing therapy.[26] Possible detrimental events that may occur during transition periods include relapse of psychotic symptoms due to inadequate antipsychotic treatment,[27,28] neuroleptic withdrawal syndromes including rebound dyskinesia, insomnia, nausea, vomiting, anxiety, and agitation,[26] and adverse drug–drug interactions characterized by excessive sedation, confusion, motor uncoordination, and cognitive worsening due to adding a newly initiated atypical drug.[29] It is likely that the larger the dosage, the greater the discontinuation symptoms. In our study, indeed, failure in switching to monotherapy was associated with larger antipsychotic dosage at baseline. In particular, excessive dose at baseline (≥1000 mg chlorpromazine equivalent) was strongly associated with failure in switching to monotherapy. Although there may be a portion of patients that really need polypharmacy,[30] more gradual switching and intense education about antipsychotics might have further increased the number of patients who were successfully switched to monotherapy.

In conclusion, the present study obtained rigorous evidence that switching from conventional antipsychotics to risperidone reduces symptom severity, antiparkinsonian medication, and polypharmacy in Japanese patients with chronic schizophrenia. This indicates that such switching is an effective strategy in the management of chronic schizophrenia, although there might be a portion of patients, particularly those who receive an excessive dosage of antipsychotics, who

are unable to tolerate switching to monotherapy with risperidone.

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ABSTRACT: Duchenne muscular dystrophy (DMD) is a devastating muscle disorder that is characterized by progressive muscle necrosis, fibrosis, and fatty infiltration. To examine the temporospatial pathological changes, a noninvasive evaluation method such as magnetic resonance imaging (MRI) is needed. The aim of this study was to precisely assess muscle necrosis and inflammation based on a sequence of T2-weighted imaging (T2WI), gadolinium-enhanced imaging, and selective fat suppression, chemical shift selective T2-weighted imaging (CHESS-T2WI), on a 3.0-Tesla MRI unit in 3-month-old and 7-year-old dogs with canine X-linked muscular dystrophy ($CXMD_J$), a suitable animal model for DMD. The results show that CHESS-T2WI was more sensitive and useful from the early to late stages of $CXMD_J$ than T2WI or contrast enhancement imaging in the evaluation of muscle necrosis, because these latter sequences can be influenced by fatty infiltration or interstitial connective tissues.
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EVALUATION OF DYSTROPHIC DOG PATHOLOGY BY FAT-SUPPRESSED T2-WEIGHTED IMAGING

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Duchenne muscular dystrophy (DMD) is a severe X-linked muscle disease characterized by progressive skeletal muscle atrophy and weakness.[1] DMD is caused by mutations in the *dystrophin* gene, which encodes the cytoskeletal protein dystrophin.[2] A loss of dystrophin accompanied by a deficiency of dystrophin–glycoprotein complex (DGC) from the sarcolemma leads to progressive degeneration of striated muscle.[3,4] In dystrophic skeletal muscles, muscle fiber necrosis with inflammation is followed by muscle regeneration, but the muscle is finally replaced by fibrous or fatty tissue.[5,6] For this devastating disorder, various therapeutic approaches, such as gene therapy, stem cell–based cell therapy, or pharmaceutical agents have been proposed and explored using various DMD animal models.

The X-linked muscular dystrophy (*mdx*) mouse and Golden Retriever muscular dystrophy (GRMD) dog are the most commonly used DMD animal models.[7,8] *mdx* mice show extensive necrosis followed by regeneration, but their phenotypes are milder than those of DMD due to the absence of apparent fibrosis and fatty infiltration.[7,9,10] The phenotypes of striated muscle in the GRMD dog are clinically and pathologically more similar to that of DMD,[8,11,12] but it is very difficult to maintain this animal model due to the severe phenotype. We have therefore established a Beagle-based colony of canine X-linked muscular dystrophy in Japan ($CXMD_J$).[13] We have found that the clinical and pathological findings in $CXMD_J$ are similar to but milder than those in GRMD.[14,15]

A method of noninvasive temporospatial assessment is required to investigate muscle involvement and, especially, to evaluate therapeutic

Abbreviations: ANOVA, analysis of variance; CE, contrast enhancement ratio; CHESS, chemical shift selective; CT, computed tomography; $CXMD_J$, canine X-linked muscular dystrophy in Japan; DGC, dystrophin–glycoprotein complex; DMD, Duchenne muscular dystrophy; EDL, extensor digitorum longus; FDS, flexor digitorum superficialis; FITC, fluorescein isothiocyanate; GC, gastrocnemius; Gd-DTPA, gadolinium diethylenetriamine pentaacetic acid; GMRD, Golden Retriever muscular dystrophy; MRI, magnetic resonance imaging; PCr, phosphocreatine; Pi, inorganic phosphate; ROI, region of interest; SNR, signal-to-noise ratio; STIR, short-tau inversion recovery; SI, signal intensity; TC, tibialis cranialis

Key words: chemical shift selective fat-suppressed T2-weighted imaging; Duchenne muscular dystrophy; dystrophic dog; magnetic resonance imaging; myopathy

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interventions. Computed tomography (CT), which shows high temporal and spatial resolution, has been used to detect selective muscle involvement, such as atrophy or fatty tissue replacement, in patients suffering from DMD,[16,17] but it requires ionizing radiation and has limited sensitivity for soft tissues.[18] Magnetic resonance imaging (MRI) produces high-resolution images with good contrast among soft tissues,[19] and therefore it has been used to evaluate skeletal muscle involvement in DMD[20] and in *mdx* mice.[21] In the early stages of dystrophy, the T1 relaxation time is prolonged due to muscle degeneration and regeneration together with an increase in muscle water concentration, and it is decreased owing to fat infiltration in the advanced stage.[22] As the main magnetic field increases, however, the capacity to differentiate tissues on the basis of T1 relaxation time may decrease.[23] On the other hand, the T2 relaxation time is prolonged in necrotic as well as fatty and connective tissue[19]; therefore, it can hardly distinguish necrosis from fat replacement or fibrosis during the dystrophic process. To selectively detect necrotic changes, MR contrast agents, such as gadolinium diethylenetriamine pentaacetic acid (Gd-DTPA), have been used extensively,[24–26] but these agents may also enhance blood vessels and the interstitium,[27] and may cause severe adverse effects, such as anaphylaxis,[28,29] which are critical for DMD patients. Thus, a safer imaging protocol is needed to distinguish necrotic lesions from fatty degeneration or fibrosis in the dystrophic skeletal muscle of DMD and $CXMD_J$.

To discriminate necrosis from fatty infiltration, one of the fat suppression sequences may be useful. As a fat suppression sequence, short-tau inversion recovery (STIR) MR imaging was used to detect muscle edema in DMD.[6] However, STIR suppresses the signal from any tissue or fluid that has a short T1 relaxation time, and therefore it does not selectively suppress the fat signal.[30,31] In contrast, chemical shift selective (CHESS) imaging, another fat suppression sequence, is a technique that selectively saturates fat magnetization by applying a 90° pulse matching with the fat resonance frequency and therefore leads to a highly selective suppression of fat signals. Moreover, the signal-to-noise ratio (SNR) of CHESS is better than that of STIR at a higher magnetic field. The sequence of CHESS combined with T2-weighted imaging (CHESS-T2WI) has been used to diagnose disorders such as lipomatous tumor or temporomandibular arthrosis.[32–34] The method, however, has not been applied to evaluation of the dystrophic changes seen in DMD or the animal models to date.

We, therefore, examined dystrophic dog muscle by CHESS-T2WI to determine whether this sequence is more useful for finding necrosis and inflammatory change than the conventional sequences of T2WI or contrast imaging.

METHODS

Animals. We used three 3-month-old normal male dogs (II-2308MN, II/III-3911MN, and II-4202MN), three littermate $CXMD_J$ male dogs (II-2302MA, II/III-3903MA, and II-4204MA), one 7-year-old normal male dog (00-174MN), and two 7-year-old $CXMD_J$ male dogs (II-C04MA and II-C12MA). II-2308MN, II-4202MN, II-2302MA, and II-4204MA were produced by mating a second-generation (G2) carrier female[13] and G2 affected male. II/III-3911MN and II/III-3903MA were the offspring of a G2 carrier female and a third-generation (G3) affected male. We obtained II-C04MA and II-C12MA by mating first-generation (G1) carrier female dogs and pure-bred normal male Beagles. 00-174MN was a pure-bred normal Beagle. All dogs were part of the breeding colony at the General Animal Research Facility, National Institute of Neuroscience, National Center of Neurology and Psychiatry (Tokyo, Japan), or the Chugai Research Institute for Medical Science, Inc. (Nagano, Japan). Ages, body weights, and serum creatine kinase values at the time of MRI of each dog are shown in Table 1. This study was carried out according to the guidelines provided by the Ethics Committee for the Treatment of Middle-sized Laboratory Animals of the National Center of Neurology and Psychiatry (Approval Nos. 18-02, 19-02, and 20-02).

MR Scanning and Image Analysis. General anesthesia was induced by an intravenous injection of thiopental sodium (20 mg/kg) before MRI scanning and was maintained by inhalation of isoflurane (2.0–3.0%). We examined lower leg muscles of these dogs by superconducting 3.0-Tesla MRI (Magnetom Trio; Siemens Medical Solutions, Erlangen, Germany) with a human extremity coil 18 cm in diameter. The MRI pulse sequences used were T1-weighted imaging (T1WI), T2WI, chemical shift selective T1-weighted imaging (CHESS-T1WI), CHESS-T2WI, gadolinium-enhanced T1-weighted imaging (Gd-T1WI), chemical shift selective gadolinium-enhanced T1-weighted imaging (CHESS-

Table 1. Clinical profiles of normal and dystrophic male dogs used in this study.

	Age (mo)	BW (kg)	Serum CK (IU/L)
Normal dogs			
II-2308MN	3	6.8	197
II/III-3911MN	3	7.7	318
II-4202MN	3	5.8	274
00-174MN	87	13.7	83
CXMDJ dogs			
II-2302MA	3	7.2	30,200
II/III-3903MA	3	6.6	22,300
II-4204MA	3	6.0	28,800
II-C04MA	85	11.5	6500
II-C12MA	94	11.6	1602

Body weight (BW) and serum creatine kinase (CK) values were measured on the day of MRI examination.

Gd-T1WI), and multi-echo T2WI for calculation of T2 relaxation time. In contrast-enhanced images, we injected 0.2 ml/kg of the gadolinium-based MR contrast agent Gd-DTPA (Magnevist; Bayer Schering Pharma, Berlin, Germany) for each sequence. In 3-month-old dogs, we scanned the images for 26 minutes, about 5 minutes after the intravenous injection. On the other hand, we took the images for 13 minutes in 7-year-old dogs at 25 minutes after the injection in order to minimize the risk of anesthesia on the cardiac involvement seen in advanced $CXMD_J$.[15] CHESS was employed to assess necrotic and inflammatory changes more precisely. The acquisition parameters for T1WI, CHESS-T1WI, Gd-T1WI, and CHESS-Gd-T1WI were based on spin echo: repetition time (TR)/echo time (TE) = 500/7.4 ms; slice thickness = 4 mm; field of view = 18 × 18 cm; matrix = 256 × 256; and NEX = 3. The parameters for T2WI and CHESS-T2WI were chosen based on fast spin echo: TR/TE = 4000/85 ms; slice thickness = 4 mm; field of view = 18 × 18 cm; matrix = 256 × 256; turbo-factor = 9; and NEX = 3. The parameters for multi-echo T2WI were selected based on spin echo: TR = 2000; TE = 11.8–118.0 (10 echoes); slice thickness = 4 mm; field of view = 28 × 28 cm; matrix = 256 × 256; and NEX = 2. We were able to clearly distinguish each lower leg muscle by each sequence. Representative cross-sectional images and anatomical locations of lower leg muscles by CHESS-T1WI in a 7-year-old normal dog are shown in Figure 1.

For quantitative analysis of the images, the manufacturer's software (Syngo MR2004A; Siemens Medical Solutions, Erlangen, Germany) was used. Flow artifacts were slight, but regions of interest (ROIs) were selected to avoid flow artifacts and large vessels as follows: three circular ROIs were picked in both right tibialis cranialis (Rt. TC) and extensor digitorum longus (Rt. EDL) muscles of the 3-month-old dogs. ROIs were also selected in the Rt. TC of the 7-year-old dogs and a normal dog. Then, T2 relaxation time or signal intensities (SIs) of CHESS-T1WI, CHESS-Gd-T1WI, and CHESS-T2WI were measured in these ROIs. Signal-to-noise ratios (SNRs) of each ROI were calculated by the equation: $SNR = SI/SD_{air}$, where SD_{air} was the standard deviation (SD) of background noise.[35] The contrast enhancement (CE) ratio was calculated using the SNR of CHESS-T1WI ($SNR_{precontrast}$) and SNR of CHESS-Gd-T1WI ($SNR_{postcontrast}$) by the following equation: $CE = SNR_{postcontrast}/SNR_{precontrast}$. We used the means of the quantitative values at three points of ROIs for statistical analysis.

Statistical Analysis. The T2 relaxation time, CE ratio, and SNR of CHESS-T2WI were evaluated using a one-way analysis of variance (ANOVA) to determine differences among the groups. When a significant difference was found with one-way ANOVA, intergroup comparisons were undertaken using Fisher's protected least significant difference test. All values are expressed as mean ± SE, and statistical significance was recognized at $P < 0.05$.

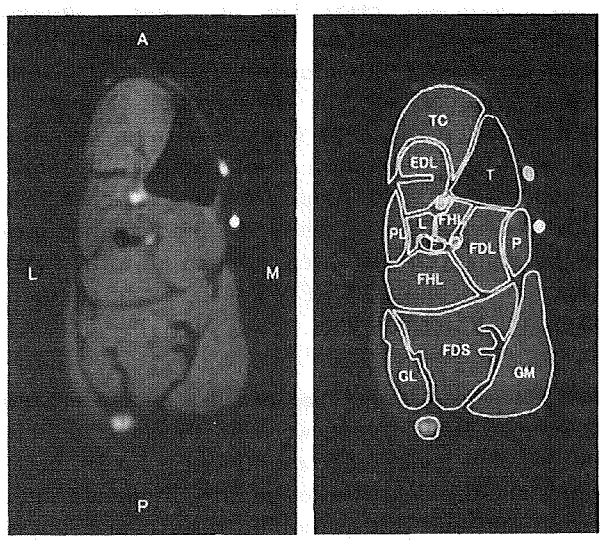

FIGURE 1. Cross-sectional images and anatomical orientation of right lower leg muscles of a 7-year-old normal dog in CHESS-T1WI. A 7-year-old normal dog (00-174MN) was used for this study. T, tibia; F, fibula; TC, tibialis cranialis; EDL, extensor digitorum longus; FHL, flexor hallucis longus; FDL, flexor digitorum longus; FDS, flexor digitorum superficialis; GM, gastrocnemius medialis; GL, gastrocnemius lateralis. A, anterior; P, posterior; L, lateral side; M, medial side.

Histopathology. We performed muscle biopsies of the right TC and right EDL on a 3-month-old normal dog (II-2308MN) and a $CXMD_J$ dog (II-2302MA), and right TC on a 7-year-old normal dog (00-174MN) and a $CXMD_J$ dog (II-C04MA) after MRI scanning. The muscle samples were snap-frozen in liquid nitrogen cooled by isopentane. Hematoxylin and eosin (H&E) staining was performed on serial 10-μm transverse cryostat sections. Anti-IgG immunofluorescence staining was performed on 6-μm serial cryostat sections incubated with fluorescein isothiocyanate (FITC)-conjugated polyclonal sheep anti-canine IgG (1:200; AbD Serotec, Oxford, UK) overnight. To examine fatty infiltration in the muscle, 6-μm serial frozen sections from normal and $CXMD_J$ dogs at 7 years of age were stained with oil red O.

RESULTS

MRI Findings of Lower Leg Muscles in 3-Month-Old Normal Dogs. First, we acquired muscle images of three 3-month-old normal dogs by T1WI, T2WI, CHESS-T1WI, CHESS-T2WI, Gd-T1WI, and CHESS-Gd-T1WI. Representative cross-sectional images of II-2308MN are shown in Figure 2A. In normal dogs, the lower leg muscles showed a homogeneous signal intensity in these sequences, but the muscles that contain mainly slow-twitch fibers, such as the gastrocnemius (GC) and flexor digitorum superficialis (FDS) (Fig. 2A, e and i), showed slight hyperintensity on T2WI and CHESS-T2WI when compared with muscles that contain mainly fast-twitch fibers such as TC and EDL (Fig. 2A, e and i). These findings were consistent with the previous T2 relaxation time study of rabbit muscles.[36] Gd-T1WI (data not shown) or CHESS-Gd-T1WI (Fig. 2A, g) showed homogeneous and slight enhancement when compared with T1WI (Fig. 2A, a) or CHESS-T1WI (Fig. 2A, c), respectively, in all normal dogs.

MRI Findings of Lower Leg Muscles in 3-Month-Old Dystrophic Dogs. Next, we tried to detect muscle involvement in three 3-month-old $CXMD_J$ dogs. Clinically, the dogs showed mild to moderate muscle atrophy and gait or mobility disturbance. These clinical findings are compatible with our previous study of $CXMD_J$dogs.[14] Representative cross-sectional images of II-2302MA are shown in Figure 2A.

All lower leg muscles of the three $CXMD_J$ showed no change on T1WI and CHESS-T1WI (Fig. 2A, b and d), but almost all lower leg muscles, especially EDL and GC, revealed remarkable hyperintensity on T2WI when compared with the images of normal dogs. We should note that the hyperintensity on TC was rather slight compared with that of the other lower leg muscles (Fig. 2A, f). We found that contrast agent uptake by Gd-T1WI (data not shown) or CHESS-Gd-T1WI (Fig. 2A, h) was increased in the areas where hyperintensity was recorded by T2WI. These findings suggest the necrotic and/or inflammatory changes that were shown in a previous study of *mdx* mice.[21] Hyperintensity was also clearly indicated by CHESS-T2WI in these regions, where hyperintensity on T2WI and contrast agent uptake in Gd-T1WI or CHESS-Gd-T1WI were noted (Fig. 2A, j).

Comparison of MR Signal Intensities of 3-Month-Old Normal and Dystrophic Dogs. To quantitatively evaluate MRI findings of three normal and three $CXMD_J$ dogs, we measured T2 relaxation time, CE based on comparison between SNR of CHESS-T1WI and CHESS-Gd-T1WI, and SNR of CHESS-T2WI. In TC, the T2 relaxation time of $CXMD_J$ dogs was significantly prolonged (49.8 $\pm$ 2.3 ms) when compared with that of normal dogs (39.9 $\pm$ 1.2 ms) ($P = 0.0004$). Moreover, T2 relaxation time of EDL was significantly prolonged in $CXMD_J$ (58.6 $\pm$ 3.1 ms) when compared with not only that in normal dogs (40.0 $\pm$ 0.5 ms) but also that of TC in $CXMD_J$ dogs ($P < 0.0001$ and $P = 0.0008$, respectively) (Fig. 2B, a).

Similarly, the effect of contrast enhancement in TC and EDL of $CXMD_J$ (1.659 $\pm$ 0.077 and 1.936 $\pm$ 0.127-fold) was significantly increased in comparison with that of normal dogs (1.511 $\pm$ 0.009 and 1.528 $\pm$ 0.015 fold) ($P = 0.0413$ and $P = 0.0002$, respectively), but the effect in TC of $CXMD_J$ was more prominent in EDL of $CXMD_J$ ($P = 0.0019$) (Fig. 2B, b).

In TC, the SNR of CHESS-T2WI was significantly increased in $CXMD_J$ (102.3 $\pm$ 12.1) when compared with that of normal dogs (70.0 $\pm$ 3.6) ($P = 0.0019$). Moreover, the SNR of CHESS-T2WI was significantly increased in EDL of $CXMD_J$ (146.0 $\pm$ 11.7) when compared with not only that of normal dogs (69.2 $\pm$ 2.9) but also that in TC of $CXMD_J$ dogs ($P < 0.0001$ and $P = 0.0003$, respectively), indicating EDL was more affected than TC in the early stage of $CXMD_J$ (Fig. 2B, c).

Histopathological Findings in Lower Leg Muscles of 3-month-old Normal and Dystrophic Dogs. To determine the relationship between MRI findings and

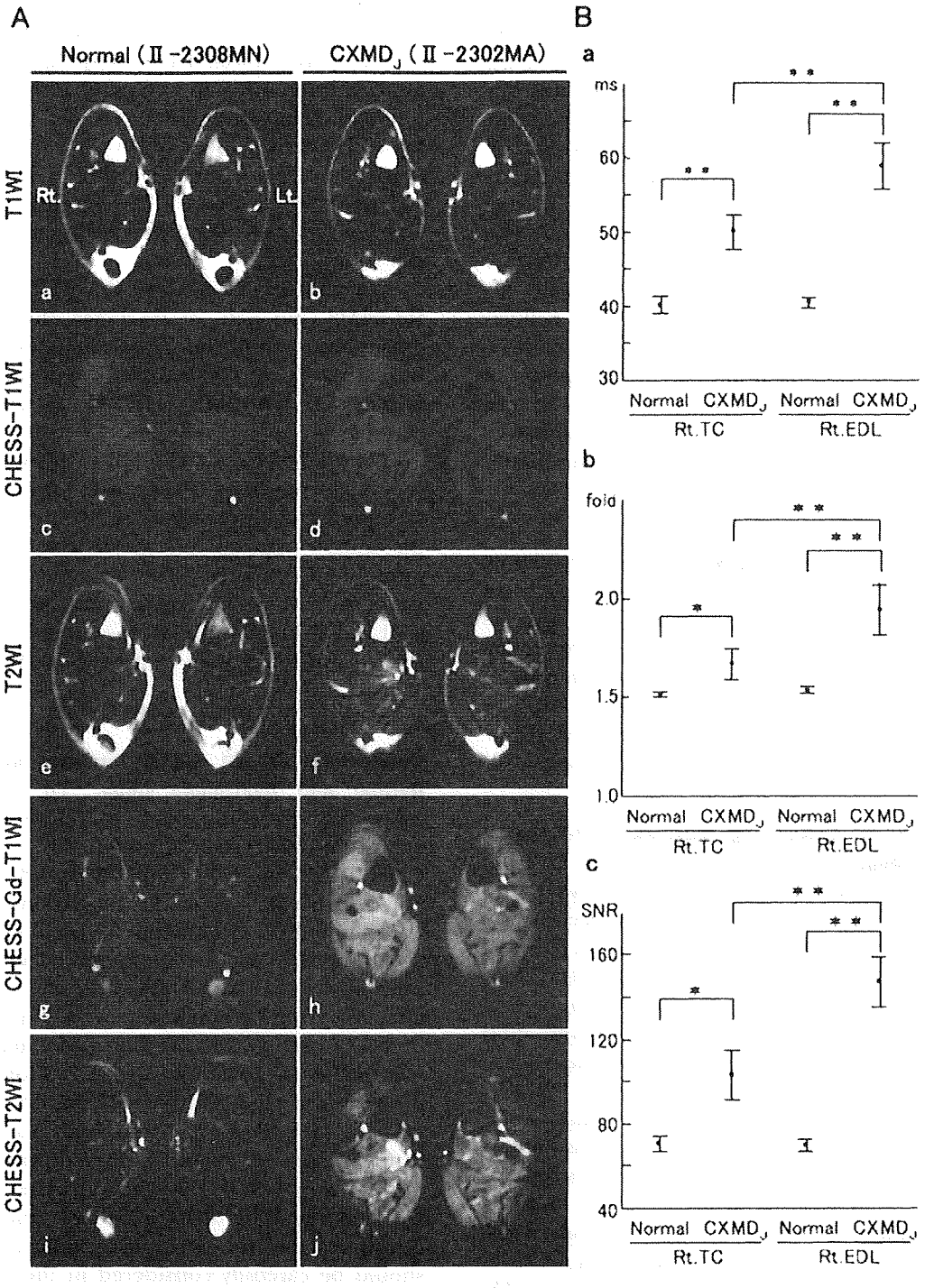

FIGURE 2. Cross-sectional MR images in lower leg muscles of 3-month-old normal and dystrophic dogs and comparison of three quantitative values in Rt. TC and Rt. EDL. **(A)** Representative images of normal (II-2308MN) and dystrophic (II-2302MA) dogs are shown. T1WI **(a, b)**, CHESS-T1WI **(c, d)**, T2WI **(e, f)**, CHESS-Gd-T1WI **(g, h)**, and CHESS-T2WI **(i, j)**. Rt, right side; Lt, left side. **(B)** Comparison of T2 relaxation time **(a)**, contrast enhancement (CE) **(b)**, and SNR of CHESS-T2WI **(c)** between three 3-month-old normal (II-2308MN, II/III-3911MN, and II-4202MN) and three littermate dystrophic (II-2302MA, II/III-3903MA, and II-4204MA) dogs are shown. Rt. TC, right side tibialis cranialis; Rt. EDL, right side extensor digitorum longus. Error bar: mean ± SD; $^*P < 0.05$; $^{**}P < 0.01$.

morphological changes of $CXMD_J$ lower leg muscles, we biopsied the Rt. TC and Rt. EDL of a normal dog (II-2308MN) and a $CXMD_J$ dog (II-2302MA) and carried out histopathological examinations. In TC of the $CXMD_J$, we found necrotic fibers, regenerating myofibers with central nuclei, a slight increase in cellular infiltration, and moderate variation in fiber size (Fig. 3A, c). Immunostaining with anti-IgG antibody, which is a marker for muscle necrosis,[37] revealed a slight degree of IgG uptake in the cytoplasm (Fig. 3A, d). On the other hand, EDL of the $CXMD_J$ showed many necrotic and hypercontracted fibers, severe cellular infiltration, and an increase in interstitial connective tissue (Fig. 3B, c). Moreover, the cytoplasm of this muscle showed a severe degree of IgG uptake (Fig. 3B, d). The necrotic and inflammatory changes in the muscle corresponded to the higher SNR on CHESS-T2W images.

MRI Findings of Lower Leg Muscles in a 7-year-old Normal Dog. Next, we obtained muscle images of a 7-year-old normal dog, 00-174MN (Fig. 4A). As shown in Figure 4A, a, c, e, g, and i, the lower leg muscles showed a homogeneous signal intensity in each sequence. Homogeneous but slight contrast enhancement was found on Gd-T1WI and CHESS-Gd-T1WI, as seen in 3-month-old normal dogs.

MRI Findings of Lower Leg Muscles in 7-Year-Old Dystrophic Dogs. We performed muscle MRI on two 7-year-old $CXMD_J$ dogs. II-C04MA showed muscle weakness and atrophy, gait disturbance, macroglossia, arthrogryposis, and dysphagia, but the dog could still rise and walk. Another dog, II-C12MA, was found to have difficulty in rising at the age of about 6.5 years. These two $CXMD_J$ showed mild clinical symptoms and signs despite their ages, which is sometimes seen in less affected GRMD.[8] We have previously reported that the clinical severity in Beagle-crossed dystrophic dogs was milder than that in GRMD,[14] in accordance with a separate report from another facility.[8]

MRI indicated muscle atrophy in both dogs, but the degree of muscle atrophy was more striking in II-C12MA than that in II-C04MA. Figure 4A, b, d, f, h, and j shows representative cross-sectional images of II-C04MA. On T1WI, almost all lower leg muscles of both dogs, in particular TC, EDL, and GC, revealed diffuse hyperintensity regions (Fig. 4A, b), although FHL and FDL did not show remarkable change compared with the normal dog. On CHESS-T1WI, these hyperintense regions were considerably suppressed, suggesting fat infiltration with progression of the disease (Fig. 4A, d), which was reported in a previous MRI study of DMD.[19]

On T2WI, II-C04MA showed slight and moderate hyperintensity in TC and other lower leg muscles, respectively (Fig. 4A, f). On the other hand, II-C12MA also had an area that showed remarkable T2 hyperintensity with T1 hyperintensity, but there is no area of T2 hyperintensity without T1 hyperintensity, with the exception of FHL (data not shown).

On CHESS-T2WI, FDL, GC, FHL, and FDS of II-C04MA and FHL of II-C12MA showed hyperintensity, but significant signal changes were found in neither TC of II-C04MA nor almost all lower leg muscles of II-C12MA (Fig. 4A, j, and data not shown).

On CHESS-Gd-T1WI of II-C04MA, the CHESS sequence considerably suppressed the hyperintense fat signal, but the contrast agent greatly enhanced the muscle regions left in all lower leg muscles, especially EDL, FHL, FDL, GC, and FDS (Fig. 4A, h). Contrast agent uptake was also found on CHESS-Gd-T1WI of II-C12MA, but the degree of uptake was lower than that of II-C04MA (data not shown).

Comparison of MR Signal Intensity in 7-Year-Old Normal and Dystrophic Dogs. To quantitatively assess the MRI findings of $CXMD_J$ dogs, we calculated the T2 relaxation time, CE, and SNR of CHESS-T2WI in TC of one 7-year-old normal dog and two $CXMD_J$ dogs. T2 relaxation time of TC was moderately prolonged in both $CXMD_J$ dogs (46.4 and 47.4 ms) when compared with that in a normal dog (37.0 ms) (Fig. 4B, a). Similarly, the effect of contrast enhancement was also increased in TC of each $CXMD_J$ (1.439- and 1.465-fold) relative to that of a normal dog (1.229-fold) (Fig. 4B, b). However, the SNR of CHESS-T2WI in TC of both $CXMD_J$ dogs (77.7 and 75.0) was not significantly increased when compared with that of a normal dog (74.7) (Fig. 4B, c). The discrepancy between the SNR of CHESS-T2WI and T2 relaxation times, and the effect of contrast enhancement should be carefully considered in the examination of affected skeletal muscle morphology.

Histopathological Findings in Lower Leg Muscles of 7-year-old Normal and Dystrophic Dogs. To determine the relationship between MRI findings and morphological changes in $CXMD_J$ lower leg muscles, we biopsied the Rt. TC of 00-174MN and

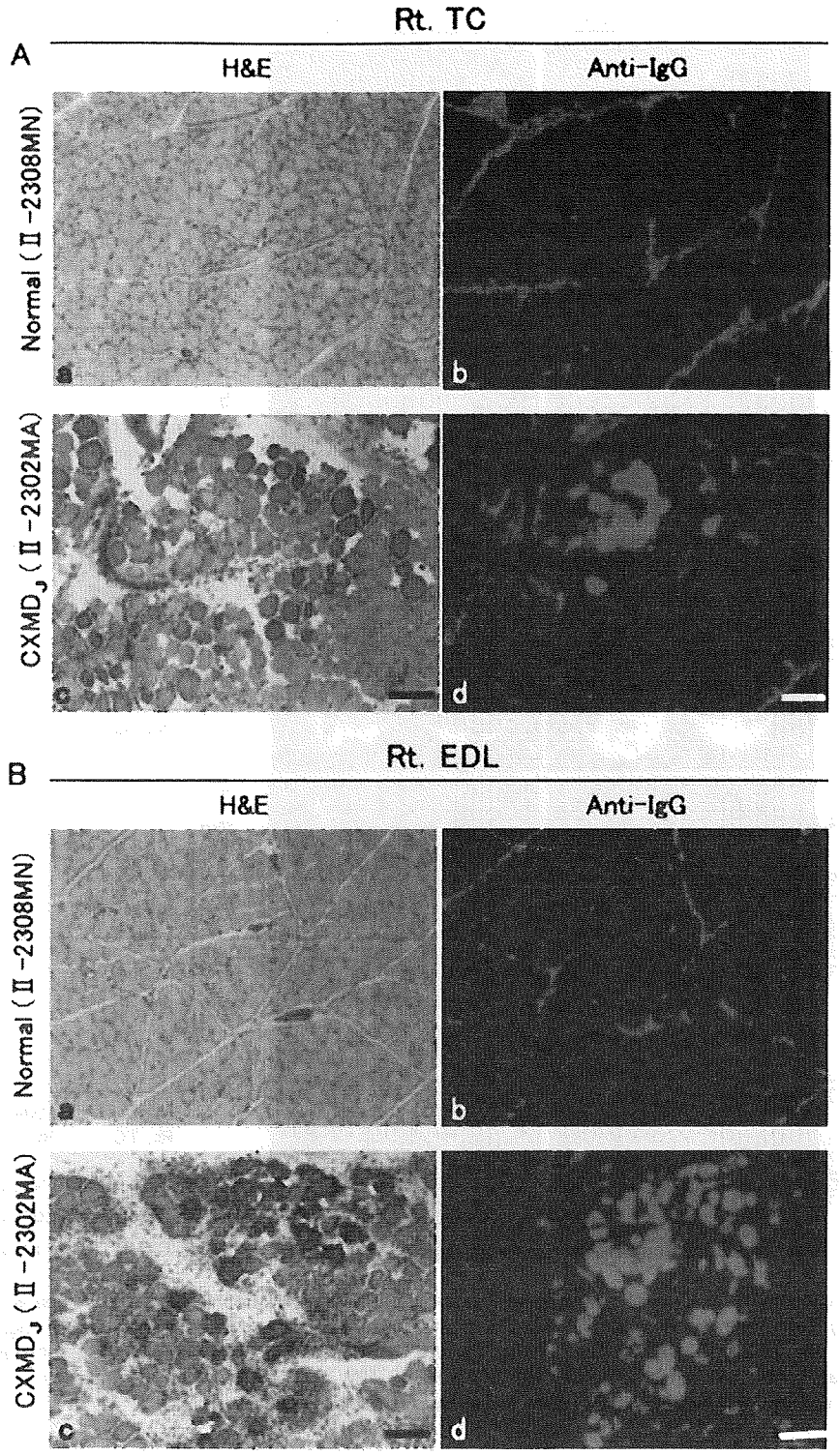

FIGURE 3. Histopathological examinations in Rt. TC and Rt. EDL of 3-month-old normal and dystrophic dogs.**(A)** Hematoxylin and eosin (H&E) staining **(a, c)** and IgG immunostaining **(b, d)** in Rt. TC of 3-month-old normal (II-2308MN) **(a, b)** and dystrophic (II-2302MA) dogs **(c, d)**. **(B)** H&E **(a, c)** and IgG immunostaining **(b, d)** in Rt. EDL of 3-month-old normal (II-2308MN) **(a, b)** and dystrophic (II-2302MA) dogs **(c, d)** are also shown. Bar = 100 μm.

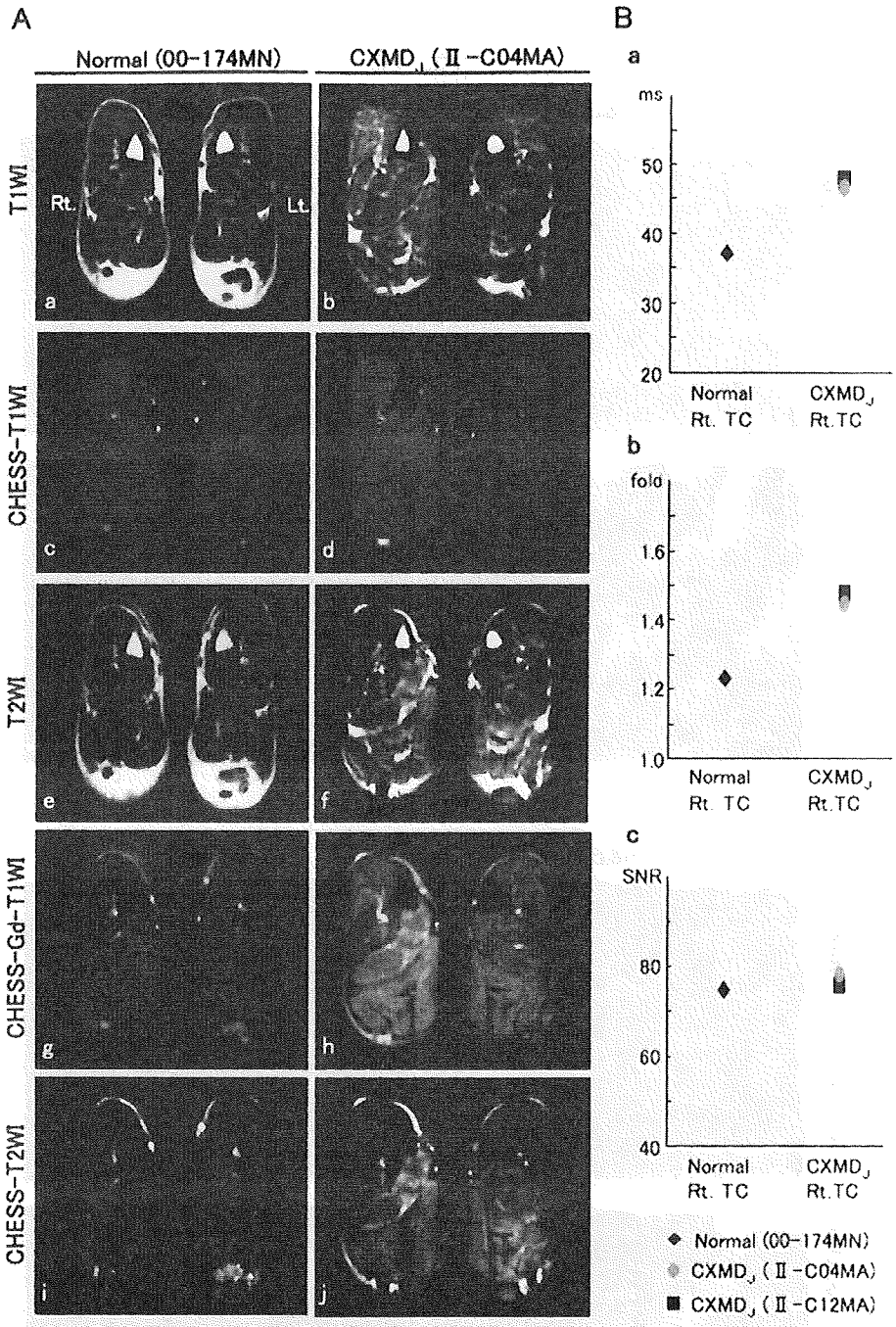

FIGURE 4. Cross-sectional MRI of lower leg muscles of 7-year-old normal and dystrophic dogs and comparison of three quantitative values in Rt. TC. **(A)** Representative images of 7-year-old normal (00-174MN) and dystrophic (II-C04MA) dogs are shown. T1WI **(a, b)**, CHESS-T1WI **(c, d)**, T2WI **(e, f)**, CHESS-Gd-T1WI **(g, h)**, and CHESS-T2WI **(i, j)**. Rt, right side; Lt, left side. **(B)** Comparisons of T2 relaxation time **(a)**, contrast enhancement (CE) **(b)**, and CHESS-T2W SNR **(c)** between the muscles in 7-year-old normal (00-174MN) and dystrophic (II-C04MA and II-C12MA) dogs are shown. TC, tibialis cranialis.

II-C04MA and carried out histopathological examinations. In CXMD$_J$ dogs, we found a few degenerated and many regenerated fibers with central nuclei and a moderate degree of fiber size variation together with fibrotic changes (Fig. 5d). IgG accumulation was found in only a few fibers, but it was extensively distributed in the interstitial tissues (Fig. 5e). Oil red O staining revealed definite fatty infiltration in the CXMD$_J$ dogs (Fig. 5f).

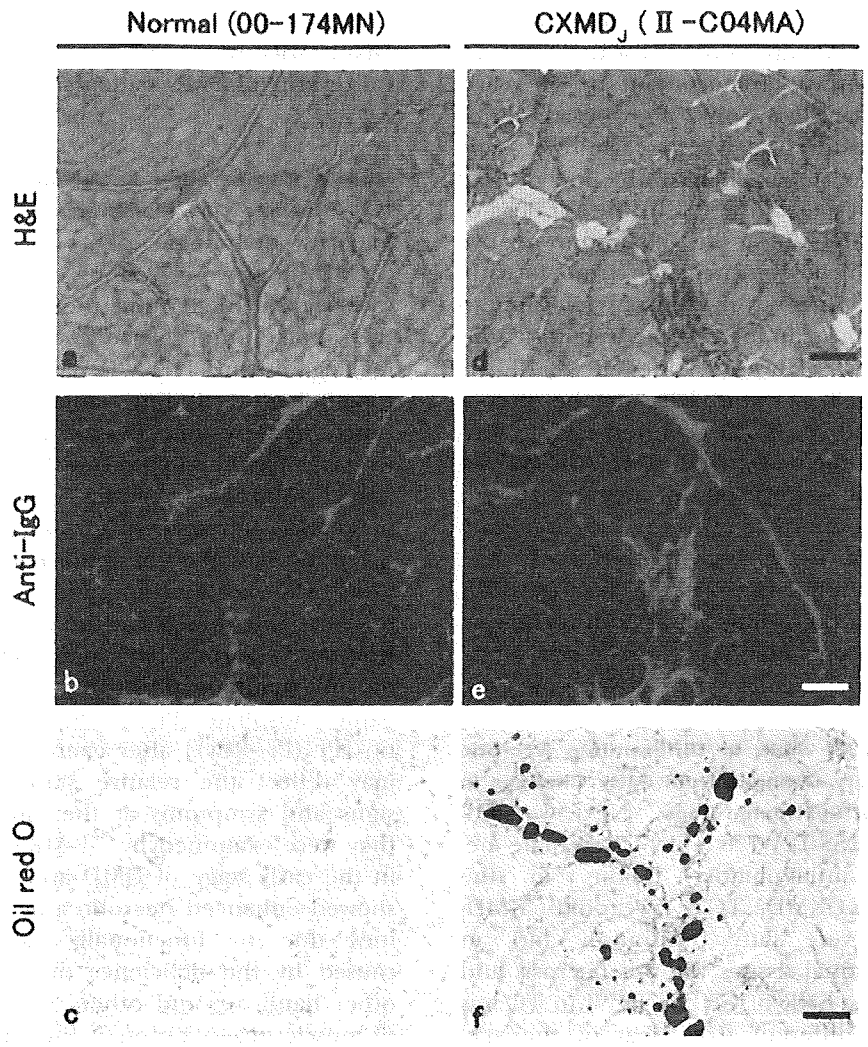

FIGURE 5. Histopathology in Rt. TC of 7-year-old normal and dystrophic dogs. We performed hematoxylin and eosin (H&E) staining **(a, d)**, IgG immunostaining **(b, e)**, and oil red O staining **(c, f)** on tissues of 7-year-old normal (00-174MN) **(a–c)** and dystrophic (II-C04MA) dogs **(d–f)**. Bar = 100 μm.

DISCUSSION

Previous studies have attempted to noninvasively evaluate involvement of striated muscle in DMD patients or the animal models by various MRI sequences; however, a method for more accurate and precise assessment of acute-phase responses such as muscle necrosis and/or inflammation is necessary, because it is difficult to distinguish between these lesions and fat infiltration and/or fibrosis by conventional MRI sequences. Among dystrophic processes, especially in muscle necrosis, the intramuscular water concentration and extracellular components are increased, with the water imbalance having been induced by the deficiency of sarcolemmal membrane integrity.[19,26,38] CHESS-T2WI may be one of the tools to solve this problem, because the sequence can selectively cancel fat tissue signals.

CHESS-T2WI Is Useful to Evaluate Dystrophic Skeletal Muscle Involvement in Early Stage. First, we tried to detect necrotic and/or inflammatory lesions in the early stage of CXMD$_J$ by using a CHESS-T2WI sequence. EDL of a 3-month-old CXMD$_J$ dog showed massive and severe necrosis and inflammatory cell infiltration in the pathological analyses, but TC of the dog revealed localized and moderate necrosis and inflammation. The SNR of CHESS-T2WI in EDL showed a significant increase compared with that in TC. These findings suggest that there is a correlation between SNR of CHESS-T2WI and the degree of necrotic and inflamma-