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Clinical features of essential thrombocythaemia with coexisting JAK2 V617F and CALR mutations: a multicentre retrospective study in China

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Abstract

JAK2 V617F, CALR and MPL mutations are treated as mutually exclusive in essential thrombocythaemia (ET), and testing usually stops once JAK2 V617F is found. Coexisting mutations cluster at a low JAK2 allele burden, but whether they define a distinct phenotype in Chinese patients is unresolved. We compared 24 Chinese patients with coexisting JAK2 V617F and CALR mutations, nine of them identified from published reports, with 110 patients with isolated JAK2 V617F and 21 with an isolated CALR mutation, and analysed phenotype, allele burdens, revised IPSET-thrombosis categories, treatment and transformation with permutation testing, Holm-Bonferroni correction and competing-risk analysis. Co-mutated patients had higher haemoglobin (12 g/L, 95% confidence interval 3–23) and more splenomegaly (55.0% versus 24.3%), both borderline after correction (adjusted P = .051 and .054), together with a lower JAK2 V617F burden (22.4% versus 27.0%; below 5% in 3 of 13 versus 3 of 65). Two thirds (66.7%) were classified as high risk because the revised International Prognostic Score of thrombosis counts JAK2 positivity alone. Thrombotic risk could not be assessed, since a thrombosis field was recorded for only six CALR-mutated controls. Transformation was more frequent in co-mutated patients in both analyses (12.5% and 20.0% versus 3.6%), but the significance was not stable (P = .111 and .038). Coexisting mutations therefore mark a phenotype that leans towards JAK2-mutated ET without a confirmed excess of thrombotic or transformative risk, and CALR exon 9 should be tested whenever JAK2 V617F is present.

Introduction

Essential thrombocythaemia (ET) is the commonest BCR::ABL1-negative myeloproliferative neoplasm, and its diagnosis rests on demonstrating clonality, which in practice means detecting a driver mutation [1–4]. JAK2 V617F, MPL and CALR exon 9 mutations are treated as mutually exclusive [5–7]. All three converge on JAK-STAT activation through MPL, which is why most laboratories stop testing once JAK2 V617F has been detected [8–10].

That framework is challenged at a low allele burden and by reports of coexisting mutations in ET [11]. Between 10% and 14% of patients with a JAK2 burden below 5% carried a CALR mutation [12], and the same gradient appears in Danish, Russian and Korean cohorts [9, 13, 14]. Single-cell sequencing places the two mutations in separate clones in some patients [15]. Because JAK2-mutated and CALR-mutated ET run different clinical courses [16], the first question is whether co-mutated patients follow the JAK2 phenotype, the CALR phenotype or one between the two. The second is whether revised IPSET-thrombosis still ranks thrombotic risk when the JAK2 clone is small, since the score records JAK2 status as present or absent [17, 18].

Chinese cohorts have described CALR mutations, the relation between JAK2 V617F burden and phenotype, and thrombotic risk in JAK2-mutated disease [19–21]. Coexisting mutations have not been examined in a controlled Chinese comparison, and paired allele burdens are rarely reported. We therefore combined four Chinese centres with a systematic literature search, giving 24 patients with coexisting

JAK2 V617F and CALR mutations and 131 single-mutation controls. The controls comprised 110 patients with isolated JAK2 V617F and 21 with isolated CALR mutation. We assessed clinical phenotype, paired allele burdens, revised IPSET categories, treatment response and transformation, with correction for multiple comparisons and competing-risk analysis. Because nine of the co-mutated patients came from published reports, every primary comparison was also run in the institutional subgroup.

Materials and methods

Study design and participants

This retrospective multicentre study compared patients with ET and one driver mutation with those with coexisting JAK2 V617F and CALR mutations (co-mutated) and with published cases. Consecutive patients seen between 1 January 2018 and 31 December 2025 were screened at four centres (Online Resource 1, Methods S2 and Table S2). Eligibility required age 18 years or older, WHO 2022 and ICC 2022 criteria [3, 4], three-gene testing and baseline data. Exclusions were BCR::ABL1-positive disease, reactive thrombocytosis, other myeloid neoplasms and missing data. The analysis set was 155 patients: 24 co-mutated (15 institutional, nine published), 110 with isolated JAK2 V617F and 21 with isolated CALR mutation (Fig. 1). All five primary comparisons were repeated in institutional patients only (Table 4).

Literature search and case selection

Two investigators independently searched CNKI, VIP, Wanfang Data and SinoMed from inception to 31 December 2025 using disease, gene and coexistence terms (Online Resource 1, Methods S1 and Table S1). Reports with confirmed co-positivity and extractable data were included; reviews, case-free abstracts, non-ET phenotypes and duplicates were excluded. Disagreements were resolved by a third investigator.

Data collection, definitions and molecular testing

Two investigators independently abstracted the variables in Tables 1 to 3.

Table 1
Baseline clinical and laboratory characteristics of the three groups.

Variable	Co-mutated (n = 24)	Isolated JAK2 V617F (n = 110)	Isolated CALR mutation (n = 21)	Statistic and effect size	P (BH q)
Age, years	62.5 (51.0, 67.8)	65.0 (43.0, 76.0)	62.0 (50.0, 71.0)	$H = 0.789$; $\varepsilon^2 = 0.005$	.680
Male sex, n (%)	14 (58.3)	47 (42.7)	10 (47.6)	$\chi^2 = 1.855$; $V = 0.11$	.407
Age 60 years or older, n (%)	16 (66.7)	67 (61.5)	11 (52.4)	$\chi^2 = 0.990$; $V = 0.08$	.604
Leucocyte count, $\times 10^9$/L	9.7 (7.6, 12.2)	8.9 (6.6, 12.0)	7.9 (6.6, 11.0)	$H = 1.117$; $\varepsilon^2 = 0.007$	.583
Haemoglobin, g/L	141.0 (130.3, 155.0)	130.0 (112.0, 144.0)	135.0 (121.0, 145.0)	$H = 6.175$; $\varepsilon^2 = 0.040$	.045 (.147)
Platelet count, $\times 10^9$/L	881.5 (653.8, 1102.3)	735.0 (576.0, 906.0)	878.0 (702.0, 1075.0)	$H = 5.658$; $\varepsilon^2 = 0.037$	.055 (.147)
Splenomegaly, n/N (%)	11/20 (55.0)	26/107 (24.3)	6/20 (30.0)	$\chi^2 = 7.681$; $V = 0.23$	.022 (.115)
Thrombosis, n/N (%)	8/24 (33.3)	21/110 (19.1)	0/6 (0)	$\chi^2 = 4.011$; $V = 0.17$	.130 (.298)
Bleeding, n (%)	0 (0)	2 (1.8)	0 (0)	$\chi^2 = 0.837$; $V = 0.07$	1.000
Follow-up, months	32.0 (23.0, 45.0) (n = 21)	17.0 (9.0, 37.0) (n = 65)	15.0 (11.5, 31.3) (n = 14)	$H = 2.983$; $\varepsilon^2 = 0.030$	.225

Note: continuous variables are median (P25, P75), compared by the Kruskal-Wallis test with ε^2 reported; categorical variables are n (%), with the denominator shown for each variable, compared by the χ^2 test with Cramér's V (V). P values are from Monte Carlo permutation tests; q values in parentheses are Benjamini-Hochberg adjusted values for the exploratory family of 16 comparisons. Holm-adjusted P values for the primary comparisons (co-mutated versus isolated JAK2 V617F) were .051 (haemoglobin), .054 (splenomegaly), .167 (platelets), .221 (thrombosis) and .221 (transformation), obtained by applying the Holm-Bonferroni correction to the unrounded raw P values rather than to the rounded values printed in the tables. Values in parentheses give the number of patients with a recorded value. One patient with isolated JAK2 V617F lacked age and baseline blood counts, so continuous variables and the age category for that group rest on 109 patients, whereas the remaining categorical variables use the full group of 110.

Table 2
Molecular features.

Variable	Co-mutated	Isolated JAK2 V617F	Isolated CALR mutation	Effect size (95% CI)	P
JAK2 V617F burden, %	22.4 (6.2–33.5) (n = 13)	27.0 (18.4–42.7) (n = 65)	—	median difference − 9.4 (− 20.4 to 0.2); r = 0.33	.061
CALR burden, %	32.2 (14.6–38.9) (n = 14)	—	22.9 (20.1–26.8) (n = 12)	median difference + 6.9 (− 7.8 to 16.8); r = − 0.26	.276
JAK2 V617F burden below 5%, n/N (%)	3/13 (23.1)	3/65 (4.6)	—	risk difference 18.5 pp (1.5–45.8)	.055
CALR subtype, n	type 1/type 1-like 14; type 2/type 2-like 6; other 4; unclassified 0	—	type 1 7; type 2 3; other 2; unclassified 9	V = 0.54 all cases; 0.00 classified cases	.003 all cases; 1.000 classified cases
Additional variants present, n/N (%)	6/16 (37.5)	30/110 (27.3)	4/21 (19.0)	V = 0.10	.503
Two or more additional variants, n (%)	0 (0)	19 (17.3)	0 (0)	V = 0.22	.007

Note: values in parentheses give the number of patients with an evaluable value. Allele burdens were missing in many patients, so the comparisons include only those with a recorded value. The analysis of additional variants includes only patients who underwent targeted panel testing; eight co-mutated patients were not tested. The CALR subtype comparison was computed twice, first with unclassified results counted as a category and then restricted to patients with a classified variant, because nine of the 21 CALR-mutated controls were reported only as positive and every co-mutated patient was classified. P values are Monte Carlo permutation values (5,000 replicates, fixed seed), except where Fisher's exact P is indicated. pp, percentage points.

Table 3
Risk stratification, treatment and disease outcome.

Variable	Co-mutated (n = 24)	Isolated JAK2 V617F (n = 110)	Isolated CALR mutation (n = 21)	Effect size	P (adjusted)
High-risk revised IPSET-thrombosis, n (%)	16 (66.7)	70 (63.6)	0 (0)	V = 0.71	.0002 (q = .003)
Hydroxycarbamide plus interferon, n (%)	6 (25.0)	9 (8.2)	1 (4.8)	V = 0.18	.054 (q = .147)
Platelets 450 × 10⁹/L or below at 3 months (recorded values), n/N (%)	7/14 (50.0)	53/94 (56.4)	16/21 (76.2)	—	.198
Same endpoint, missing values counted as not achieved, n/N	7/24	53/110	16/21	—	.006
Platelets 450 × 10⁹/L or below at last follow-up (recorded values), n/N (%)	11/19 (57.9)	40/82 (48.8)	4/15 (26.7)	—	.171
Disease transformation, n (%)	3 (12.5)	4 (3.6)	1 (4.8)	V = 0.14	.253 (q = .404)
36-month cumulative incidence of transformation (competing risk), % (95% CI)	0.0 (0.0–0.0)	10.3 (1.7–21.7)	10.0 (0.0-33.3)	—	.600

Note: IPSET, International Prognostic Score of thrombosis in WHO-essential thrombocythaemia. P values are Monte Carlo permutation values (5,000 replicates, fixed seed), except where Fisher's exact P is indicated, and the adjusted values are Holm-Bonferroni for the five prespecified primary comparisons with Benjamini-Hochberg q values for the exploratory comparisons. The IPSET distribution cannot include the very-low or intermediate categories in either JAK2-positive group, because JAK2 mutation status is a component of the score. The 3-month platelet endpoint was available in a subset of patients, and the sensitivity analyses given in the Results treat missing values first as failure and then at the extreme bounds.

Table 4
Prespecified bias analysis: the five primary comparisons in the pooled and institutional-only co-mutated groups.

Comparison	Pooled (co-mutated n = 24 versus isolated JAK2 V617F n = 110)	Institutional only (co-mutated n = 15 versus isolated JAK2 V617F n = 110)	Direction retained
Haemoglobin, g/L	141.0 (130.3–155.0) versus 130.0 (112.0-144.0); median difference 12 (95% CI 3–23), P = .010; Holm-adjusted P = .051	145.0 (133.0-155.0) versus 130.0; median difference 16 (95% CI 5–27), P = .006	Yes
Platelet count, ×10⁹/L	881.5 (653.8-1102.3) versus 735.0 (576.0-906.0); median difference 125 (95% CI 0-261), P = .056; Holm-adjusted P = .167	819.0 (632.5-1117.5) versus 735.0; median difference 103 (95% CI − 55 to 275), P = .181	Yes, same direction, not significant in either
Splenomegaly, n/N (%)	11/20 (55.0) versus 26/107 (24.3); risk difference 30.7 pp (95% CI 8.1–51.2), Fisher P = .014; Holm-adjusted P = .054	8/14 versus 26/107; Fisher P = .022	Yes
Thrombosis, n/N (%)	8/24 (33.3) versus 21/110 (19.1); risk difference 14.1 pp (95% CI − 3.4 to 35.0), Fisher P = .171; Holm-adjusted P = .221	5/15 versus 21/110; Fisher P = .306	Yes, same direction; not significant in either analysis
Transformation, n (%)	3/24 (12.5) versus 4/110 (3.6); risk difference 8.8 pp (95% CI − 0.9 to 27.5), Fisher P = .111; Holm-adjusted P = .221	3/15 versus 4/110; Fisher P = .038	Yes, same direction; significant only in the institutional-only analysis

Note: the institutional-only analysis was prespecified because nine of the 24 co-mutated patients came from published reports. Age did not differ in the institutional subgroup (63.0 [60.5–71.0] versus 65.0 [43.0–76.0] years; median difference 1 year, 95% CI − 7 to 10, P = .848). The JAK2 V617F burden in the institutional subgroup was 22.4% (6.2–26.5, n = 9), a median difference of − 10.2 percentage points (95% CI − 22.1 to 1.0, P = .068). pp, percentage points.

Co-mutation meant JAK2 V617F with a CALR exon 9 mutation in the same sample and no MPL mutation. Thrombosis meant an arterial or venous event confirmed by imaging, before or after diagnosis (definitions in Online Resource 1, Methods S4). Platelet control meant a count of 450 × 10⁹/L or below, not the ELN/IWG-MRT response criteria [22]. Transformation meant post-ET myelofibrosis, accelerated or blast phase or acute myeloid leukaemia.

Three centres used one platform hosted at Beijing GoBroad Boren Hospital; samples from Heping Hospital were analysed by Beijing Haisite Medical Laboratory. JAK2 V617F was quantified by allele-specific real-time PCR; CALR exon 9 by fragment analysis with Sanger or next-generation sequencing,

classified as type 1/type 1-like, type 2/type 2-like or other; MPL exon 10 and other variants by targeted next-generation sequencing; and karyotype by G-banding (Online Resource 1, Methods S3 and Tables S3-S5).

Statistical analysis and ethics

Continuous variables are given as median (interquartile range), compared by Kruskal-Wallis and Mann-Whitney tests with Hodges-Lehmann median differences and rank-biserial correlations; categorical variables as counts and percentages with denominators, using χ^2 or Fisher's exact tests with risk differences and Cramér's V. P values for the continuous comparisons and for the three-group χ^2 tests are Monte Carlo permutation values (5,000 replicates, fixed seed), and P values for the 2 × 2 categorical comparisons are Fisher's exact values; the Holm-Bonferroni adjustment for the five prespecified primary comparisons and the Benjamini-Hochberg q values for the exploratory comparisons were both computed from the unrounded raw P values, so that the adjusted values shown in the tables can be reproduced from the unrounded raw values. Transformation was analysed by Aalen-Johansen cumulative incidence with death as the competing event, and missing data were not imputed, with missing-as-failure and extreme-bound sensitivity analyses (Online Resource 1, Methods S4). Analyses used SPSS 26.0; reporting follows STROBE [23] and PRISMA 2020 [24]. The study followed the Declaration of Helsinki and was approved by the research ethics committee of each participating centre (Peking University International Hospital, 2023-KY-0070-03; Peking University Shougang Hospital, IRBK-2024-022; Beijing GoBroad Boren Hospital, 20241101-TY; Heping Hospital Affiliated to Changzhi Medical College, IRB-2024-012). Written informed consent was waived at every centre because only de-identified data collected during routine clinical care were analysed. The nine patients taken from previously published reports were studied from the de-identified data given in those reports, which are cited individually in Online Resource 1.

Results

Patients and follow-up

The analysis included 155 patients with BCR::ABL1-negative ET: 24 with coexisting JAK2 V617F and CALR mutations (nine from published reports, 15 from the participating centres), 110 with isolated JAK2 V617F and 21 with isolated CALR mutation. Median follow-up among patients with a recorded duration was 32.0 (23.0–45.0) months (n = 21), 17.0 (9.0–37.0) months (n = 65) and 15.0 (11.5–31.3) months (n = 14) respectively (H = 2.983, P = .210).

Baseline clinical and laboratory features

Age, leucocyte count, sex distribution and the proportion aged 60 years or older were similar across groups (Table 1). Median age was 62.5 (51.0-67.8), 65.0 (43.0–76.0) and 62.0 (50.0–71.0) years (H =

0.789, ε^2 = 0.005, P = .680). Median leucocyte count was 9.7 (7.6–12.2), 8.9 (6.6–12.0) and 7.9 (6.6–11.0) × 10^9/L (H = 1.117, ε^2 = 0.007, P = .583), and the proportion of men was 58.3%, 42.7% and 47.6% (χ^2 = 1.855, Cramér's V = 0.11, P = .407).

Haemoglobin differed across the three groups: 141.0 (130.3–155.0), 130.0 (112.0-144.0) and 135.0 (121.0-145.0) g/L (H = 6.175, ε^2 = 0.040, P = .045). Pairwise testing located the difference in the co-mutated group, which stood 12 g/L above the JAK2 V617F group (95% CI 3–23, P = .010) and 10 g/L above the CALR group (95% CI -3 to 23, P = .160). After Holm correction the contrast with isolated JAK2 V617F was borderline (adjusted P = .051).

Platelet counts moved in the same direction without reaching significance: 881.5 (653.8-1102.3), 735.0 (576.0-906.0) and 878.0 (702.0-1075.0) × 10^9/L (H = 5.658, ε^2 = 0.037, P = .055). The median difference between co-mutated and JAK2-mutated patients was 125 × 10^9/L (95% CI 0-261, P = .056).

Splenomegaly was recorded more often in co-mutated patients: 11 of 20 (55.0%) versus 26 of 107 (24.3%) with isolated JAK2 V617F and 6 of 20 (30.0%) with isolated CALR mutation (χ^2 = 7.681, Cramér's V = 0.23, P = .022). Against isolated JAK2 V617F the risk difference was 30.7 percentage points (95% CI 8.1–51.2, Fisher P = .014), with exact intervals of 31.5–76.9% and 16.5–33.5%; the adjusted difference was again borderline (Holm-adjusted P = .054).

Thrombosis had occurred in 8 of 24 co-mutated patients (33.3%), 21 of 110 with isolated JAK2 V617F (19.1%) and none of the six CALR-mutated patients in whom the field was documented (χ^2 = 4.011, Cramér's V = 0.17, P = .130). The risk difference against isolated JAK2 V617F was 14.1 percentage points (95% CI -3.4 to 35.0, Fisher P = .171). Bleeding was uncommon (none, two patients [1.8%] and none; P = 1.000). A thrombosis field was recorded for all co-mutated and all JAK2 V617F-mutated patients but for only six of the 21 CALR-mutated patients, so the three-group comparison cannot be interpreted as a thrombotic risk estimate. We therefore treat thrombotic risk in this cohort as unassessed rather than as increased or unchanged.

Molecular features

Among patients with a recorded value, the JAK2 V617F burden was lower in the co-mutated group: 22.4% (6.2–33.5, n = 13) versus 27.0% (18.4–42.7, n = 65) in patients with isolated JAK2 V617F. The Hodges-Lehmann median difference was − 9.4 percentage points (95% CI -20.4 to 0.2), with a rank-biserial correlation of 0.33 (P = .061). A burden below 5% was found in 3 of 13 co-mutated patients (23.1%) and 3 of 65 patients with isolated JAK2 V617F (4.6%). The risk difference was 18.5 percentage points (95% CI 1.5–45.8, Fisher P = .055).

The CALR burden was numerically higher in co-mutated than in CALR-mutated patients (32.2% [14.6–38.9], n = 14 versus 22.9% [20.1–26.8], n = 12). Few patients were evaluable and the interval was wide, so the difference was not statistically significant (median difference 6.9 percentage points, 95% CI -7.8 to 16.8, P = .276).

Every co-mutated patient had a classified CALR subtype, whereas nine of the 21 patients with isolated CALR mutation were reported only as positive. Co-mutated patients carried type 1/type 1-like mutations in 14 cases, type 2/type 2-like in six and other variants in four; the corresponding numbers among classified CALR-mutated patients were seven, three and two. Treating unclassified results as a separate category produced an apparent difference in subtype distribution (P = .003), but among patients with a classified subtype the two distributions were exactly proportional (14:6:4 and 7:3:2; $\chi^2 = 0.00$, Cramér's V = 0, P = 1.000). The apparent difference therefore reflects subtyping completeness rather than a different distribution of variants, and we no longer report it as a molecular difference between groups. No co-mutated patient carried an MPL exon 10 mutation.

Additional myeloid variants were detected in 6 of 16 tested co-mutated patients (37.5%), 30 of 110 patients with isolated JAK2 V617F (27.3%) and 4 of 21 with isolated CALR mutation (19.0%). The difference did not reach significance (P = .503, Cramér's V = 0.10). The number of additional variants did differ (P = .007, Cramér's V = 0.22): two or more were found only among patients with isolated JAK2 V617F (19 patients, 17.3%), and none of the co-mutated or CALR-mutated patients. Eight co-mutated patients had not undergone panel testing.

Thrombotic risk stratification

Revised IPSET categories differed across groups (P = .0002, Cramér's V = 0.71), but the difference lay entirely with the CALR-mutated patients, all of whom were very low risk (10 of 21) or intermediate risk (11 of 21). Co-mutated and JAK2 V617F-mutated patients were indistinguishable, with 16 of 24 (66.7%) and 70 of 110 (63.6%) classified as high risk (risk difference 2.4 percentage points, 95% CI -19.2 to 20.4, Fisher P = 1.000). The score is built from age, thrombosis history and JAK2 mutation status, so the very-low and intermediate categories cannot occur in either JAK2-positive group. The comparison is therefore a property of the score rather than independent evidence about risk, and we report it only to describe how the tool behaves in these patients.

Treatment and haematological response

All three groups were treated mainly with hydroxycarbamide, alone or with aspirin (16 of 24, 77 of 110 and 18 of 21). Combination treatment with interferon was more frequent in co-mutated patients (25.0%, 8.2% and 4.8%; Cramér's V = 0.18, P = .054).

At three months, a platelet count of 450 × 10^9/L or below was recorded in 7 of 14 co-mutated patients (50.0%), 53 of 94 patients with isolated JAK2 V617F (56.4%) and 16 of 21 CALR-mutated patients (76.2%). The difference was not significant ($\chi^2 = 3.298$, P = .198). Recording was uneven: the three-month value was missing in 10 of 24 co-mutated patients (41.7%), 16 of 110 patients with isolated JAK2 V617F (14.5%) and none of the CALR-mutated patients. Counting missing values as treatment failure produced a difference (7 of 24, 53 of 110 and 16 of 21; P = .006). An extreme-bound analysis counted the missing co-mutated values as successes and all missing values in the other groups as failures. That

analysis also produced a difference (17 of 24, 53 of 110 and 16 of 21; P = .018). The endpoint is therefore sensitive to assumptions about the missing values.

At last follow-up, platelet control among patients with a recorded value was 11 of 19 (57.9%), 40 of 82 (48.8%) and 4 of 15 (26.7%) (P = .171). Median platelet counts at three months were 461.0 (392.3–541.0), 432.0 (352.0-513.8) and 356.0 (323.0-436.0) × 10^9/L (P = .110). At last follow-up they were 435.0 (352.5–517.0), 452.5 (346.8–548.0) and 513.0 (454.5–566.0) × 10^9/L (P = .138).

Disease transformation

Transformation occurred in three of 24 co-mutated patients (12.5%; two to myelofibrosis, one to acute myeloid leukaemia), four of 110 patients with isolated JAK2 V617F (3.6%, all acute myeloid leukaemia) and one of 21 CALR-mutated patients (4.8%, myelofibrosis). The difference was not significant (P = .253, Cramér's V = 0.14; risk difference against isolated JAK2 V617F 8.8 percentage points, 95% CI -0.9 to 27.5, Fisher P = .111).

Among 100 patients with an evaluable follow-up duration (21 co-mutated, 65 with isolated JAK2 V617F and 14 with isolated CALR mutation; two, four and one transformation and one, one and no death without transformation respectively), the competing-risk analysis gave a 36-month cumulative incidence of transformation of 0.0% in co-mutated patients (bootstrap 95% CI 0.0–0.0). The estimates were 10.3% in patients with isolated JAK2 V617F (95% CI 1.7–21.7) and 10.0% in CALR-mutated patients (95% CI 0.0-33.3). The omnibus permutation test gave P = .600, and the difference between co-mutated and JAK2 V617F-mutated patients was − 10.3 percentage points (P = .244). The interval in the co-mutated group is degenerate because only two events fell inside the 36-month window, so it is uninformative rather than reassuring, and both evaluable transformations occurred after 36 months. Read together, the crude proportions and the cumulative incidence do not support a claim of increased short-term transformation risk.

Sensitivity analyses and data completeness

Because nine of the 24 co-mutated patients came from published reports, all five primary comparisons were repeated in the institutional subgroup and both sets of estimates are given in Table 4. Age did not differ between groups in this subgroup (63.0 [60.5–71.0] versus 65.0 [43.0–76.0] years; median difference 1 year, 95% CI -7 to 10, P = .848). Haemoglobin remained higher in co-mutated patients (145.0 [133.0-155.0] versus 130.0 [112.0-144.0] g/L; median difference 16 g/L, 95% CI 5–27, P = .006), platelet counts moved in the same direction without reaching significance (819.0 [632.5-1117.5] versus 735.0 [576.0-906.0] × 10^9/L; median difference 103 × 10^9/L, 95% CI -55 to 275, P = .181), and splenomegaly was more frequent (8 of 14 versus 26 of 107; Fisher P = .022). Thrombosis remained non-significant (5 of 15 versus 21 of 110; P = .306). The transformation comparison pointed in the same direction as the pooled analysis and became statistically significant (3 of 15, 20.0%, versus 4 of 110, 3.6%; Fisher P = .038), so what depends on the published cases is the significance of the result rather than its direction. Because the two estimates rest on three and four events, no conclusion about transformation risk is drawn here. In the same subgroup the JAK2 V617F burden was 22.4% (6.2–26.5, n = 9), a median

difference of -10.2 percentage points against patients with isolated JAK2 V617F (95% CI -22.1 to 1.0, P = .068).

Completeness varied by variable. A thrombosis field was recorded for all 24 co-mutated and all 110 JAK2 V617F-mutated patients but only six of 21 CALR-mutated patients; spleen size was recorded for 20 of 24 co-mutated patients and the three-month platelet count for 14 of 24. Allele burdens were evaluable in 13 of 24 and 14 of 24 co-mutated patients (JAK2 and CALR respectively), 65 of 110 patients with isolated JAK2 V617F and 12 of 21 CALR-mutated patients. Karyotype and lactate dehydrogenase were available for only 10 co-mutated patients and were not compared between groups.

Discussion

Coexisting JAK2 V617F and CALR mutations in this cohort of 155 Chinese patients with ET identified a group whose phenotype resembled JAK2-mutated disease. Haemoglobin was 12 g/L higher than in patients with isolated JAK2 V617F (95% CI, 3–23). Splenomegaly was more than twice as common (55.0% versus 24.3%; risk difference, 30.7 percentage points), and both differences were borderline after correction (adjusted P = .051 and .054). The JAK2 V617F burden was lower in co-mutated patients (22.4% versus 27.0%). A burden below 5% was recorded in 3 of 13 co-mutated patients with an evaluable value and in 3 of 65 patients with isolated JAK2 V617F (P = .055). Two findings bound how far this phenotype can be taken. A thrombosis field was recorded for only six of the 21 CALR-mutated controls, so the thrombotic comparison cannot be read as a risk estimate. The direction was towards more events in co-mutated patients (33.3% versus 19.1%), as reported for ET generally [37]. Transformation went the same way, with no significant pooled excess (12.5% versus 3.6%, P = .111) and a significant excess once the published cases were excluded (3 of 15 versus 4 of 110, P = .038); only the significance differed. Both evaluable co-mutated events occurred after 36 months, so the 36-month cumulative incidence was 0% versus 10.3%. The cohort therefore describes a recognisable phenotype rather than a confirmed high-risk subtype. That reading depends on the nine patients taken from published reports, so all five primary comparisons were also run in institutional patients alone (Table 4). The three phenotype comparisons kept their direction, thrombosis remained non-significant in both analyses, and only the statistical significance of the transformation comparison changed.

Comparison with previous reports

The allele-burden pattern in our cohort matches previous reports. Coexisting mutations are rare when the JAK2 V617F burden is high and become more common at a low burden. This gradient has been described in French, Danish and Korean cohorts and in large sequencing series [9, 12–14], and reviewed in relation to phenotype [25]. Our data reproduce that gradient in Chinese patients. The phenotype we observed is also familiar: JAK2 mutations associate with higher haemoglobin and leucocyte counts, whereas CALR mutations associate with higher platelet counts and slower treatment responses [9]. CALR subtype and allele burden refine this picture rather than change it. Type 1 and type 2 variants differ in fibrotic tendency [31], a CALR burden of 60% or more shortens myelofibrosis-free survival [32], and a

burden below 20% marks a more indolent course [33]. A meta-analysis of 15 studies found lower thrombotic risk for CALR type 1 (OR 0.65, 95% CI 0.46–0.91) and CALR type 2 (OR 0.45, 0.32–0.62) than for JAK2 V617F. Risk was higher for type 1 than for type 2 (OR 1.50, 1.00-2.24) [34]. In our cohort, the subtype distributions of the two groups were identical once the comparison was limited to patients with a classified variant. The difference we first observed was therefore an artefact of incomplete subtyping rather than a biological signal.

A low JAK2 V617F burden should not be read as low risk. In a series of 11 patients with coexisting driver mutations, two clones grew side by side during treatment [27], and single-cell sequencing has placed JAK2 and CALR or MPL in separate subclones [15]. Low-level mutations may also reflect age-related clonal haematopoiesis [28]. The mechanism supports this caution, because JAK2 V617F clonal haematopoiesis promotes arterial thrombosis through platelet activation [29], and JAK-STAT activation in non-malignant cells contributes both to the disease phenotype and to thrombosis [30]. Our higher thrombotic proportion alongside a low JAK2 burden points the same way, but the cohort is too small to settle the question.

Clinical implications

The practical consequences follow less from the phenotype than from how these patients are tested, classified and followed. Testing comes first: any JAK2 V617F-positive patient with ET should have CALR exon 9 tested with the assay sensitivity stated, particularly when the burden is below 5%. Nearly a quarter of our co-mutated patients would otherwise be reported as JAK2-mutated only, and germline JAK2 variants should be considered when there is a family history [40]. Risk classification needs the same caution, because revised IPSET-thrombosis assigns high risk on JAK2 positivity and age alone, whatever the allele burden. Two thirds of our co-mutated patients (66.7%) were thus classified as high risk despite a median JAK2 burden of 22.4%. The score should therefore be one input to the decision rather than the decision itself. The measured burden, spleen size, previous thrombosis and cardiovascular risk factors belong in the assessment [26].

Treatment response adds a further caution. Platelet control at three months was least frequent in co-mutated patients (50.0% versus 56.4% versus 76.2%), and hydroxycarbamide with interferon was used more often in this group (25.0% versus 8.2% versus 4.8%). Later responses have been reported in CALR-mutated ET [9], so three months of counts is too short a window to declare treatment failure. Two of five values were missing, and follow-up should cover the spleen and early signs of fibrotic progression. Interferon reduces the CALR allele burden and can induce molecular responses in CALR-mutated ET [35], and ruxolitinib is an option after hydroxycarbamide failure [36], although neither has been studied in co-mutated patients.

Strengths and limitations

Several constraints bound these data. Nine of the 24 co-mutated patients came from published Chinese reports. Transformation is the one comparison whose statistical significance changed with their removal (P = .038 institutional only versus .111 pooled) although its direction did not, and because both

estimates rest on three and four events we draw no conclusion about transformation risk. Haemoglobin, platelet count and splenomegaly kept their direction in both analyses (Table 4). The five prespecified primary comparisons had Holm-adjusted P values of .051, .054, .167, .221 and .221, so the phenotype differences are best described as suggestive. Missing data were uneven: the three-month platelet count was missing in 41.7% of co-mutated patients, only six CALR-mutated controls had a recorded thrombosis field, and nine had no CALR subtype. Transformation risk in ET tracks with age, leucocytosis, karyotype and high-molecular-risk mutations [38], and MPL mutation, splenomegaly and ASXL1 mutation have each been linked to fibrotic progression [2, 26, 39]. Our follow-up is too short to address these risks, and crude proportions of transformation invite the mistaken impression of early excess risk. The study also rests on a single time point in one tissue, without clonal architecture data. Transformation and death times came from medical records rather than adjudicated review, treatment was not randomised, and molecular results were taken from the reporting laboratories rather than re-run centrally. Three of the four centres shared one platform, but Heping Hospital used a separate reference laboratory, so assay-level differences cannot be excluded for that centre.

Coexisting JAK2 V617F and CALR mutations are uncommon in ET but identifiable, and in this Chinese cohort they defined a phenotype that leans towards JAK2-mutated disease without a confirmed excess of thrombotic or transformative risk. In practice, CALR should be tested whenever JAK2 V617F is present, particularly at a low allele burden. The revised IPSET category should be one element of an assessment that also includes the measured allele burden and the spleen. Prospective multicentre cohorts in China, with centralised molecular testing, clonal architecture analysis and serial allele-burden measurement, are needed to establish whether coexistence alters thrombotic risk and how these patients should be treated.

Conclusions

In Chinese patients with ET, coexisting JAK2 V617F and CALR mutations are associated with a lower JAK2 burden, a relatively higher CALR burden, higher haemoglobin and more frequent splenomegaly. The phenotype therefore leans towards the JAK2 pattern. Thrombotic risk could not be assessed in this cohort, and an excess of transformative risk was not confirmed. Revised IPSET-thrombosis assigns high risk on JAK2 positivity alone, which limits its usefulness in these patients. CALR exon 9 testing belongs in the work-up of JAK2-positive ET, especially at a low allele burden, and spleen status and allele burden should inform follow-up. Prospective multicentre Chinese cohorts with centralised testing, complete CALR subtyping, clonal architecture analysis and serial allele-burden measurement are the next step.

Declarations

Competing Interests

The authors declare no competing interests.

Ethics Approval

The study was approved by the research ethics committee of each participating centre: Peking University International Hospital (2023-KY-0070-03), Peking University Shougang Hospital (IRBK-2024-022), Beijing GoBroad Boren Hospital (20241101-TY) and Heping Hospital Affiliated to Changzhi Medical College (IRB-2024-012). Nine of the 24 co-mutated patients were identified from previously published Chinese case reports and were analysed only from the patient-level, de-identified data given in those reports, each of which is cited and listed individually in Online Resource 1, Table S2; no new human participants were studied for these cases and no separate ethics approval was therefore required. Their inclusion in this multicentre comparison is an aggregate re-analysis of previously reported observations and does not constitute duplicate publication of the original reports.

Consent to Participate

Written informed consent was waived at all four centres because de-identified data collected during routine clinical care were analysed and no patient was contacted.

Consent for Publication

Not applicable; the manuscript contains no identifiable individual data.

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Author Contribution

Liu Jianxin: conceptualisation, methodology, formal analysis, writing - original draft. Dong Lu: data curation, investigation. Hu Wenqing: data curation, investigation. Ma Xiaocai: resources, data curation. Shen Xuliang and Zheng Qinlong: resources, supervision, writing - review and editing. Zhang Meixiang: conceptualisation, supervision, funding acquisition, writing - review and editing.

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Data Availability

The de-identified dataset and the analysis scripts are available from the corresponding author on reasonable request. The supplementary material, including the search strategy, the patient flow, the testing platforms and the per-case source information, is provided as Online Resource 1.

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Figures

Figure 1. Data screening flow

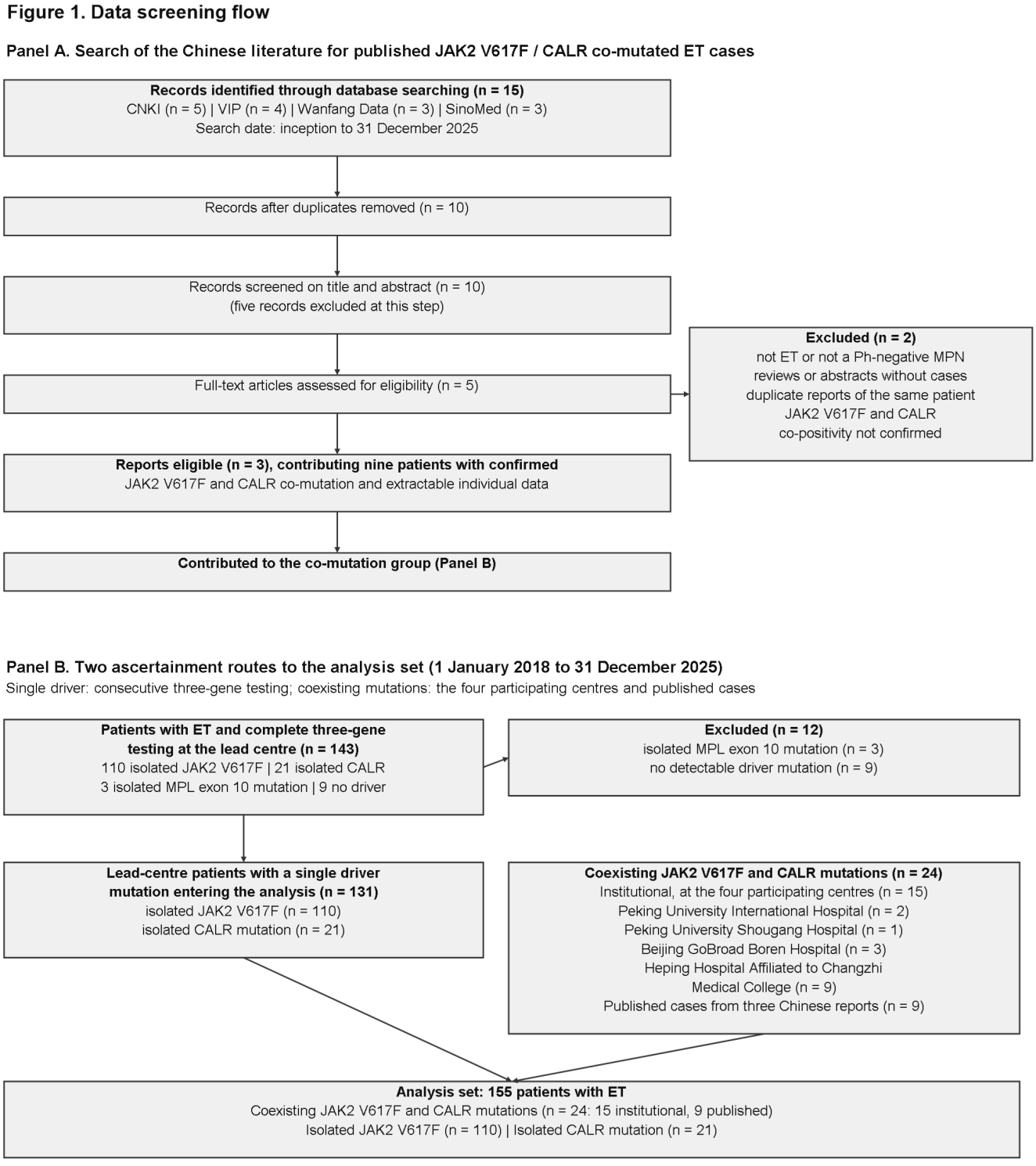


Figure 1

Study flow. (A) Search of the Chinese literature (CNKI, VIP, Wanfang Data and SinoMed). Fifteen records were identified, five duplicates were removed, ten records were screened on title and abstract, five full texts were assessed, two were excluded and three reports contributing nine patients with confirmed JAK2 V617F and CALR co-mutation were included. (B) Two ascertainment routes to the analysis set. The lead centre's consecutive registry contained 143 patients with ET and complete three-gene testing, of whom 110 had isolated JAK2 V617F, 21 an isolated CALR mutation, three an isolated MPL exon 10 mutation and nine no detectable driver mutation. The 12 patients with an isolated MPL mutation or with no detectable driver mutation were excluded, leaving 131 patients with a single driver mutation; all single-driver controls came from the lead centre. Fifteen patients with coexisting JAK2 V617F and CALR mutations were identified at the participating centres: nine at Heping Hospital Affiliated to Changzhi Medical College, three at Beijing GoBroad Boren Hospital, two at Peking University International Hospital and one at Peking University Shougang Hospital. These 15 patients together with the 131 single-driver controls formed the institutional cohort of 146 patients with ET. Adding the nine published cases gave the 155 patients analysed: 24 with coexisting mutations, 110 with isolated JAK2 V617F and 21 with an isolated CALR mutation.

Supplementary Files

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