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White Blood Cell Counts and Future Relapse in Ulcerative Colitis under Low-Dose Thiopurine Treatment in Real-World Practice: A 3-Year Japanese Multi-Center Retrospective Cohort Study

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Keywords
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Abstract
Introduction: Whether white blood cell (WBC) counts are predictors for the effectiveness of thiopurine treatment in ulcerative colitis (UC) has been inconclusive in

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previous studies with small sample sizes. We investigated the association between WBC counts and future relapses in UC patients in a large-scale multi-center study. ***Methods:*** This retrospective cohort study enrolled a total of 723 UC patients in remission from 33 hospitals and followed up for 3 years. Relapse was defined as a need for treatment intensification. The risk of relapse was compared among patients with the baseline WBC counts <3,000/μL (*N* = 31), 3,000–4,000/μL (*N* = 167), 4,000–5,000/μL (*N* = 241), and ≥5,000/μL (*N* = 284) using a Cox regression model analysis. Moreover, exploratory analyses were conducted to identify other factors predicting relapse. ***Results:*** During a median follow-up period of 1,095 (interquartile range, 1,032–1,119) days, relapse occurred in 17.2% (125/723). In a crude analysis, WBC counts were not associated with relapse; hazard ratios (HRs) (95% confidence interval [CI]) were 1.50 (0.74–3.06), 1.02 (0.66–1.59), and 0.67 (0.43–1.05) in WBC <3,000/μL, 3,000–4,000/μL, and 4,000–5,000/μL groups, respectively (WBC ≥5,000/μL group, as reference). Multivariable-adjusted analyses showed similar results; HRs (95% CI) were 1.21 (0.59–2.49), 1.08 (0.69–1.69), and 0.69 (0.44–1.07), in <3,000/μL, 3,000–4,000/μL, and 4,000–5,000/μL groups, respectively. In the exploratory analyses, thiopurine use <1 year and a mean corpuscular volume <90 fL were predictors for relapse. ***Discussion/Conclusion:*** WBC counts were not predictors for future relapses in patients with UC treated with thiopurine as a maintenance therapy.

Introduction

The incidence of ulcerative colitis (UC), a chronic gut inflammatory disease, has been increasing worldwide [1]. The therapeutic goal for UC is to maintain remission and therefore to improve the quality of life, to prevent irreversible structural and functional damage, and to reduce the risk of colitis-associated neoplasm, resulting in the improvement of long-term prognoses [1].

Thiopurines, including azathioprine (AZA) and 6-mercaptopurine (6-MP), are an efficacious drug as maintenance therapy particularly in patients with steroid-dependent disease or those who are intolerant to 5-aminosalicylic acid (5-ASA) [2–4]. A meta-analysis in quiescent UC patients showed that AZA reduced the risk of relapse (relative risk, 0.60; 95% confidence interval [CI], 0.37–0.95) [5]. Another study revealed that the number need to treat of AZA to prevent a relapse was 5, and AZA reduced the risk of relapse by 23% compared to a placebo [6]. Thiopurines are incorporated into the DNA of white blood cells (WBCs) as a metabolite. One of the plausible mechanisms of thiopurine-induced leukocytopenia has been reported that apoptosis of T-lymphocytes is promoted by the incorporation of deoxy thioguanosine, a thiopurine derivative, into replicating DNA [7]. Meanwhile, the metabolic pathways of thiopurines are complicated and the enzymatic activity, which is involved in the drug metabolization, varies among the individuals. Thus, the optimal doses of thiopurines are different among patients. In this respect, the therapeutic drug monitoring of thiopurine is important.

Currently, the 6-thioguanine nucleotide (6-TGN) concentration in red blood cells is used for thiopurine monitoring [8]. However, 6-TGN-based thiopurine monitoring has several problems. First, large inter- and intra-individual variability is pointed out in the 6-TGN level [9, 10]. Second, the measurement of 6-TGN level is not available universally in certain countries including Japan, and the cost is expensive [11]. Finally, it has been recently revealed that thiopurine-induced leukopenia occurs independent of the 6-TGN level in patients harboring a single-nucleotide polymorphism (SNP) of p.Arg139Cys, in the *nucleotide diphosphate-linked moiety X-type motif 15* (*NUDT15*) gene [12, 13]. Thus, leukopenia other than the 6-TGN level could be a dose-limiting factor of thiopurine in patients with the *NUDT15* genetic variant. The frequency of p.Arg139Cys SNP in the *NUDT15* gene is estimated at approximately 20% in Japanese inflammatory bowel disease (IBD) patients, and it is more common in Asians than Caucasians [14–16]. In consideration of the mechanism of thiopurines, including lymphocyte apoptosis and the frequency of the *NUDT15* genetic variant, the usefulness of 6-TGN levels in therapeutic drug monitoring seems to be limited in Asian IBD patients. Therefore, we focused on the WBC counts, which are decreased as the result of thiopurine-induced apoptosis as a potential indicator for thiopurine monitoring.

Several studies have studied the question whether WBC counts are useful markers in optimizing the dose of thiopurines. However, the argument has been inconclusive due to the small sample size or no adjustment of potential confounders [17–21]. Furthermore, thiopurine is often used at a lower dose in Japan than in Western countries [22]. Therefore, we conducted a large-scale multi-center observational study to investigate the association between WBC counts and future relapses in Japan.

Materials and Methods

Study Design and Participants

A multi-center retrospective cohort study was conducted among 33 academic centers and community general hospitals in Japan. We included UC patients who visited our hospitals between April 1, 2016, and June 30, 2016, and who met all of the three following criteria: (1) patients of age 16 years or older, (2) patients who were administered AZA or 6-MP for more than 6 months and at the same dosage for the latest 1 month, and (3) patients in clinical remission. Clinical remission was assessed by the patient-reported outcome (PRO)-2 score, consisting of the rectal bleeding and stool sub-scores [23]. Clinical remission was defined as both "rectal bleeding sub-score = 0" and "stool sub-score ≤1" [24]. Patients with any following criterion were excluded: (1) proctitis, (2) after colectomy, (3) treated for UC other than thiopurine and/or 5-ASA (e.g., topical corticosteroids, systemic corticosteroids, tacrolimus, or Janus kinase inhibitors) within 1 month before the baseline, (4) under maintenance therapy with biologics, (5) past history of hematologic disorder, (6) pregnant woman, (7) after total gastrectomy, or (8) after ileocecal resection. The patients were followed up for 3 years.

WBC Counts at Baseline

The WBC counts at baseline were defined as the lowest WBC counts between April 1, 2016, and June 30, 2016. The study patients were categorized into four groups according to the WBC counts at baseline: (1) WBC <3,000/μL, (2) 3,000≤ WBC <4,000/μL, (3) 4,000≤ WBC <5,000/μL, and (4) WBC ≥5,000/μL.

Outcomes

The primary outcome was the time to relapse, defined as the requirement of induction therapy for UC with the worsening of clinical symptoms [24]. The induction therapy included systemic corticosteroids, tacrolimus, cyclosporin A, leukocytapheresis, granulocyte and monocyte apheresis, biologic agents, Janus kinase inhibitor, indigo naturalis, any investigational new drug, or colectomy. The addition of topical 5-ASA and/or corticosteroids, and the dose escalation of 5-ASA or thiopurine were not regarded as relapses because the relapses which did not require systemic induction therapy seemed not to be clinically relevant. The patients were followed up until relapse or 3 years after the baseline date, regardless of whether thiopurine was discontinued or not. If a colectomy was performed due to the reason except for UC flare (e.g., UC-associated neoplasm), it was not regarded as relapse and the observation was censored at the time of colectomy. In case the patient transferred to another hospital or suspended hospital visit for more than 6 months or died due to any reason, it was also regarded as censored. The secondary outcomes were the proportion of severe adverse event (SAE), defined as any adverse event requiring admission.

Covariates

Disease extension was categorized as extensive colitis, left-sided colitis, and others. The smoking status was categorized as current smoker or non-current smoker. The dose of thiopurine was categorized into three groups by daily dosage: low, AZA ≤50 mg or 6-MP ≤30 mg; medium, 50< AZA <100 mg or 30< 6-MP <60 mg; and high, AZA ≥100 mg or 6-MP ≥60 mg. The duration of thiopurine use was categorized as <1 year, 1–2 years, and ≥2 years.

Statistical Analyses

The baseline patient characteristics are expressed as the number (%) or median (interquartile ranges). The time to relapse was depicted in Kaplan-Meier plots. For the primary analysis, we used Cox regression models to estimate hazard ratios (HRs) and 95% CI for the association between the baseline WBC counts and relapse. Because *p* value-based covariate selection is not recommended to select confounders, we prespecified covariates for the multivariable analyses based on biological and clinical knowledge [25]. We adjusted the following potential confounders: age, sex, smoking status, disease extension, the duration of thiopurine use, dose of thiopurine, and concomitant use of 5-ASA.

We also conducted three sensitivity analyses. First, we treated patients who discontinued thiopurine due to reasons other than adverse events (e.g., patient's wish) as censored. Second, we categorized patients by different cut-off points of WBC counts at baseline: (1) WBC <3,500/μL, (2) 3,500≤ WBC <4,500/μL, (3) 4,500≤ WBC <5,500/μL, and (4) WBC ≥5,500/μL. Third, we conducted a subgroup analysis in patients under high-dose thiopurine (see Covariates section for the definition) treatment. The subgroup patients were stratified by mean corpuscular volume (MCV) at baseline: (1) MCV ≥100 fL (elevated MCV) and (2) MCV <100 fL (not-elevated MCV). After stratification by MCV, time to relapse was analyzed with log-rank test among the four baseline WBC count groups.

For the secondary analysis, we investigated the safety of thiopurine treatment. The reasons for thiopurine discontinuation and the SAEs were described in each baseline WBC group. We also conducted exploratory analyses to search other factors predicting relapse using Cox regression models.

The missing values were complemented by multiple imputation using chained equations. Stata version 17 (StataCorp, College Station, TX, USA) was used for all statistical analyses.

Results

Study Population and Baseline Characteristics

A total of 723 UC patients were included for analyses in this study. The baseline characteristics of the patients are shown in Table 1. The patients were divided into four groups according to the WBC counts at baseline: (1) WBC <3,000/μL (N = 31), (2) 3,000≤ WBC <4,000/μL (N = 167), (3) 4,000≤ WBC <5,000/μL (N = 241), and (4) WBC ≥5,000/μL (N = 284). More than half of the patients were administered thiopurine at a low dose and for more than 2 years in all groups.

Primary Analysis: WBC Counts at Baseline and Relapse

Relapse was observed in 17.2% of patients (125/723) during the median follow-up time of 1,095 (interquartile range, 1,032–1,119) days. Additionally, the relapse rate was 6.53 per 100 person-years. The proportion of relapse in each group (WBC <3,000/μL, 3,000≤ WBC <4,000/μL, 4,000≤ WBC <5,000/μL, and WBC ≥5,000/μL) were

Table 1. Baseline patient characteristics

	WBC <3,000 (*N* = 31)	3,000≤ WBC <4,000 (*N* = 167)	4,000≤ WBC <5,000 (*N* = 241)	WBC ≥5,000 (*N* = 284)
Sex, female, *N* (%)	9 (29.0)	74 (44.3)	93 (38.6)	113 (39.8)
Age, median [IQR], years	41 [31–55]	49 [36–63]	46 [31–58]	45 [34–55]
Disease extension, *N* (%)				
Extensive	24 (77.4)	126 (75.5)	180 (74.7)	213 (75.0)
Left sided	7 (22.6)	39 (23.3)	59 (24.5)	63 (22.2)
Others	0 (0)	2 (1.2)	2 (0.8)	8 (2.8)
Smoking status, *N* (%)				
Current smoker	0 (0)	7 (4.2)	21 (8.7)	33 (11.6)
Non-current smoker	20 (64.5)	122 (73.1)	169 (70.1)	178 (62.7)
Missing data	11 (35.5)	38 (22.8)	51 (21.2)	73 (25.7)
Dose of thiopurine[a], *N* (%)				
Low	17 (54.8)	108 (64.7)	143 (59.3)	175 (61.6)
Medium	10 (32.3)	29 (17.4)	51 (21.2)	69 (24.3)
High	4 (12.9)	30 (18.0)	47 (19.5)	40 (14.1)
Duration of thiopurine use, *N* (%)				
≥2 years	18 (58.1)	116 (69.5)	185 (76.8)	204 (71.8)
1–2 years	5 (16.1)	31 (18.6)	31 (12.9)	43 (15.1)
<1 year	7 (22.6)	17 (10.2)	23 (9.6)	37 (13.0)
Missing data	1 (3.2)	3 (1.8)	2 (0.8)	0 (0)
Concomitant use of 5-ASA, *N* (%)	27 (87.1)	149 (89.2)	214 (88.8)	256 (90.1)
Neutrophil counts, median [IQR], μL	1,444 [1,170–1,725]	2,139 [1,890–2,343]	2,753 [2,381–3,030]	4,049 [3,339–4,751]
Lymphocyte counts, median [IQR], μL	805 [686–1,025]	930 [792–1,164]	1,190 [968–1,402]	1,450 [1,091–1,820]
MCV, median [IQR], fL	99 [95–105]	96 [93–100]	95 [91–99]	92 [88–96]
CRP, median [IQR], mg/L	0.5 [0.2–1.0]	0.3 [0.1–0.7]	0.4 [0.2–1.0]	0.6 [0.2–1.3]

The values are described as *N* (%) or median [IQR]. [a]Thiopurine doses are categorized into three groups: low, AZA ≤50 mg or 6-MP ≤30 mg; medium, 50< AZA <100 mg or 30< 6-MP <60 mg; and high, AZA ≥100 mg or 6-MP ≥60 mg. 5-ASA, 5-aminosalicylic acid; 6-MP, 6-mercaptopurine; AZA, azathioprine; CRP, C-reactive protein; IQRs, interquartile ranges; MCV, mean corpuscular volume; WBC, white blood cell.

29.0% (9/31), 19.2% (32/167), 12.9% (31/241), and 18.7% (53/284), respectively (Table 2). Figure 1 shows the Kaplan-Meier plots. The result of the primary analysis, HR and 95% CI for the association between WBC counts at the baseline and relapse, is shown in Table 2. After adjusting for likely confounders, the WBC counts at baseline were not associated with relapse; adjusted HR (WBC ≥5,000/μL as reference) in WBC <3,000/μL, 3,000≤ WBC <4,000/μL, and 4,000≤ WBC <5,000/μL were 1.21 (95% CI, 0.59–2.49), 1.08 (95% CI, 0.69–1.69), and 0.69 (95% CI, 0.44–1.07), respectively.

Sensitivity Analyses

We conducted three sensitivity analyses. First, we regarded patients who discontinued thiopurine due to the reason except for adverse events as censored at the time of thiopurine withdrawal. The baseline WBC counts were not associated with relapse in 3 years in this sensitivity analysis (online suppl. Table 1; for all online suppl. material, see https://doi.org/10.1159/000535889). Second, we changed the cut-off point of the baseline WBC counts: WBC <3,500/μL, 3,500≤ WBC <4,500/μL, 4,500≤ WBC <5,500/μL, and WBC ≥5,500/μL. After adjusting the likely confounders, the baseline WBC counts were not associated with future relapse (online suppl. Table 2). Third, we conducted a subgroup analysis in patients under high-dose thiopurine treatment. After stratification by MCV, the baseline WBC counts were not associated with relapse rate (p = 0.970, elevated MCV group; p = 0.132, not-elevated MCV group) (online suppl. Table 3).

Table 2. WBC counts at baseline and relapse

	Relapse proportion, %	Relapse rate, /100 PY	Crude		Multivariable-adjusted	
			HR (95% CI)	*p* value	aHR (95% CI)	*p* value
WBC <3,000	29.0	10.7	1.50 (0.74–3.06)	0.26	1.21 (0.59–2.49)	0.61
3,000≤ WBC <4,000	19.2	7.3	1.02 (0.66–1.59)	0.92	1.08 (0.69–1.69)	0.72
4,000≤ WBC <5,000	12.9	4.8	0.67 (0.43–1.05)	0.08	0.69 (0.44–1.07)	0.10
WBC ≥5,000	18.7	7.1	Reference	–	Reference	–

Cox regression models were used to estimate the aHR of relapse. Age, sex, smoking status, disease extension, duration of thiopurine use, dose of thiopurine, and concomitant use of 5-ASA were used as covariates. aHR, adjusted hazard ratio; CI, confidence interval; HR, hazard ratio; PY, person-years.

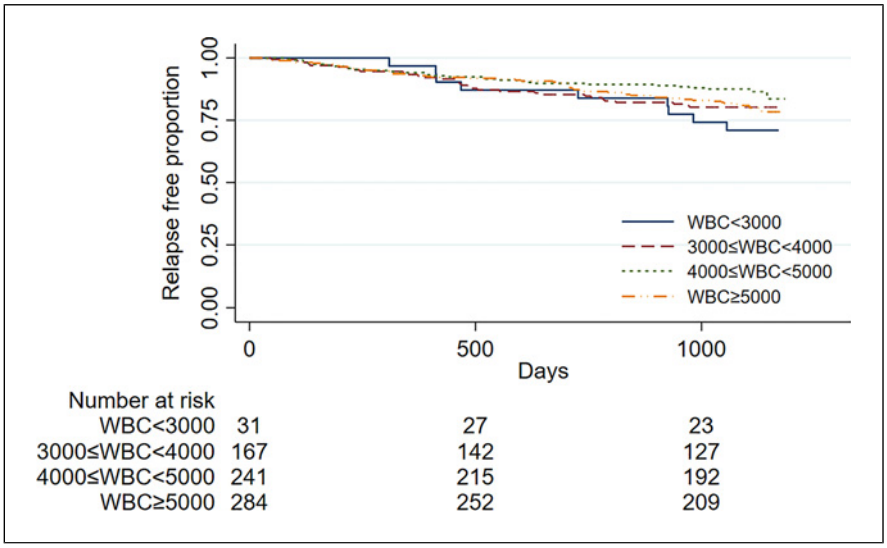

Fig. 1. Kaplan-Meier plots of time to relapse. Patients were categorized into four groups according to the WBC counts at the baseline. WBC, white blood cell.

Secondary Analysis: WBC Counts at Baseline and Safety

The reasons for thiopurine discontinuation and details of adverse events are shown in Table 3. The proportion of SAEs in each baseline WBC group, WBC <3,000/μL, 3,000≤ WBC <4,000/μL, 4,000≤ WBC <5,000/μL, and WBC ≥5,000/μL, was 3.2% (1/31), 1.2% (2/167), 1.7% (4/241), and 1.4% (4/284), respectively. All the SAEs were infectious disease or malignant neoplasm.

Exploratory Analyses to Search for Predictive Factors of Relapse

Table 4 shows the results of exploratory analyses to search for predictive factors of relapse other than WBC counts. In multivariable analyses, the duration of thiopurine use <1 year was identified as a predictor for relapse; multivariable-adjusted HRs in the groups of 1–2 years and <1 year (≥2 years group as reference) were 1.34 (95% CI, 0.83–2.18) and 2.54 (95% CI, 1.62–4.00), respectively. Moreover, the MCV < 90 fL at baseline was also identified as a predictor for relapse; multivariable-adjusted HRs in the groups of 90≤ MCV <100 fL and MCV <90 fL (MCV ≥100 fL group as reference) were 1.08 (95% CI, 0.64–1.81) and 2.08 (95% CI, 1.15–3.75), respectively.

Discussion

In the present study, the WBC counts of UC patients in remission under treatment with thiopurine were not associated with future relapse during 3 years. The result was also confirmed by two sensitivity analyses. Importantly, this study was conducted in a Japanese population in which the *NUDT15* genetic variant was more common than those in Western countries. As far as we know, our

Table 3. Reasons for thiopurine discontinuation and details of adverse events

	WBC <3,000 (*N* = 31)	3,000≤ WBC <4,000 (*N* = 167)	4,000≤ WBC <5,000 (*N* = 241)	WBC ≥5,000 (*N* = 284)
Thiopurine discontinuation, *N* (%)	1 (3.2)	15 (9.0)	25 (10.4)	32 (11.3)
Reasons for thiopurine discontinuation, *N*				
Leukocytopenia	1	2	1	–
Alopecia	–	–	–	1
Anemia	–	–	1	–
Pancreatitis or hyperamylasemia	–	–	1	–
Infectious disease	–	1	–	4
Nausea	–	1	2	1
Malignant neoplasm	–	–	3	2
Clinical ineffectiveness	–	–	–	2
Patient's wishes	–	8	15	22
Others	–	3	2	–
SAEs, *N* (%)	1 (3.2)	2 (1.2)	4 (1.7)	4 (1.4)
Infectious disease	1	1	1	1
Malignant neoplasm	–	1	3	3

The reason for thiopurine discontinuation and details of severe adverse events are described. Severe adverse event is defined as an adverse event accompanied with admission. Values are described as *N* (%). SAEs, severe adverse events.

study is the largest so far to investigate whether WBC counts predict the efficacy of maintenance therapy with thiopurine.

Previous studies showed inconsistent results regarding whether lower WBC counts in UC patients were associated with a lower risk of future relapse. Campbell et al. [17] divided patients with IBD treated with AZA (UC, *N* = 94; Crohn's disease [CD], *N* = 109) into two groups – those in whom neutrophil counts were decreased less than 2,500/μL or not and reported that the relapse rate was similar between two groups in a retrospective study. However, the potential confounders were not adjusted in this study. Meanwhile, Fraser et al. [18] reported that among IBD patients who were treated with AZA for more than 6 months and in clinical remission (UC, *N* = 232; CD, *N* = 192), those with the lowest WBC counts less than 5,000/μL were less likely to relapse than those with WBC counts more than 5,000/μL. However, the details about adjusted confounders were uncertain and the cut-off value was high. Park et al. [20] conducted a retrospective study in Korean IBD patients, including UC, CD, and Behçet's disease, and reported that the relapse was less in patients with the lowest WBC counts during the observation period <4,000/μL than those with that ≥4,000/μL. However, this was a single-center study with a small sample size (UC, *N* = 45; CD, *N* = 68; Behçet's disease, *N* = 83), and the definition of relapse was ambiguous because it was based on only laboratory data or symptoms without using a unified disease activity index (e.g., partial Mayo score and PRO-2 score). Thus, the evidence from the previous studies is insufficient to conclude the association between leukocytopenia and the efficacy of thiopurine treatment as a maintenance therapy.

The present study overcomes the limitations of the past studies with the following strengths. First, as far as we know, this is the largest multi-center study (723 UC patients from 33 academic centers and community general hospitals) to investigate the association between WBC counts and the efficacy of maintenance therapy of thiopurines. Second, we classified the patients into four groups according to the baseline WBC counts and accounted for the degree of leukocytopenia in evaluating the treatment efficacy. We also conducted a sensitivity analysis using different values of WBC count cut-off. In addition, we systematically extracted the WBC counts by the lowest value during the predefined 3 months because WBC is likely to fluctuate due to various reasons other than UC. Third, we used a well-established PRO-2 score to determine clinical remission and defined relapse as a requirement of induction therapy to reduce the risk of outcome misclassification.

The present study may reflect the current situation of thiopurine use in Japan, which reveals the relatively low

Table 4. Exploratory analyses for prediction of relapse

	Crude		Multivariable-adjusted	
	HR (95% CI)	*p* value	aHR (95% CI)	*p* value
Age (continuous)	0.99 (0.97–1.00)	<0.01	0.99 (0.98–1.00)	0.17
Female	1.11 (0.78–1.58)	0.57	0.96 (0.66–1.39)	0.83
Current smoking	0.67 (0.33–1.38)	0.28	0.64 (0.32–1.32)	0.23
Disease extension				
Extensive	Reference	–	Reference	–
Left sided	0.97 (0.64–1.47)	0.89	1.06 (0.69–1.62)	0.79
Others	0.50 (0.07–3.60)	0.49	0.41 (0.06–3.01)	0.38
Concomitant use of 5-ASA	0.92 (0.53–1.60)	0.76	0.95 (0.54–1.67)	0.85
Dose of thiopurine[a]				
Low	Reference	–	Reference	–
Medium	1.05 (0.69–1.61)	0.81	1.16 (0.75–1.79)	0.51
High	0.81 (0.48–1.35)	0.42	0.86 (0.51–1.46)	0.58
Duration of thiopurine				
≥2 years	Reference	–	Reference	–
1–2 years	1.56 (0.97–2.51)	0.07	1.34 (0.83–2.18)	0.24
<1 year	3.02 (1.96–4.66)	<0.01	2.54 (1.62–4.00)	<0.01
MCV				
≥100 fL	Reference	–	Reference	–
90–100 fL	1.05 (0.63–1.75)	0.84	1.08 (0.64–1.81)	0.79
<90 fL	2.18 (1.29–3.68)	<0.01	2.08 (1.15–3.75)	0.02

Cox regression models were used to identify the predictors for relapse as the exploratory analyses. In the multivariable-adjusted analyses, baseline WBC counts, age, sex, smoking status, disease extension, concomitant use of 5-ASA, dose of thiopurine, duration of thiopurine use, and MCV were used as covariates. [a]Thiopurine doses are categorized into three groups: low, AZA ≤50 mg or 6-MP ≤30 mg; medium, 50< AZA <100 mg or 30< 6-MP <60 mg; and high, AZA ≥100 mg or 6-MP ≥60 mg. 5-ASA, 5-aminosalicylic acid; 6-MP, 6-mercaptopurine; aHR, adjusted hazard ratio; AZA, azathioprine; CI, confidence interval; HR, hazard ratio; MCV, mean corpuscular volume.

dosage compared to the standard dose in Western countries (2–2.5 mg/kg/day of AZA) [3]. More than half of the patients in the present study were treated with thiopurine at a low dosage (AZA ≤50 mg/day or 6-MP ≤30 mg/day) in any of the WBC groups. Meanwhile, only 10–20% of patients were treated at high dosage (AZA ≥100 mg/day or 6-MP ≥60 mg/day). The dose of thiopurine in Japan was low, even though a relatively high prevalence of p.Arg139Cys SNP in *NUDT15* is considered. Nevertheless, the previous studies confirmed the efficacy of low-dose thiopurines in Japanese UC patients [22, 26]. One possible explanation for this finding is the low activity of thiopurine S-methyltransferase in Japanese patients [27] and high prevalence of *NUDT15* polymorphisms. In fact, Komiyama et al. [28] found that the mean 6-TGN concentration was within the therapeutic range (342.3 pmol/8 × 10^8 red blood cells) under low-dose thiopurine treatment (mean dose, 29.8 mg/day as 6-MP) among 134 Japanese IBD patients. In another study, Andoh et al. [29] investigated the relationship between the thiopurine dose and the 6-TGN level in 83 Japanese IBD patients and revealed that the mean 6-TGN concentration was above therapeutic threshold although most of the patients were on low-dose thiopurines. In a nationwide Japanese randomized controlled trial to investigate the efficacy of thiopurine with adalimumab for CD, mean 6-TGN concentration was above therapeutic threshold although more than half of the patients used low-dose thiopurines [30]. These findings suggest that low-dose thiopurines may be sufficient for Japanese patients. Unfortunately, we did not measure 6-TGN levels in this study, and further studies are warranted to investigate the relationship among WBC counts, 6-TGN level, and future relapse. Furthermore, the mechanism of therapeutic efficacy with low-dose thiopurine for Japanese patients is to be explored in the future study.

In the present study, the proportion of relapse within 3 years was 17.2% (125/723) and the relapse rate was 6.53 per 100 person-years, which was observed to be lower than that in previous studies [31]. One of the possible

reasons is that relapse was defined as a requirement of induction therapy for UC with worsening of clinical symptoms, except for the addition of topical therapy. We focused on the drug efficacy of thiopurine to prevent relapse, which requires systemic immunosuppressive therapy (e.g., systemic corticosteroid, biologics), whereas the minor exacerbation controlled only by topical therapy or dose adjustment of thiopurine was not defined as relapse. This stringent criteria of relapse in this study might have reduced the absolute number of relapses.

In the exploratory analyses, the duration of thiopurine use less than 1 year and MCV less than 90 fL was identified as the possible predictive factor for relapse within 3 years. Since patients treated with molecular targeted therapy were excluded in this study, the long-term use of thiopurine meant that the patient was clinically stable for a long time under thiopurine monotherapy. This could be a plausible reason why UC patients with thiopurine use <1 year were more likely to relapse than those with thiopurine use ≥1 year. In regard to MCV, a systematic review of 15 studies concluded that although changes in MCV during thiopurine treatment may be an index reflecting the intracellular metabolism of thiopurine, whether MCV is useful as a prognostic factor of clinical remission or not is inconclusive because of the following reasons: the inconsistency of the study results, small sample sizes in most studies, and other concomitant immunosuppressive therapy [32]. Among 15 studies in this systematic review, multivariable-adjusted analysis in patients with UC was conducted only in five studies [33–37], with the largest patient number of 168 in the report by Kopylov et al. [37]. However, in this study, the adjusted covariates were only age and hematologic laboratory data (e.g., WBC, MCV, lymphocyte counts), which were selected based on p values in the univariable analysis; thus, the adjustment seemed to be insufficient. In the other four studies, the adjustment of covariates was also insufficient and the sample sizes were small (N <150). In our study, the covariates in the multivariable analysis were predefined based on the clinically more relevant factors, but not on p values [25] as we have explained in the Methods section. As far as we know, the number of patients in the present study was the largest among similar studies previously reported [32, 38, 39]. Our study overcame the limitations in the past studies and revealed that an MCV less than 90 fL was predictive of future relapse within 3 years.

The present study has several limitations. First, the remission at inclusion was not defined according to endoscopic findings, but only PRO-2. Thus, confounding by the endoscopic activity was not able to be adjusted. Second, the duration of thiopurine use at baseline varies among patients. However, we included the duration of thiopurine use as a covariate in multivariable analyses and adjusted this potential confounder. Third, the WBC counts before starting thiopurine as well as the changes of them due to thiopurine use were not available. Fourth, almost all the patients in this study were Japanese; therefore, the result should be interpreted in consideration of ethnicity. Fifth, the actual medication adherence of thiopurine which could be confounding could not be considered due to retrospective study design. However, regular prescription was confirmed in each case and patients who discontinued hospital visit for more than 6 months were treated as censored. Finally, the data on body weight and the 6-TGN level (a standard index in Western countries) were unavailable. In conclusion, this largest nationwide cohort study showed that the WBC counts are not predictive for future relapse in UC patients with thiopurine in Japan, where low-dose thiopurine is prevalent.

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Statement of Ethics

This study was conducted in accordance with the Declaration of Helsinki. This study protocol was reviewed and approved by the Research Ethics Committee of Kitasato University Kitasato Institute Hospital or the Ethics Committees at each of the participating sites. The detailed information about the affiliations of the Institutional Review Board and the approval numbers are provided in the supplementary material. Written informed consent was not required for this study in accordance with local or national guidelines.

Conflict of Interest Statement

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Author Contributions

H.K., H.Y., T.K., K.M., N.A., and S.K. conceived of and designed the study; H.K., K.M., S.K., K.T., T.F., K.T., J.U., S.S., Y.H., T.T., H.I., T.E., R.O., O.H., K.M., Y.Y., T.K., K.Y., K.O., K.A., T.K., K.M., S.N., T.N., M.M., T.T., S.O., N.N., T.K., T.Y., K.K., T.S., and N.H. acquired the data; H.K., H.Y., and T.K. performed statistical analyses, interpreted the data, and drafted the manuscript; all authors performed critical revision of the manuscript for important intellectual content; H.K., H.Y., T.K., A.A., and T.H. supervised this study.

Data availability statement

All data generated or analyzed during this study are included in this article and its supplementary material files. Further inquiries can be directed to the corresponding author.

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