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RESEARCH ARTICLE

A globally applicable "triple A" risk model for essential thrombocythemia based on Age, Absolute neutrophil count, and Absolute lymphocyte count

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Abstract

We examined the individual prognostic contribution of absolute neutrophil (ANC), lymphocyte (ALC), and monocyte (AMC) counts, on overall (OS), leukemia-free (LFS), and myelofibrosis-free (MFFS) survival in essential thrombocythemia (ET). Informative cases ($N = 598$; median age 59 years; females 62%) were retrospectively accrued from a Mayo Clinic database: *JAK2* 59%, *CALR* 27%, triple-negative 11%, and *MPL* 3%; international prognostic scoring system for ET (IPSET) risk high 21%, intermediate 42%, and low 37%; 7% (37/515) had abnormal karyotype and 10% (21/205) adverse mutations (*SF3B1/SRSF2/U2AF1/TP53*). At median 8.4 years, 163 (27%) deaths, 71 (12%) fibrotic, and 20 (3%) leukemic transformations were recorded. Multivariable analysis resulted in HR (95% CI) of 16.5 (9.9–27.4) for age > 70 years, 3.7 (2.3–6.0) for age 50–70 years, 2.4 (1.7–3.3) for ANC $\geq 8 \times 10^9$/L, and 1.9 (1.4–2.6) for ALC $< 1.7 \times 10^9$/L. The corresponding HR-based scores were 4, 2, 1, and 1, resulting in an new 4-tiered AgeAncAlc (AAA; triple A) risk model: high (5–6-points; median survival 8 years; HR 30.1, 95% CI 17.6–54), intermediate-2 (4 points; median 13.5 years; HR 12.7, 95% CI 7.1–23.0), intermediate-1 (2–3 points; median 20.7 years; HR 3.8, 95% CI 2.3–6.4) and low (0–1 points; median 47 years). The AAA model (Akaike Information Criterion [AIC] 621) performed better than IPSET (AIC 647) and was subsequently validated by an external University of Florence ET cohort ($N = 485$). None of the AAA variables predicted LFS while ALC $< 1.7 \times 10^9$/L was associated with inferior MFFS ($p = .01$). Adverse mutations ($p < .01$) and karyotype ($p < .01$) displayed additional prognostic value without disqualifying the prognostic integrity of the AAA model. This study proposes a simple and globally applicable survival

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model for ET, which can be used as a platform for further molecular refinement. This study also suggests a potential role for immune-related biomarkers, as a prognostic tool in myeloproliferative neoplasms.

1 | INTRODUCTION

Essential thrombocythemia (ET) is included in the international consensus classification (ICC) category of myeloproliferative neoplasms (MPN) and subcategory of *JAK2* mutation-prevalent MPN, along with polycythemia vera (PV), primary myelofibrosis (PMF), and MPN, unclassifiable (MPN-U).[1] These clinicopathologic entities share similar genetic and bone marrow (BM) morphologic characteristics; the former involves mutually exclusive mutations in *JAK2*, *CALR*, or *MPL* (so-called driver mutations) and the latter variable degrees of trilineage myeloproliferation with corresponding changes in BM cellularity, BM fibrosis, and megakaryocytic proliferation and atypia.[2] Diagnosis of ET requires the presence of thrombocytosis (platelet count ≥450 × 10^9/L) and absence of both erythrocytosis (exclusion of PV) and BM fibrosis and PMF-characteristic megakaryocyte morphology (exclusion of PMF, prefibrotic and overt fibrotic stage).[2,3] Driver mutations in MPN lack diagnostic specificity and their distribution in ET is similar to that of PMF: *JAK2* ~60%, *CALR* 20%, and *MPL* 3%; 15%–20% of patients do not express any of the three driver mutations and are referred to as "triple-negative."[4]

The clinical course of patients with ET is relatively indolent but might be interrupted by thrombohemorrhagic complications, microvascular disturbances, recurrent miscarriage, and, in a small percentage of patients, progression into myelofibrosis (post-ET MF) or acute myeloid leukemia (AML), also referred to as "blast phase MPN."[5–7] Although life-expectancy in ET is inferior to that of the control population,[8] overall survival is generally considered favorable with estimated median of 18 years[9] and exceeding 35 years for patients aged 40 years or younger.[10] There are currently two survival risk models in ET; the international prognostic score for ET (IPSET) considers three variables (age ≥ 60 years, leukocyte count ≥11 × 10^9/L, prior thrombosis),[11] in order to delineate high (median survival 13.8 years), intermediate (median 24.5 years), and low (median not reached) risk groups. The more recent mutation-enhanced international prognostic model for ET (MIPSS-ET)[12] also considers 3 variables (age > 60 years, male gender, adverse mutations including *SF3B1*, *SRSF2 U2AF1 TP53*), in order to delineate high (median 8.3 years), intermediate (median 14.1 years), and low (median 34.4 years) risk groups. In this study, we describe absolute lymphocyte count (ALC) <1.7 × 10^9/L as an independent predictor of shortened survival in ET, thus allowing for the development of a novel survival model that is based on ALC, absolute neutrophil count (ANC) and Age (AAA).

2 | MATERIALS AND METHODS

This study was conducted under institutional review board approved minimum risk protocols that allowed retrospective collection and analysis of patient data from the Mayo Clinic (Rochester, MN) and the University of Florence (Florence, Italy). Study patients were retrospectively accrued from the MPN database of the respective institutions after confirmation of diagnosis according to the 2016 World Health Organization (WHO) diagnostic criteria for ET[13] and availability of information on ANC (reference range 1.56–6.45 × 10^9/L), ALC (0.95–3.07 × 10^9/L), and AMC (0.26–0.81 × 10^9/L). Data were collected retrospectively corresponding to the time of first referral, which in the majority of cases was at the time of or within the first year of diagnosis and before initiation of cytoreductive therapy. All patients were followed until death or last follow-up, as assessed by medical records or through direct contact with patients or their physicians. Treatments received, often at the discretion of the treating physician, at any time during the disease course, were reviewed and documented. Conventional criteria were used for definitions of major complications, including leukemic or fibrotic transformation.[13] Laboratory methods used in DNA sequencing have previously been published.[14] Cytogenetics data were analyzed using standard techniques and reported in conformity with the International System for Human Cytogenetic Nomenclature criteria.[15]

Statistical analyses considered clinical and laboratory data collected at the time of initial diagnosis/referral. Receiver operating characteristic (ROC) curve analysis was utilized to curate the optimal cutoff points for age, ANC, and ALC. Differences in the distribution of continuous variables between categories were compared using the Mann–Whitney or Kruskal–Wallis test. Categorical variables were compared using the χ^2 test. Cox regression analysis was applied in order to identify risk factors for overall (OS), leukemia-free (LFS), and myelofibrosis-free (MFFS) survival. The Kaplan–Meier method was used to construct time-to-event curves, which were compared by the log-rank test. The *p*-values of <.05 were considered significant. The triple A (AAA) prognostic model was developed using hazard ratio (HR)-based risk point allocation and predictive accuracy was compared with that of IPSET,[11] using Akaike Information Criterion (AIC). The AAA risk model derived from Mayo Clinic patients was validated by its application to an external cohort from the University of Florence. The JMP® Pro 13.0.0 software from SAS Institute, Cary, NC (Mayo cohort), and IBM SPSS Statistics for Windows, version 24, IBM Corp., Armonk, NY (Italian cohort), were used for all calculations.

3 | RESULTS

3.1 | Patients

A total of 598 Mayo patients constituted the core study group (median age 59 years, range 18–90; 62% females). Driver mutation distribution was 59% *JAK2*, 27% *CALR*, 3% *MPL*, and 11% triple negative (Table 1).

TABLE 1 Presenting features and postdiagnosis events in 598 Mayo Clinic and 485 University of Florence patients with essential thrombocythemia.

Variables	Mayo Clinic patients ($N = 598$)	University of Florence patients ($N = 485$)
Age in years, median (range)	59 (18–90)	61 (18–93)
Males, *N* (%)	230 (38)	183 (37)
Leukocytes $\times 10^9$/L, median (range)	8.4 (2.7–29.8)	8.2 (3.9–23.4)
Absolute neutrophil count $\times 10^9$/L, median (range)	5.5 (1.3–23.3)	5.2 (1.7–19.1)
Absolute lymphocyte count $\times 10^9$/L, median (range)	1.8 (0.34–6.1)	2.0 (0.5–5.4)
Absolute monocyte count $\times 10^9$/L, median (range)	0.54 (0–4.5)	0.5 (0–1.8)
Hemoglobin in g/dL, median (range)	13.9 (11.1–16.4)	14 (11–16.4)
Platelets $\times 10^9$/L, median (range)	800 (451–3460)	714 (451–2088)
JAK2 mutation, *N* (%)	353 (59)	322 (66.4)
CALR mutation, *N* (%)	161 (27)	87 (18)
MPL mutation, *N* (%)	19 (3)	21 (4.3)
Triple Negative, *N* (%)	65 (11)	55 (11.3)
Palpable Splenomegaly, *N* (%)	71 (12)	61 (12.8)
Cardiovascular risk factors, *N* (%) *HTN diabetes tobacco use*	308 (55) *N* evaluated = 562	246 (52.2) *N* evaluated = 471
Microvascular symptoms, *N* (%)	132 (23) *N* evaluated = 575	164 (34.3) *N* evaluated = 478
Thrombosis at or prior to diagnosis, *N* (%)	124 (21)	93 (19.2)
Venous thrombosis at or prior to diagnosis, *N* (%)	59 (10)	31 (6.4)
Arterial thrombosis at or prior to diagnosis, *N* (%)	79 (13)	67 (13.8)
Major hemorrhage at or prior to diagnosis, *N* (%)	32 (5) *N* evaluated = 592	11 (2.3) *N* evaluated = 479
IPSET risk category, *N* (%)		
High	125 (21)	87 (18)
Intermediate	252 (42)	228 (47)
Low	221 (37)	170 (35)
Abnormal karyotype, *N* (%)	37 (7) *N* evaluated = 515	7 (6) *N* evaluated = 116
Adverse mutations, *N* (%) *SF3B1 SRSF2 TP53*	21 (10) *N* evaluated = 205	13 (9.2) *N* evaluated = 142
Deaths, *N* (%)	163 (27)	87 (17.9)
Years to death or last follow-up, median (range)	8.4 (0–47)	8.4 (0–36.4)
Progression to myelofibrosis, *N* (%)	71 (12)	43 (8.9)
Years to myelofibrosis progression, median (range)	8.2 (0–31)	10.1 (1.5–28.8)
Progression to acute myeloid leukemia (AML), *N* (%)	20 (3)	12 (2.5)
Years to AML progression, median (range)	13.7 (1.9–36.6)	10.3 (3–24.2)
Any thrombosis after diagnosis, *N* (%)	110 (18)	95 (19.6)
Arterial thrombosis after diagnosis, *N* (%)	92 (15)	59 (12.2)
Venous thrombosis after diagnosis, *N* (%)	42 (7)	43 (8.9)
Years to postdiagnosis venous thrombosis, median (range)	8.8 (0.1–31)	3 (0–24.2)
Years to postdiagnosis arterial thrombosis, median (range)	4.7 (0.04–37)	4.2 (0–26.2)
Major bleeding events after diagnosis, *N* (%)	*N* evaluated = 592 69 (12)	*N* evaluated = 474 34 (7.2)
Treatment documented		
Aspirin, *N* (%)	495 (90)	420 (87)
Cytoreductive, *N* (%)	453 (81)	388 (80)
Hydroxyurea, *N* (%)	426 (76)	378 (78)
Anagrelide, *N* (%)	139 (25)	55 (11)
Interferon, *N* (%)	34 (6)	21 (4)
Causes of death, *N* (%)		
Total deaths	163 (27)	87 (18)
Cardiovascular, *N* (% of known causes)	24 (25)	14 (23)
Disease progression to myelofibrosis or AML, *N* (% of known causes)	22 (23)	16 (27)
Infection, *N* (% of known causes)	11 (12)	3 (5)
Bleeding, *N* (% of known causes)	10 (10)	2 (3)
Second malignancies, *N* (% of known causes)	5 (5)	7 (12)
Other, *N* (% of known causes)	23 (24)	21 (35)
Unknown cause, *N* (% of all deaths)	68 (42)	24 (28)

IPSET risk category rate was high 21%, intermediate 42%, and low 37%. Baseline median (range) values were 13.9 g/dL (11.1–16.4) for hemoglobin, 8.4×10^9/L (2.7–29.8) for leukocyte count, and 800×10^9/L (451–3460) for platelet count. Palpable splenomegaly was documented in 71 (12%) patients and cardiovascular (CV) risk factors in 308 (55%). Cytogenetic information was available in 515 patients and revealed abnormal karyotype in 37 (7%). Among 205 patients with DNA sequence information, 21 harbored at least one adverse mutation, including *SF3B1*, *SRSF2*, *U2AF1*, and *TP53*. Thrombosis history at or before diagnosis was present in 124 (21%) patients and major bleeding history in 32 (5%). At a median follow-up of 8.4 years (range 0–47), 163 (27%) deaths, 71 (12%) progressions to post-ET myelofibrosis, 20 (3%) leukemic transformations, and 92 (15%) arterial and 42 (7%) venous thromboses were recorded (Table 1). Treatment included aspirin in 495 (90%) and cytoreductive therapy in 453 (81%) patients (Table 1). Causes of death information were available in 95 (58%) of the 163 Mayo patients and included CV in 24 (25%), progression into post-ET MF or MPN-BP in 22 (23%), infections in 11 (12%), bleeding in 10 (10%), second malignancies in 5 (5%), and other in 23 (24%) patients. Additional details on the timeline of fibrotic or leukemic transformation as well as postdiagnosis thrombotic events are included in Table 1.

The validation cohort consisted of 485 Florence patients (median age 61 years, range 18–92; females 62%). Driver mutation distribution was 66% *JAK2*, 18% *CALR*, 4% *MPL*, and 11% triple-negative (Table 1). Baseline median (range) values were 14.1 g/dL (11.2–16.5) for hemoglobin, 8.23×10^9/L (3.9–20.5) for leukocyte count, and 704×10^9/L (451–2088) for platelet count. Palpable splenomegaly was documented in 61 (13%) patients and CV risk factors in 52%. At a median follow-up of 8.4 years (range 0–36), 87 (18%) deaths, 43 (9%) progressions to post-ET myelofibrosis, 12 (3%) leukemic transformations, and 74 thrombotic events, including 59 (12%) arterial and 43 (9%) venous, were recorded. Causes of death information were available in 63 (72%) of the 87 Florence patients and included CV in 14 (23%), progression into post-ET MF or MPN-BP in 16 (27%), infections in 3 (5%), bleeding in 2 (3%), second malignancies in 7 (12%), and other in 21 (35%) patients. Additional details on presenting features of patients in the Florence cohort, causes of death, and postdiagnosis events and their timelines are included in Table 1.

3.2 | Survival analysis

In the Mayo patient cohort, the following were flagged by univariable analysis as being significant in their association with survival: age, ANC, ALC, male sex, arterial thrombosis history, CV risk factors, and palpable spleen; in addition, venous thrombosis history and AMC displayed borderline significance (Table 2). Multivariable analysis of continuous variables identified age ($p < .01$), ANC ($p < .01$), and ALC ($p = .03$), but not AMC ($p = .5$), platelet count ($p = .15$), or hemoglobin level ($p = .84$), as independent predictors of survival; ROC plot-based optimal cutpoints were age > 70 years (HR 16.5, 95% CI 9.9–27.4), age 50–70 years (HR 3.7, 95% CI 2.3–6.0), ANC ≥8×10^9/L (HR 2.4, 95% CI 1.7–3.3), and ALC <1.7×10^9/L (HR 1.9, 95% CI 1.4–2.6). These ROC-determined cutoff levels for age, ANC and ALC were used in subsequent multivariable analyses, which included either clinical variables only (Table 2, top section) or both clinical and genetic variables (Table 2, bottom section). In both instances, age and ALC retained their significance while ANC was downgraded to borderline significance, when genetic variables were added to the multivariable analysis (Table 2).

In addition to age, ANC, and ALC, multivariable analysis limited to clinical variables (previously recognized by IPSET[11] and MIPSS-ET[12]) also identified CV risk factors ($p < .01$) and arterial thrombosis ($p = .02$) as independent predictors of inferior survival (Table 2, top section). A similar analysis that included genetic variables confirmed the independent adverse prognostic effect of adverse mutations ($p = .001$; HR 3.3, 95% CI 1.8–6.2) and abnormal karyotype ($p = .007$; HR 3.0, 95% CI 1.4–6.6) while significance was also sustained for age > 70 years ($p < .001$; HR 14.5, 95% CI 6.7–31.7), age 50–70 years ($p = .004$; HR 3.3, 95% CI 1.6–6.6), and ALC <1.7×10^9/L ($p < .001$; HR 2.7, 95% CI 1.6–4.7), but not ANC ($p = .08$), arterial thrombosis ($p = .3$) or CV risk factors ($p = .09$). These readings from univariable and multivariable analyses are included in Table 2, which also includes the corresponding findings from the Florence validation cohort; the latter also isolated age, ANC, and ALC, but not AMC, CV risk factors, arterial thrombosis, venous thrombosis, as the most consistent predictors of survival (Table 2, top section); it should be noted that male sex retained multivariable significance in the Florence but not Mayo cohort and borderline significance ($p = .05$) was still apparent after the addition of genetic variables to the multivariable model (Table 2, top and bottom sections).

Unlike the case with OS, LFS was not affected by age ($p = .32$), ANC ($p = .78$), or ALC ($p = .37$), in the Mayo cohort, while significant association with age was noted in the Florence cohort. On the other hand, ALC was significantly associated with MFFS ($p = .01$), in the Mayo cohort, and borderline significance was also noted in the Florence cohort ($p = .08$). In the Mayo cohort, older age was associated with both arterial ($p < .01$) and venous ($p < .01$) thrombosis-free survival while the former was also affected by ANC ($p = .01$) and history of arterial thrombosis ($p = .02$). Considering the novel and unexpected survival impact from ALC, clinical and laboratory features were compared between Mayo patients with ALC <1.7×10^9/L versus those with ALC ≥1.7×10^9/L: the former showed significant association with older age ($p = .01$), male sex ($p < 0.01$), and higher AMC ($p < .01$), but not with other variables tested, including ANC ($p = .31$), thrombosis history ($p = .38$), CV risk factors ($p = .89$), diabetes ($p = .5$) hypertension ($p = .11$), tobacco use ($p = .11$), driver mutations ($p = .74$), karyotype ($p = 0.86$), or adverse mutations ($p = .16$). In contrast, and as expected, ANC >8×10^9/L was associated with *JAK2* mutation ($p < .01$), older age ($p < .01$), CV risk factors ($p < .01$), palpable splenomegaly (.02), and history venous thrombosis at diagnosis ($p = .03$).

3.3 | Development of the AAA model

The above elaborated statistical permutations have isolated age > 70 years, age 50–70 years, ALC <1.7×10^9/L and ANC

TABLE 2 Univariable and multivariable analyses of variables at presentation for prediction of survival in 598 Mayo Clinic and 485 University of Florence patients with essential thrombocythemia and fully annotated for driver mutations.

	Mayo Clinic $N = 598$		University of Florence $N = 485$	
Clinical variables	**Univariable *p*-value**	**Multivariable *p*-value**	**Univariable *p*-value**	**Multivariable *p*-value**
Age continuous	<.001		<.001	
Age > 70 years	<.001	**<.001 HR 12.5 (7.1–21.8)**	<.001	**<.001 HR 21.8 (7.5–63.7)**
Age 50–70 years	<.001	**<.001 HR 3.3 (2.0–5.6)**	<.001	**.003 HR 4.9 (1.7–14.2)**
ANC continuous	<.001		.002	
ANC ≥8 × 10^9/L	<.001	**<.001 HR 2.7 (1.9–3.9)**	.001	**.01 HR 2.1 (1.2–3.5)**
ALC continuous	<.01		.02	
ALC <1.7 × 10^9/L	<.001	**<.001 HR 2.0 (1.4–2.9)**	<.001	**.018 HR 1.7 (1.1–2.6)**
AMC continuous	.09	0.4	.3	
Male sex	.02	0.31	.004	**.002 HR 1.9 (1.3–2.9)**
Arterial thrombosis	.003	**.02**	.5	
CV risk factors	<.001	**<.001 HR 2.3 (1.5–3.6)**	.32	
Palpable spleen	.04	0.5	.63	
Venous thrombosis	.08	0.3	.71	
Hemoglobin level	.26		.2	
Platelet count	.24		.6	
Microvascular symptoms	.9		.12	
Hemorrhage	.9		.56	
Multivariable analysis with genetic variables added to the above independently significant clinical variables				
	Univariable *p*-value	**Multivariable *p*-value**	**Univariable *p*-value**	**Multivariable *p*-value**
Abnormal karyotype *N* evaluated = 515	.02	**.007 HR 3.0 (1.4–6.6)**	.4 *N* evaluated = 116	
Adverse mutations *N* evaluated = 205	<.001	**.001 HR 3.3 (1.8–6.2)**	<.001 *N* evaluated = 132	**.01 HR 2.6 (1.2–5.5)**
JAK2 vs. *CALR*	.001	.3	.46	
JAK2 vs. *TN*	.07	.5	.55	
Age > 70 years	<.001	**<.001 HR 14.5 (6.7–31.7)**	<.001	**<.001 HR 26.5 (1.5–66.8)**
Age 50–70 years	<.001	**.004 HR 3.3 (1.6–6.6)**	<.001	**<.001 HR 5.6 (2.4–13.4)**
ANC ≥8 × 10^9/L	<.001	.08	.001	.07
ALC <1.7 × 10^9/L	<.001	**<.001 HR 2.7 (1.6–4.7)**	<.001	**.03 HR 1.7 (1.1–2.6)**
Arterial thrombosis	.003	.3	.5	
CV risk factors	<.001	.09	.32	
Male sex	.02	.13	.004	.05

Note: Bold values are denoted by statistically significant.

Abbreviations: ALC, absolute lymphocyte count; AMC, absolute monocyte count; ANC, absolute neutrophil count; CV, cardiovascular; HR, hazard ratio.

≥8 × 10^9/L, as the most prominent risk factors for survival in ET, across both the discovery and validation cohorts (Table 2); the additional variables showing significance were either cytogenetic/molecular or not consistent between the two cohorts (male sex, CV risk factors, arterial thrombosis). Accordingly, we chose age, ANC, and ALC to develop a first-line risk stratification model that is globally applicable and as a platform for further molecular refinement based on health resource access. Multivariable analysis performed on the

Mayo discovery cohort and restricted to the three parameters of interest resulted in HR (95% CI) of 16.5 (9.9–27.4) for age > 70 years, 3.7 (2.3–6.0) for age 50–70 years, 1.9 (1.4–2.6) for ALC <1.7 × 10^9/L, and 2.4 (1.7–3.3) for ANC ≥8 × 10^9/L. The corresponding values derived from the Florence cohort were 26.5 (10.5–67), 5.7 (2.4–13.5), 1.7 (1.1–2.7), and 1.8 (1.1–3.1). Subsequently, HR-based risk scores were assigned, with 4 adverse points for age > 70 years, 2 points for age 50–70 years, and one point each for ALC <1.7 × 10^9/L and ANC

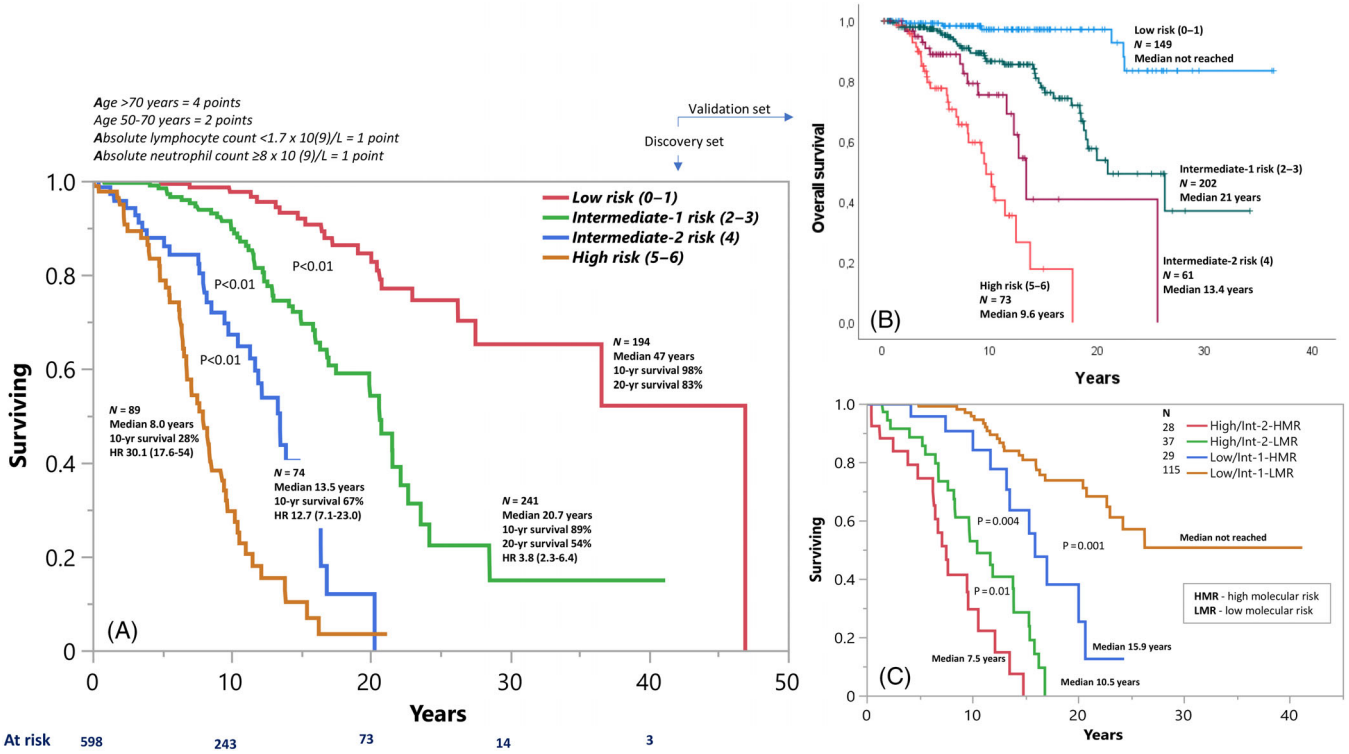

FIGURE 1 Overall survival data among (A) 598 Mayo Clinic and (B) 485 University of Florence patients with essential thrombocythemia, stratified by Age, Absolute neutrophil and Absolute lymphocyte count (AAA) risk model, and (C) 209 Mayo Clinic patients with AAA substratified by presence or absence of adverse mutations/abnormal karyotype.

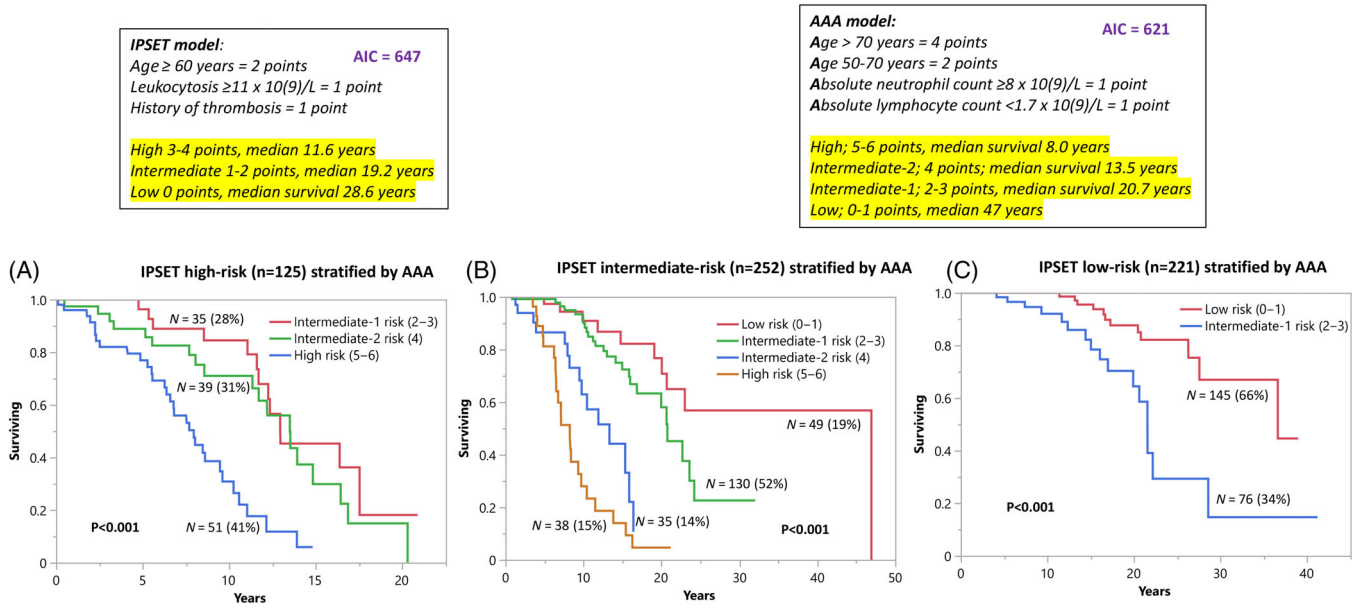

FIGURE 2 Overall survival among 598 Mayo Clinic patients with essential thrombocythemia stratified by AAA risk model in the context of the International Prognostic Score for ET (IPSET).

≥8 × 10^9/L, resulting in the development of a new 4-tiered AgeAncAlc (AAA; triple A) risk model (Figure 1A): high risk with 5–6 points (median survival 8 years; HR 30.1, 95% CI 17.6–54), intermediate-2 risk with 4 points (median survival 13.5 years; HR 12.7, 95% CI 7.1–23.0), intermediate-1 risk with 2–3 points (median survival 20.7 years; HR 3.8, 95% CI 2.3–6.4) and low risk with 0–1 points (median survival 47 years). The AAA model was subsequently validated by the Florence cohort with corresponding median survivals of 9.6, 13.4, 21, and not reached years (Figure 1B). Prognostic impact of AAA on MFFS ($p = .28$) or LFS ($p = .23$) was not significant. However, overall thrombosis-free survival was significantly different between the four AAA risk categories ($p < .001$), likely driven by differences in age distribution, and was apparent for both arterial ($p < .001$) and venous ($p = .008$) events. Figure 1C depicts the additional impact of adverse genetic features (abnormal karyotype or adverse mutations) in AAA-assigned higher and lower risk diseases.

3.4 | Comparison of AAA versus IPSET

Figure 2 illustrates performance comparisons between the AAA and IPSET survival models using the Mayo cohort ($N = 598$). Better performance was reflected by the lower AIC value of 621 for AAA versus 647 for IPSET. A graphic illustration to that effect is depicted in Figure 2A–C, where patients categorized by IPSET as low ($N = 221$; Figure 2A), intermediate ($N = 252$; Figure 2B), or high ($N = 125$; Figure 2C) risk groups were further substratified, within each IPSET category, into multiple AAA subcategories with markedly different survival data. For example, 15% of IPSET intermediate patients (expected median survival 19.2 years) were reclassified into AAA high-risk category (expected median survival 8 years) while 19% were reclassified as AAA low risk (expected median survival 47 years). Similarly, IPSET high-risk and IPSET low-risk patients were shown to include subsets of patients with significantly different survival expectations (Figure 2A,C, respectively). Conversely, 89 patients classified as high risk by AAA were subclassified into IPSET intermediate ($N = 38$) and IPSET high ($N = 51$) with superimposed survival data (median 8.3 vs. 8.0 years, respectively; $p = .56$). The same pattern was shown in the context of AAA intermediate-2 risk ($p = .71$) and low-risk ($p = .1$) categories while a substantial subset of AAA intermediate-2 risk patients were reclassified as IPSET high ($p = .005$).

4 | DISCUSSION

The novel discovery in this study is the association of lower ALC with shortened OS, and possibly MFFS, in ET. Mangaonkar et al.,[16] Saeed et al.,[17] Jacobs et al.,[18] and Silzle et al.[19] have previously reported on similar observations in a related myeloid neoplasm, myelodysplastic syndrome with ring sideroblasts (MDS-RS). In the most recent communication regarding the latter,[16] 71 patients with MDS-RS were studied and the ALC cutoff applied was 1 × 10^9/L; survival in patients with ALC <1 × 10^9/L ($N = 17$), compared with those with higher ALC ($N = 54$), was significantly worse (median 2.8 vs. 6.8 years), despite similar distribution for age, sex, and cytogenetic risk categories. ET and MDS-RS are both included in the ICC category of myeloid neoplasms[1] and are characterized by a relatively indolent clinical course.[7,20] Lymphocytopenia has also been shown to be an independent predictor of inferior survival in other hematologic malignancies[21–23] and solid cancers,[24] and some of which could be treatment-emergent.[25] Interestingly, lymphocytopenia has also been associated with increased risk of mortality among adults in the US general population[26]; the latter retrospective study included 31 178 participants (median age 45 years; 52% women) from the National Health and Nutrition Examination Survey (NHANES) where ALC was recorded at ≤1.5 in 20% and ≤1 × 10^9/L in 3% of the subjects; both degrees of lymphocytopenia were incrementally associated with increased risk of mortality (HR 1.3 and 1.8, respectively) from CV and non-CV causes. Causes of death in this study were also mostly CV but the association between ALC and MFFS suggests impact on disease biology, as well. Taken together, these observations suggest increased risk of mortality associated with biomarkers of immune dysregulation; additional studies are needed to determine whether or not lymhocytopenia is a manifestation of a generalized tumor-associated inflammatory state or is directly related to acceleration of death from expected causes.

An offshoot from the current unexpected observation linking lower ALC with increased mortality in ET was the utilization of the specific variable (ALC) in developing a new prognostic model (AAA) with better perfomance than the current standard IPSET model[11] and globally more applicable than the more recent MIPSS-ET model.[12] As vividly outlined in Figure 2, a significant number of IPSET-classified patients were reclassified by the AAA model, with significantly different survival data within each IPSET risk category. A main advantage for AAA, compared with IPSET, is its nonreliance on "thrombosis history" as a risk variable, which has been a subject of controversy because of its lack of distinction between arterial and venous events and nonreproducibility by other studies[12]; in a recent large Mayo-Florence collaborative study of patients with ET ($N = 451$), independent clinical risk factors included age > 60 years, leukocytosis ≥11 × 10^9/L, and male sex, but not thrombosis[12]; furthermore, univariable analysis restricted to the Mayo cohort failed to identify either arterial ($p = .1$) or venous ($p = .3$) thrombosis as being significant. Such inconsistencies could be in part due to cumulating history of thrombosis before diagnosis (e.g., possibly ET-unrelated) and at diagnosis (e.g., ET-related) and the possibility that AAA scoring overcomes inaccurate attribution of thrombosis to ET. The other issue with both the IPSET and MIPSS-ET survival models is the broad consideration of "leukocytosis," without accounting for its different components. To that effect, we have recently isolated ANC as the component of significance in predicting venous thrombosis in PV, and possibly ET as well.[27] The observations from this study are consistent with the assertion regarding differences in the individual contribution of ANC, AMC, and ALC, to disease outcome in MPN.[27,28] The superiority of the AAA model is also reflected in its acknowledgment of the disproportionate significance attached to age and endorsement of 3-tiered age categories (>70, 50–70, and < 50 years); a similar 3-tiered age categorization (67, 57–66, and <57 years) was also used in the development of international risk stratification model for PV.[29]

Although we are confident about the performance of our AAA survival model in ET, additional studies are needed to clarify its prognostic interaction with genetic risk factors and the impact of ALC on MFFS, LFS, and thrombosis risk. Also in need of additional attention are studies on the underlying mechanisms of immune dysregulation in ET and its impact on ALC and other immunologic and inflammatory markers. The new AAA survival model for ET offers a simple globally applicable tool, which can be used as a platform for further molecular refinement. Observations from this study suggest feasibility of the latter possibility, through demonstration of AAA-independent prognostic contribution from abnormal karyotype and adverse mutations; however, the latter observations require further scrutiny, considering the diagnostic ambiguity between ET and early/prefibrotic myelofibrosis.[3] Regardless, our observations in this study suggest potential value for immune profiling as an additional prognostic tool in MPN and reinforce the concept of associated inflammatory state in disease pathogenesis and prognosis.[30–32] In regard to therapeutic implications, one could argue for avoidance of drugs with immunosuppressive properties (e.g, JAK2 inhibitors) and focus on life style changes that enhance immune competence. Obviously, drugs that specifically target the malignant clone, if and when they become available, will not only result in disease modification, but might also attend to the root cause of tumor-associated immune dysfunction.

AUTHOR CONTRIBUTIONS

All authors reviewed and approved the article. All authors participated in the discussion and interpretation of data. Ayalew Tefferi, Giuseppe G. Loscocco, Natasha Szuber, Francesco Mannelli, Animesh Pardanani, Paola Guglielmelli, Naseema Gangat, and Alessandro M. Vannucchi participated provided direct patient care. Ayalew Tefferi, Giuseppe G. Loscocco, Faiqa Farrukh, Natasha Szuber, Francesco Mannelli, Paola Guglielmelli, Naseema Gangat, and Alessandro M. Vannucchi participated in data abstraction. Curtis A. Hanson reviewed pathology. Ayalew Tefferi, Giuseppe G. Loscocco, and AC performed the statistical analyses. Ayalew Tefferi and Giuseppe G. Loscocco prepared the tables and figures associated with the article. Ayalew Tefferi and Alessandro M. Vannucchi designed the study and oversaw the project. Ayalew Tefferi wrote the article.

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CONFLICT OF INTEREST STATEMENT

The authors declare no conflicts of interest.

DATA AVAILABILITY STATEMENT

The data that support the findings of this study are available from the corresponding author upon reasonable request.

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