Title

Reevaluation of cardiovascular risk factors for thrombotic events in 580 Japanese patients with essential thrombocythemia

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This study was approved by the Ethics Committee of the School of Medicine, Juntendo University (IRB#M12-0895) and was conducted in accordance with the 1975 Helsinki Declaration.

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Written informed consent was obtained prior to the use of patient samples and the collection of clinical records.

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CF, YH, SM, and NK planned this study, CF, YH, SM, and NK performed research and wrote the manuscript, SS, TI, YE, TO, MA, and MA supervised the study and revised the manuscript.

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Title page abstract

•The impact of cardiovascular risk factors on thrombosis in essential thrombocythemia was analyzed.

•Hypertriglyceridemia is an independent risk factor for thrombosis.

•The risk of thrombosis is higher in patients with multiple cardiovascular risk factors.

•Thrombosis in essential thrombocythemia may be prevented by lifestyle interventions, including the control of cardiovascular risk factors.

Abstract

Risk-adapted therapy is recommended to prevent thrombosis in essential thrombocythemia (ET) patients. An advanced age, a history of thrombosis, and the presence of the *JAK2*V617F mutation are well-defined risk factors for thrombosis in ET; however, the impact of cardiovascular risk (CVR) factors on thrombosis in ET remains elusive. Therefore, we herein investigated the impact of CVR factors on thrombosis in 580 ET patients who met the 2017 World Health Organization Classification diagnostic criteria. A univariate analysis identified hypertriglyceridemia and multiple CVR factors as strong risk factors for thrombosis (hazard ratio [HR]: 3.530, 95% confidence interval [CI] =1.630-7.643, P = 0.001 and HR: 3.368, 95% CI = 1.284-8.833, P = 0.014, respectively) and hyper-LDL cholesterolemia as a potential risk factor (HR: 2.191, 95% CI = 0.966-4.971, P = 0.061). A multivariate analysis revealed that hypertriglyceridemia was an independent risk factor for thrombosis (HR: 3.364, 95% CI =1.541-7.346, P = 0.002). Furthermore, poor thrombosis-free survival was observed in patients with a serum triglyceride level ≥1.2 mmol/L (HR=2.592, P = 0.026 vs. <1.2 mmol/L) or two or more CVR factors (P = 0.011 vs. no CVR factors and P=0.005 vs. one CVR factor). These results revealed the impact of CVR factors on thrombosis in ET. Since CVR factors are manageable, lifestyle interventions, such as the control of serum triglyceride levels, may effectively prevent thrombosis in ET patients.

Introduction

Essential thrombocythemia (ET) is a subtype of Philadelphia chromosome-negative myeloproliferative neoplasms (MPNs) and is characterized by thrombocytosis with the presence of megakaryocytic hyperplasia in bone marrow. Since thrombosis is one of the major and critical complications in ET, the risk stratification of thrombosis based on risk factors is beneficial for the selection of individualized treatment plans. To date, three major thrombotic risk scoring systems have been used in clinical practice: the conventional thrombosis risk classification[1], the International Prognostic Score for Essential Thrombocythemia (IPSET)-thrombosis[2], and the revised IPSET-thrombosis[3,4]. All three systems use an advanced age and a history of thrombosis, while the latter two also use the presence of the *JAK2*V617F mutation, which is one of causal factors for ET[5], as risk factors for thrombosis[6-10].

Hypertension (HT), diabetes mellitus (DM), hyperlipidemia (HL), and smoking are risk factors for cerebro-cardiovascular diseases in healthy subjects[11-14]. The relationships among thrombosis and CVR factors have been actively investigated in ET patients (Supplemental Table 1)[2,4,9,15-28]; however, the impact of CVR factors remains controversial. Cortelazzo et al. reported that none of the CVR factors (HT, DM, HL, and smoking) examined were associated with thrombosis in 100 patients with ET[15]; however, Besses et al. subsequently demonstrated that hypercholesterolemia was associated with macrovascular complications[18]. Furthermore, based on the findings of a large international study[2], CVR factors were incorporated into the risk scoring system, IPSET-thrombosis. Although the accuracy of this scoring system was confirmed in a follow-up study with a large number of ET cases[23], two major

MPN research groups showed that the impact of CVR factors on thrombosis was smaller than originally expected[3,4]; therefore, CVR factors were omitted from the newly proposed revised IPSET-thrombosis. Since it currently remains unclear whether CVR factors are risk factors for thrombosis in ET[2-4], we herein reevaluated the impact of CVR factors on thrombosis in ET in a large Japanese cohort.

Materials and methods

Patients

We enrolled 580 patients diagnosed with ET according to World Health Organization (WHO) Classification 2017 diagnostic criteria[29,30]. Data were retrospectively collected at Juntendo University Hospital between June 17, 1993, and December 2, 2020. Some patients with high hemoglobin (Hb) levels (>165 g/L in males and >160 g/L in females) were suspected of having polycythemia vera (PV); however, the results of bone marrow biopsy were not compatible with PV due to the absence of increased erythropoiesis and, thus, these patients were diagnosed with ET. The following clinical parameters and events were included in the analysis: date of diagnosis, age, sex, the presence of driver gene mutations, the white blood cell (WBC) count, Hb level, the hematocrit (Hct), platelet (Plt) count, low-density lipoprotein cholesterol (LDL-C) level, triglyceride (TG) level, the presence of CVR factors (HT, DM, hyper-LDL cholesterolemia, hypertriglyceridemia, and smoking), a history of thrombosis, and thrombotic events after diagnosis. A driver gene mutation analysis was performed as previously reported[31,32]. The present study was conducted in accordance with the Declaration of Helsinki and was approved by the Ethics Committee of Juntendo University (IRB #M12-0895).

Definition of clinical conditions

HT and HL were defined according to previous guidelines[33]. Briefly, HT was defined as blood pressure at rest higher than 140/90 mmHg. HL consisted of hyper-LDL cholesterolemia and hypertriglyceridemia (defined as a LDL-C level ≥3.6 mmol/L and TG level ≥1.7 mmol/L, respectively). The risk classification of thrombosis was estimated according to the conventional risk classification[1], IPSET-thrombosis[2], and revised IPSET-thrombosis[3,4]. Thrombotic events in the present study included cerebral infarction, transient ischemic attack, myocardial infarction, angina pectoris, peripheral arterial occlusion, pulmonary embolism, deep vein thrombosis, and other life-threatening thrombotic events. Thrombosis-free survival (TFS) was calculated as the time from the day of diagnosis to the onset of arterial and venous thrombosis. The discontinuation of patient visits and death were considered to be censored events.

Statistical analysis

The Kaplan-Meier method was used to analyze TFS, and the Log-rank test to compare TFS curves. To identify risk factors for thrombotic events, univariate and multivariate analyses using the Cox proportional hazards regression were performed after selecting clinically important variables based on previous studies.

Two-tailed tests were used for all statistical analyses of valid variables, with P <0.05 considered to be significant. EZR version 1.55 (Jichi Medical University, Saitama Medical Center, Japan) was used for statistical analyses[34]. EZR is a graphical user interface for R (The R Foundation for Statical Computing, Vienna, Austria). More precisely, it is a modified version of R commander designed to add statistical functions frequently used in biostatistics.

Results

Patient characteristics

We analyzed data obtained from 580 ET patients who met the diagnostic criteria of the WHO classification 2017 (Table 1). The number of patients in each thrombosis risk category at diagnosis is shown in Supplemental Table 2. The median observation period was 3.6 years (range, 0-27.9 years), and thrombosis occurred after diagnosis in 6.4% of patients (n = 37, 1.3/100 patient years), including arterial (n = 31, 84%) and venous (n = 6, 16%) events (Supplemental Table 3). Although available data were limited, no significant differences were observed in the incidence of thrombotic events between patients treated with (27 out of 73 patients, 37.0%) and without (10 out of 34 patients, 29.4%) cytoreductive therapy (CRT) (P = 0.516, by Fisher's exact test) or those treated with (19 out of 35 patients, 54.3%) and without (18 out of 36 patients, 50.0%) antiplatelet therapy (P = 0.814, by Fisher's exact test). Five- and 10-year TFS rates in the entire cohort were 94.3% (95% confidence interval [CI]: 91.4-96.3%) and 89.9% (95% CI: 84.7-93.3%), respectively (Figure 1a). TFS rates significantly differed among the four groups defined by the driver mutation status, and the *JAK2*V617F mutation group showed the shortest TFS, whereas the triple-negative group had only two thrombotic events in the observation period (Figure 1b). Similar to previous studies by Western groups[1-4], Japanese ET patients were accurately stratified based on the conventional (P = 0.048, Figure 1c), IPSET-thrombosis (P = 0.007, Figure 1d), and revised IPSET-thrombosis risk scoring systems (P = 0.035, Figure 1e).

Strong impact of hypertriglyceridemia on thrombosis in ET

To establish whether CVR is a risk factor for thrombosis in ET, we initially performed a univariate analysis of TFS. Hypertriglyceridemia was identified as a strong risk factor for thrombosis (hazard ratio [HR]: 3.530, 95% CI =1.630-7.643, P = 0.001) and hyper-LDL cholesterolemia as a potential risk factor (HR: 2.191, 95% CI = 0.966-4.971, P = 0.061) (Table 2). Consistent with previous findings[1-4], the presence of the *JAK2*V617F mutation (HR: 2.110, 95% CI =1.042-4.272, P = 0.038) and an advanced age (≥60 years) (HR: 2.002, 95% CI = 0.961-4.170, P = 0.064) were associated with thrombotic events. The univariate analysis revealed that none of the CVR factors had an impact on TFS or overall survival (Table 2 and Supplemental Table 4), whereas hypertriglyceridemia had a significant effect on TFS (P = 0.001). Therefore, we focused on each CVR factor (HT, DM, hyper-LDL cholesterolemia, hypertriglyceridemia, and smoking) and performed a multivariate analysis of five models incorporating an advanced age (≥ 60 years) and positivity

for the *JAK2*V617F mutation, which are known risk factors for thrombosis (Table 3). We found that hypertriglyceridemia remained an independent risk factor for thrombosis (HR: 3.364, 95% CI =1.541-7.346, P = 0.002) and hyper-LDL cholesterolemia was a potential risk factor (HR: 2.046, 95% CI = 0.895-4.676, P = 0.090). An additional analysis that focused on TG levels revealed a significantly worse TFS rate in patients with a TG level ≥1.2 mmol/L than in those with a TG level <1.2 mmol/L (HR: 2.592, P = 0.026, Figure 2, Supplemental Table 5), suggesting the impact of hypertriglyceridemia on thrombotic events in ET.

To identify risk factors for thrombosis, we divided 308 patients for whom all five CVR factors were fully available (Supplemental Table 6) into low- and high-risk groups according to the conventional thrombosis risk classification. Although no significant factors were identified in the low-risk group by the multivariate analysis, presumably due to the small number of patients with thrombosis, HRs for the *JAK2*V617F mutation and hypertriglyceridemia were high (HR: 2.672, 95% CI = 0.447-15.970, P = 0.282 and HR: 2.050, 95% CI = 0.337-12.480, P = 0.436, respectively; Supplemental Table 6A). On the other hand, hypertriglyceridemia was the only significant risk factor among CVR factors in the high-risk group (HR: 3.620, 95% CI = 1.455-9.005, P = 0.006; Supplemental Table 6B). Collectively, these results indicated a relationship between hypertriglyceridemia and thrombosis in ET.

Multiple CVR factors increased the thrombotic risk in ET patients

To further investigate the impact of CVR factors on thrombosis, we examined the effect of the CVR load on thrombosis in ET. ET patients (n = 308) with complete data on all five CVR factors were divided into three groups: 127 patients (41.2%) with no CVR factors (CVR0), 100 (32.5%) with only one CVR factor (CVR1), and 81 (26.3%) with two or more CVR factors (CVR ≥2). The frequency of thrombotic events increased with the number of CVR factors, and HR for CVR ≥2 was significantly higher in the univariate analysis (HR: 3.368, 95% CI = 1.284-8.833, P = 0.014) when CVR0 was used as a reference (Table 4). Patients with CVR ≥2 had the shortest TFS (P = 0.011 vs. CVR0 and 0.005 vs. CVR1; Figures 3a and 3b), whereas no significant differences were observed between the CVR0 and CVR1 groups (P = 0.961, Figure 3c).

To confirm the impact of CVR factors on thrombotic events, we compared the risk classification of 480 ET patients evaluable by both the IPSET-thrombosis scoring system, which considers CVR factors, and the revised IPSET-thrombosis scoring system, which does not. Fifteen patients who were differently stratified between the systems were classified as low by revised IPSET-thrombosis and high by IPSET-thrombosis (Supplemental Table 7). Among these patients, two (13.3%) developed thrombosis and both had hypertriglyceridemia and multiple CVR factors (hypertriglyceridemia plus hyper-LDL cholesterolemia [n = 1] and hypertriglyceridemia plus HT [n = 1]). These results indicate that the thrombotic risk increased in patients with hypertriglyceridemia or multiple CVR factors and that the thrombotic risk classification without considering CVR factors may overlook patients who potentially are at a higher risk of thrombosis.

Discussion

By evaluating the impact of CVR factors on thrombosis in a large ET cohort, we herein demonstrated that: 1) hypertriglyceridemia is an independent risk factor for thrombosis, and 2) the risk of thrombosis is markedly higher in patients with multiple CVR factors. The present results confirmed that CVR factors are a valuable component for the risk stratification of thrombosis. Since the treatment of HT, DM, and hyper-LDL cholesterolemia as well as the cessation of smoking are prioritized in the management and prevention of cerebro-cardiovascular diseases[12,33,35,36], additional interventions for hypertriglyceridemia may also effectively prevent thrombosis in ET with hypertriglyceridemia.

High TG levels were identified as a thrombotic risk factor for stroke in an 18-year prospective study on 9087 Japanese individuals aged between 40 and 69 years[37]. Concomitant with this finding, cohort analyses revealed that hypertriglyceridemia may cause thrombosis in non-ET individuals of several ethnicities[38,39]. *In vitro* analyses of blood samples from patients with CVR factors showed that hypertriglyceridemia induced Plt activation and shortened the time to thrombus formation[40]. These findings strongly support our hypothesis that hypertriglyceridemia is an independent risk factor for thrombosis. In addition, we found that TFS was significantly shorter in ET patients with a TG level ≥1.2 mmol/L (Figure 2). Even though the present results were retrospectively obtained from a single ethnic cohort at one center and there are currently no large cohort analyses of the relationship between hypertriglyceridemia and thrombosis in ET patients of other ethnicities, this study implies that target TG levels need to be more strictly controlled in ET patients than in non-ET patients. Diet may affect TG levels; however, the influence of diet was considered to be minimal in the present study for the following reasons: 1) it was unlikely that non-fasting patients were biased toward either the thrombosed or nonthrombosed group that was analyzed; and 2) TG levels have been associated with a risk of ischemic cardiovascular disease regardless of the fasting status[41].

In the present study, no significant differences were observed in thrombotic events between CVR0 and CVR1 (Figure 3c); however, the risk of thrombosis was significantly higher with CVR ≥2 than with CVR0 and CVR1 (Figures 3a and 3b). These results suggest that the risk of thrombosis in patients with multiple CVR factors is overlooked or underestimated by the revised IPSET-thrombosis scoring system, which does not adopt CVR factors (Supplemental Table 7). Therefore, the original IPSET-thrombosis scoring system[2], which includes CVR factors and the *JAK2*V617F mutation, is more suitable for Japanese ET patients than the revised IPSET-thrombosis scoring system[3,4].

The main limitation of the present study was its retrospective nature. CVR factors and treatment information, including CRT, antiplatelet therapy, and the treatment of CVR factors with lipid-lowering medications, were not recorded in all subjects. Furthermore, the number of thrombotic events was small, presumably due to the short median follow-up of 3.6 years. In addition, the present study was solely conducted on Japanese patients with ET; therefore, it currently remains unclear whether the results obtained are applicable to other ethnic groups. Nevertheless, hypertriglyceridemia and multiple CVR factors were

extracted as risk factors for thrombosis.

In conclusion, we identified hypertriglyceridemia as a thrombotic risk factor in Japanese ET patients. We also showed that ET patients with multiple CVR factors were at a higher risk of thrombosis. Since the majority of CVR factors are manageable, thrombosis in ET may be prevented by lifestyle interventions, including the control of serum TG levels.

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Table 1 Patient backgrounds

Characteristics at diagnosis	Number of patients or value	Evaluable patients
Age, median (range)	62 (8-93)	580
Female, n (%)	337 (58.5)	576
Male, n (%)	239 (41.5)	576
WBC, median; $\times 10^9$/L (range)	8.9 (3.2-28.8)	579
Hb, median; g/L (range)	138 (78-182)	580
Hct, median; % (range)	42.0 (22.9-57.9)	576
Plt, median; $\times 10^9$/L (range)	824 (450-3817)	580
*JAK2*V617F mutation, n (%)	299 (51.6)	580
CALR mutation, n (%)	145 (25.0)	580
Type 1, n (%)	98[a] (16.9)	580
Type 2, n (%)	47 (8.1)	580
MPL mutation, n (%)	23 (4.0)	580
W515K, n (%)	9 (1.6)	580
W515L, n (%)	11 (1.9)	580
Others, n (%)	3[a] (0.5)	580
Triple-negative, n (%)	114 (19.7)	580
History of thrombosis, n (%)	82 (14.1)	568
Cardiovascular risk factors[b], n (%)	181 (58.8)	308
Hypertension, n (%)	99 (30.2)	328
Diabetes mellitus, n (%)	31 (9.5)	327
Hyper-LDL cholesterolemia, n (%)	69 (23.5)	294
LDL level, median; mmol/L (range)	2.7 (1.1-5.9)	292
Hypertriglyceridemia, n (%)	76 (24.5)	310
Triglyceride level, median; mmol/L (range)	1.2 (0.3-5.0)	310
Smoking, n (%)	31 (9.7)	319

LDL, low-density lipoprotein

[a] One patient harbored *CALR* and *MPL*S505N mutations

[b] Cardiovascular risk factors were defined as cases with at least one of hypertension, diabetes mellitus, hyper-LDL cholesterolemia, hypertriglyceridemia, and/or current smoking

Table 2 Univariable and multivariable analyses of predictors of thrombosis-free survival

	Thrombotic	Univariable			Multivariable		
Variables	events (n, %)	HR	95% CI	P value	HR	95% CI	P value
Age ≥ 60 years	25, 8.1%	2.002	0.961-4.170	<u>0.064</u>	1.470	0.619-3.494	0.3831
Sex (male)	16, 6.7%	1.091	0.565-2.105	0.796			
WBC ≥11×10^9/L	6, 4.1%	0.613	0.255-1.469	0.272			
Plt ≥1500×10^9/L	All patients who developed thrombosis had a Plt count of 1500×10^9/L or lower						
Plt ≥1000×10^9/L	12, 6.9%	1.053	0.528-2.100	0.883			
*JAK2*V617F mutation	26, 8.7%	2.110	1.042-4.272	**0.038**	1.674	0.634-4.418	0.298
CALR mutation	5, 3.4%	0.448	0.175-1.151	0.095			
MPL mutation	4, 17.4%	2.053	0.722-5.835	0.177			
History of thrombosis	8, 9.8%	1.578	0.721-3.456	0.254	1.223	0.536-2.793	0.632
Cardiovascular risk factors	20, 11.0%	1.919	0.764-4.822	0.166	1.982	0.823-4.769	0.127
Hypertension	12, 12.1%	1.798	0.830-3.895	0.137			
Diabetes mellitus	4, 12.9%	2.175	0.745-6.351	0.155			
Hyper-LDL cholesterolemia	9, 13.0%	2.191	0.966-4.971	<u>0.061</u>			
Hypertriglyceridemia	14, 18.4%	3.530	1.630-7.643	**0.001**			
Smoking	4, 12.9%	1.370	0.469-3.999	0.565			

CI, confidence interval; *HR*, hazard ratio; *LDL*, low-density lipoprotein

P values <0.05 are highlighted in bold

P values ≥0.05 and <0.1 are underlined

Table 3 Multivariable analysis of predictors of thrombosis-free survival including individual cardiovascular risk factors

Variables	HR	95% CI	P value
Model 1			
Age ≥60 years	1.558	0.646-3.760	0.324
*JAK2*V617F mutation	1.357	0.592-3.113	0.471
Hypertension	1.495	0.656-3.409	0.339
Model 2			
Age ≥60 years	1.698	0.731-3.946	0.218
*JAK2*V617F mutation	1.302	0.563-3.009	0.537
Diabetes mellitus	2.000	0.671-5.960	0.214
Model 3			
Age ≥60 years	1.687	0.724-3.931	0.226
*JAK2*V617F mutation	1.301	0.543-3.114	0.555
Hyper-LDL cholesterolemia	2.046	0.895-4.676	<u>0.090</u>
Model 4			
Age ≥60 years	1.476	0.636-3.429	0.365
*JAK2*V617F mutation	1.243	0.539-2.867	0.610
Hypertriglyceridemia	3.364	1.541-7.346	**0.002**
Model 5			
Age ≥60 years	1.835	0.803-4.970	0.150
*JAK2*V617F mutation	1.511	0.657-3.476	0.332
Smoking	1.640	0.550-4.893	0.375

CI, confidence interval; *HR*, hazard ratio; *LDL*, low-density lipoprotein

P values <0.05 are highlighted in bold

P values ≥0.05 and <0.1 are underlined

Table 4 Correlations between thrombotic events and the number of cardiovascular risk factors

Number of cardiovascular risk factors	Number of patients (%)	Number of thrombotic events (%)	Univariable		
			HR	95% CI	P value
0	127 (41.2)	6 (4.7)	1.000 (Reference)		
1	100 (32.5)	6 (6.0)	0.947	0.302-2.966	0.925
≥2	81 (26.3)	14 (17.3)	3.368	1.284-8.833	**0.014**

CI, confidence interval; *HR*, hazard ratio

P values <0.05 are highlighted in bold

Fig.1

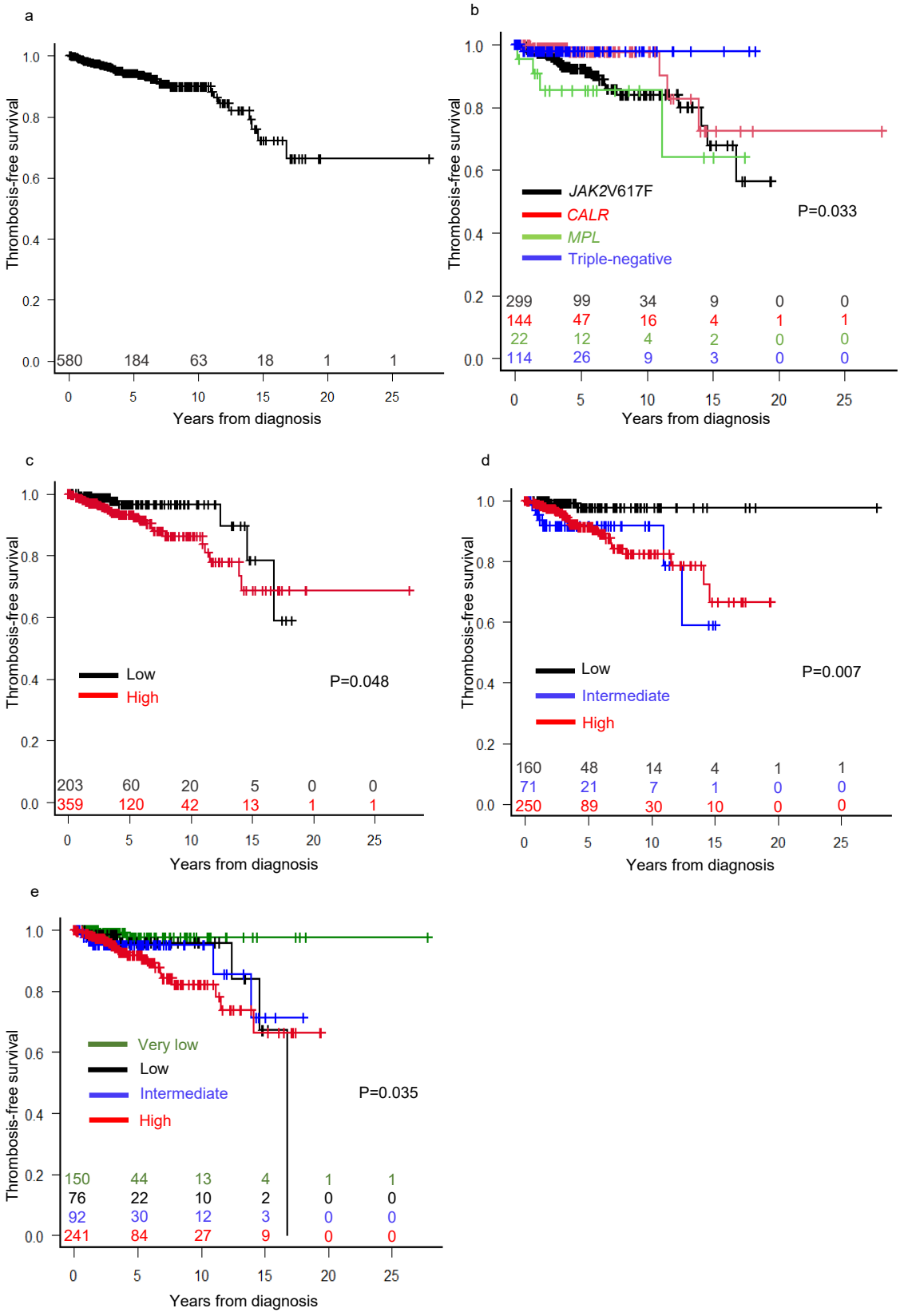
a
Thrombosis-free survival
1.0
0.8
0.6
0.4
0.2
0.0
580 184 63 18 1 1
0 5 10 15 20 25
Years from diagnosis
b
Thrombosis-free survival
JAK2V617F
CALR
MPL
Triple-negative
P=0.033
299 99 34 9 0 0
144 47 16 4 1 1
22 12 4 2 0 0
114 26 9 3 0 0
0 5 10 15 20 25
Years from diagnosis
c
Thrombosis-free survival
Low
High
P=0.048
203 60 20 5 0 0
359 120 42 13 1 1
0 5 10 15 20 25
Years from diagnosis
d
Thrombosis-free survival
Low
Intermediate
High
P=0.007
160 48 14 4 1 1
71 21 7 1 0 0
250 89 30 10 0 0
0 5 10 15 20 25
Years from diagnosis
e
Thrombosis-free survival
Very low
Low
Intermediate
High
P=0.035
150 44 13 4 1 1
76 22 10 2 0 0
92 30 12 3 0 0
241 84 27 9 0 0
0 5 10 15 20 25
Years from diagnosis

Fig.2

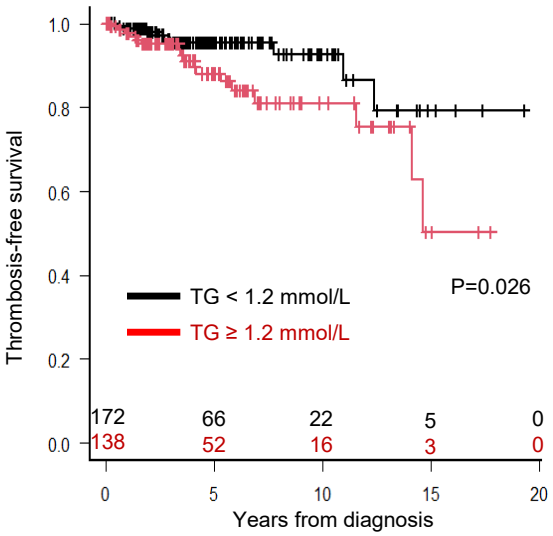
Thrombosis-free survival
1.0
0.8
0.6
0.4
0.2
0.0
TG < 1.2 mmol/L
TG ≥ 1.2 mmol/L
P=0.026
172 66 22 5 0
138 52 16 3 0
0 5 10 15 20
Years from diagnosis

Fig.3

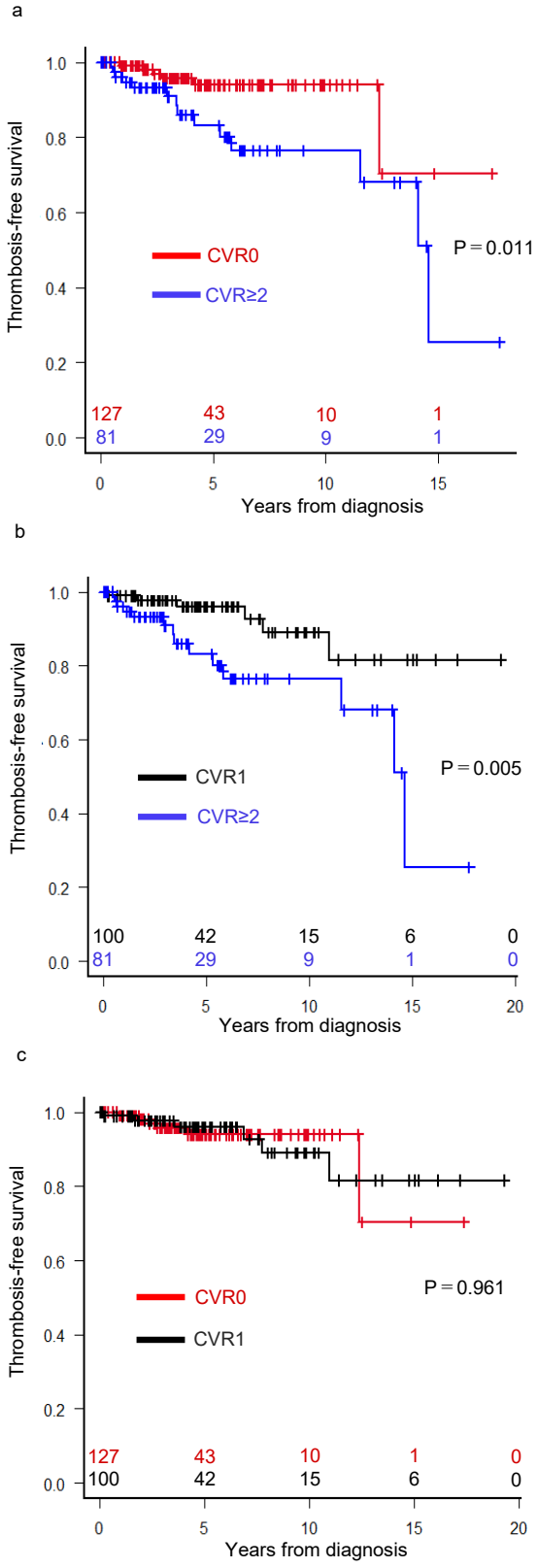
a
Thrombosis-free survival
1.0
0.8
0.6
0.4
0.2
0.0
CVR0
CVR≥2
P = 0.011
127
81
43
29
10
9
1
1
0
5
10
15
Years from diagnosis
b
Thrombosis-free survival
CVR1
CVR≥2
P = 0.005
100
81
42
29
15
9
6
1
0
0
0
5
10
15
20
Years from diagnosis
c
Thrombosis-free survival
CVR0
CVR1
P = 0.961
127
100
43
42
10
15
1
6
0
0
0
5
10
15
20
Years from diagnosis

Figure legends

Fig. 1

Kaplan-Meier curves showing thrombosis-free survival (TFS) in the entire cohort (a), and TFS among patients with a different driver mutation status: *JAK2*V617F (black), *CALR* exon 9 frameshift mutations (red), *MPL* S204P and W515L/K/A (green), and triple-negative (blue) (b); patients classified by the use of the conventional risk classification for thrombosis: low (black) and high (red) (c); patients classified by IPSET-thrombosis: low (black), intermediate (blue), and high (red) (d); and by revised IPSET-thrombosis: very low (green), low (black), intermediate (blue), and high (red) (e). $P < 0.05$ was defined as significant

Fig. 2

Kaplan-Meier curves showing thrombosis-free survival of patients with a triglyceride (TG) level ≥1.2 mmol/L (red) or <1.2 mmol/L (black). $P < 0.05$ was defined as significant

Fig. 3

Kaplan-Meier curves comparing the thrombosis-free survival of patients with multiple cardiovascular risk factors (CVR2, blue) versus those without CVR (CVR0, red) (a); CVR2 versus patients with one CVR (CVR1, black) (b); and CVR0 versus CVR1 (c). $P < 0.05$ was defined as significant