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High *JAK2V617F variant allele frequency* is associated with coronary artery but not aortic valve calcifications in patients with Philadelphia-negative myeloproliferative neoplasms

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Abstract

Background: Patients with Philadelphia-negative myeloproliferative neoplasms (MPNs) have a higher burden of cardiac calcifications compared to the general population. It is not known whether the *JAK2V617F* mutation is associated with increased cardiac calcification.

Aim: To investigate if a higher *JAK2V617F* variant allele frequency (*VAF*) is associated with severe coronary atherosclerosis and the presence of aortic valve calcification (AVC).

Methods: Patients with MPNs were examined by cardiac computer tomography to establish coronary artery calcium score (CACS) and AVC score. The first *VAF* after diagnosis was registered. Severe coronary atherosclerosis was defined as a CACS >400 and AVC was defined as an AVC score >0.

Results: Among 161 patients, 137 were *JAK2V617F* mutation-positive, with a median *VAF* of 26% (interquartile range 12%–52%). A *VAF* in the upper quartile range was associated with a CACS >400 [odds ratio (OR) 15.96, 95% confidence interval [CI] 2.13–119.53, $p = .0070$], after adjustment for cardiovascular risk factors and MPN subtype. An association was not found for the presence of AVC (OR 2.30, 95% CI 0.47–11.33, $p = 0.31$).

Conclusion: In patients with MPNs, there is a significant association between having a *VAF* in the upper quartile (>52%), and severe coronary atherosclerosis, defined as a CACS >400. The presence of AVC is not associated with *VAF*.

KEYWORDS
aortic valve disease, atherosclerosis, coronary, essential, Janus kinase 2, multidetector computed tomography, myelofibrosis, myeloproliferative disorders, polycythemia vera, thrombocythemia

Novelty Statements

What is the new aspect of your work?

We are the first to investigate whether higher *JAK2V617F variant allele frequency* in patients with myeloproliferative neoplasms (MPNs) is associated with severe coronary atherosclerosis or the presence of aortic valve calcifications (AVCs).

What is the central finding of your work?

A *JAK2V617F variant allele frequency* in the upper quartile range (>52%) is associated with severe coronary atherosclerosis, but not with AVCs.

What is (or could be) the specific clinical relevance of your work?

JAK2V617F variant allele frequency might be a useful clinical tool in selecting patients with MPNs with increased risk of atherosclerotic disease, and if these patients get prophylactic treatment earlier, their risk of cardiovascular disease caused by atherosclerotic plaques might decrease.

1 | INTRODUCTION

The classic Philadelphia-negative myeloproliferative neoplasms (MPNs), essential thrombocythaemia (ET), polycythaemia vera (PV), and myelofibrosis (MF) are caused by acquired mutations in the pluripotent stem cells in the bone marrow, resulting in the proliferation of one or more cell types of the myeloid cell linage.[1] The three driver mutations, *JAK2V617F*, *CALR*, and *MPL* leads to activation of the JAK–STAT pathway, resulting in an increased number of mature blood cells, and increased release of pro-inflammatory and pro-thrombotic cytokines.[1] The chronic inflammation in MPNs is maintained by the continuous activation of leucocytes and platelets, resulting in a constant release of inflammatory products into the bloodstream and the tissues, activating more leucocytes and platelets.[2] As a result, one of the major complications of MPNs are thrombotic events, but the pathophysiology of thrombosis in MPNs is multifactorial and complex.[3,4] Inflammation and atherosclerosis are closely linked, and leucocyte activation is recognized as a key player in the development of atherosclerosis, the leading cause of thrombosis in the coronary arteries.[5,6] We have previously shown that patients with MPNs have a higher prevalence of severe coronary atherosclerosis, defined as a coronary artery calcium score (CACS) >400, as well as a higher prevalence of calcifications of the aortic valve (AVC).[7] However, it is not known whether the *JAK2V617F* mutation is associated with a higher burden of cardiac calcifications in patients with MPNs. The primary aim of this study was to investigate if a higher *JAK2V617F* variant allele frequency (*VAF*) is associated with severe coronary atherosclerosis and the presence of AVC.

2 | METHODS

2.1 | Study cohort and examination program

We recruited patients with MPNs from a haematological outpatient clinic in Eastern Denmark, between 2016 and 2018.[7] Eligible patients with a diagnosis of PV, ET, or MF according to the WHO 2008 criteria of MPN classification were included.[8] All patients were above 18 years old and excluded if they were pregnant, unable to understand the written information material, or if their MPN disease was not classified as ET, PV or MF. Information on diabetes mellitus (DM), hypertension, hypercholesterolemia, prior cerebral stroke, ischaemic heart disease (IHD), medication, smoking, and alcohol consumption was registered to outline the patients' cardiovascular risk profile. Systolic and diastolic blood pressure were measured with the patient in a supine position, after 15 min of rest. Body mass index (BMI) was calculated from self-reported values of height and weight, using the standard BMI equation [weight (kg)/height (m)^2]. Routine blood samples were analysed according to normal clinical practice. In *JAK2V617F*-positive patients, the first registered *VAF* after diagnosis (*Initial-JAK2*) was recorded and a follow-up *VAF* (*Followup-JAK2*) was measured at the time of enrolment. *VAF* was determined by quantitative polymerase chain reaction with a routine sensitivity of 0.1% mutated alleles.[9]

Hypertension was defined as the use of antihypertensive medication or blood pressure >140/90 mmHg. Hypercholesterolemia was defined as total cholesterol >5.0 mmol/L or the use of lipid-lowering treatment. Obesity was defined as a BMI >30 kg/m^2.

Low-dose ECG-gated non-contrast cardiac CT was performed on a 256-Slice CT Scanner (Phillips iCT 256), using 220 ms scan time and 2.5 mm slice thickness. A semi-automatic algorithm in the Intellispace Portal and Heartbeat CS Software (Phillips Healthcare) was used to calculate the CACS and AVC scores. Areas with a density >130 Hounsfield units were automatically identified, and manually assessed by an experienced cardiologist.[10] A CACS >400 was defined as severe coronary calcification, AVC was defined as a calcium score of the aortic valve >0, and an AVC score ≥2065 in men and ≥1274 in women were defined as severe AVC.[11,12]

2.2 | Statistics

Continuous data were reported as mean ± standard deviation (SD) or median with interquartile range (IQR). Categorical data were reported as counts and percentages. Comparison between groups was performed

using Students' *t*-test or Mann–Whitney *U* test for continuous data and categorical data, we used χ^2 test or Fishers' test as appropriate.

Logistic regression analyses were used to assess associations between the *JAK2V617F* mutation and CACS >400 or AVC. Patients were divided into five groups; one group for each quartile of *Initial-JAK2*, and one group of patients who were *JAK2V617F* mutation negative, serving as reference group. Similar analyses were conducted on the *Followup-JAK2*. Results were presented as odds ratio (OR) with a 95% confidence interval (CI).

Multivariate models were adjusted for age, sex, prior IHD, stroke, diagnosis (ET, PV, or MF) and having >1 additional cardiovascular risk factor (hypertension, hypercholesterolemia, DM, smoking, family history of IHD or stroke, and/or obesity). In sensitivity analyses presented in the Supporting Information Material, we added leucocytosis, time from *Initial-JAK2* to study examination, or MPN-specific medical treatment to the models. We also conducted sensitivity analyses on patients without prior IHD. Results on the separate MPN subgroups are available in the Supporting Information Material.

We used SAS 9.4 for Windows (SAS Institute Inc.) for all statistical analysis, and figures were made with GraphPad Prism 9 (GraphPad Software). Two-sided *p* values were presented and a $p < .05$ was considered statistically significant.

2.3 | Ethics

The study was conducted in accordance with the Helsinki Declaration of ethical principles for medical research, and approved by the Zealand Regional Committee on Health Research Ethics (SJ-588).[13] Written informed content was obtained from all participants.

2.4 | Data availability statement

The data underlying this article cannot be shared publicly due to the privacy of individuals that participated in the study. Anonymized data will be shared on reasonable request to the corresponding author.

3 | RESULTS

Of the 197 patients screened, 18 patients did either withdraw their consent before the study invitation (13) or did not meet the inclusion criteria after all (5). Out of the 179 remaining patients, 170 were invited to the examination and 161 (mean age 65.5 years, 52% male) completed a cardiac CT (Figure 1). The *JAK2V617F* mutation was present in 137 patients (85%), the *CALR* mutation was present in 17 patients, and the *MPL* mutation was present in 2 patients (Table 1) The remaining five (3%) patients did not have any of the three main driver mutations ('triple negatives'). The median *VAF* in the first *VAF* quantification after diagnosis (*Initial-JAK2*) was 26% (IQR 12%–52%), and the median *VAF* at the time of study examination (*Followup-JAK2*) was 12% (IQR 6%–33%). Patients in the upper quartile (Q4) of *Initial-JAK2* were older, and the frequency of male patients and prior IHD was higher in this group compared with the other quartile groups (Table 1). We did not obtain a quantitative *Followup-JAK2* in 17 of the *JAK2V617F*-positive patients due to technical issues.

3.1 | CACS and the *JAK2V617F VAF*

The median CACS was 35 (IQR 0–453). When dividing the cohort into quartiles according to *VAF*, the highest median CACS was found in patients with a *VAF* in Q4, with a median CACS in the *Initial-JAK2* Q4 group of 569 (IQR 55–1863) and a median CACS in the *Followup-JAK2* Q4 group of 698 (IQR 77–1523) (Tables 2 and S1). A CACS >400 was found in 42 (26%) patients. Ten of these patients had a history of IHD, while 32 patients did not. In the *Initial-JAK2* Q4 56% ($n = 20$) had a CACS >400, and in the *Followup-JAK2* Q4 59% ($n = 17$) of patients had a CACS >400 (Tables 2 and S1). In the 21 patients who were in Q4 of both the *Initial-JAK2* and *Followup-JAK2* 15 (71%) had a CACS >400.

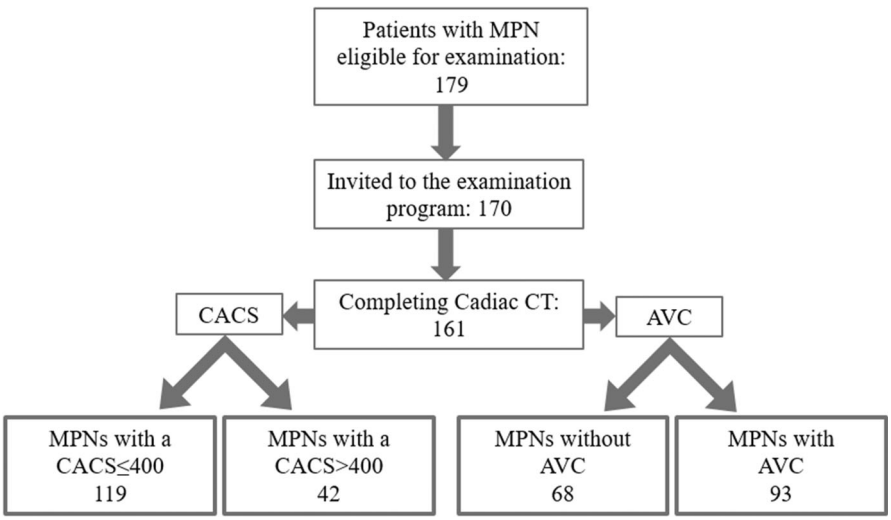

FIGURE 1 Flowchart of the MPN cohort. The cohort has also been presented in a previous study, focusing on differences in CACS and AVC between patients with MPNs and a control group from the general population.[7] AVC, aortic valve calcification; CACS, coronary artery calcium score; CT, computed tomography; MPN, myeloproliferative neoplasms.

TABLE 1 Cohort characteristics.

Initial JAK2V617F variant allele frequency[a] (%, range)	*JAK2V617F* negative 0%	*JAK2V617F* positive Q1 0.4%–11.9%	Q2 12.0%–25.9%	Q3 26.0%–51.9%	Q4 52.0%–97.0%	*p* Value
N (%)	24 (15)	33 (21)	34 (21)	34 (21)	36 (22)	.60
Age (years, mean ± SD)	63.5 ± 10.7	64.3 ± 10.6	62.5 ± 10.2	66.0 ± 11.2	70.3 ± 8.4	.0056
Sex (male), *N* (%)	12 (50)	17 (51)	13 (38)	18 (53)	24 (67)	.22
Ischaemic heart disease, *N* (%)	2 (8)	3 (9)	0	2 (6)	5 (14)	.27
Stroke, *N* (%)	2 (8)	8 (24)	12 (35)	10 (29)	6 (17)	.12
Diabetes mellitus, *N* (%)	4 (17)	1 (3)	2 (6)	1 (3)	3 (8)	.25
Hypertension, *N* (%)	14 (58)	21 (63)	27 (79)	20 (59)	20 (56)	.26
Hypercholesterolemia, *N* (%)	12 (50)	21 (64)	22 (65)	19 (56)	16 (44)	.39
Smoking (ever), *N* (%)	8 (33)	22 (67)	20 (59)	17 (50)	17 (47)	.13
Obesity (BMI > 30 kg/m^2), *N* (%)	3 (12)	8 (24)	7 (20)	5 (15)	4 (11)	.57
Essential thrombocythaemia, *N* (%)	8 (33)	10 (30)	12 (35)	5 (15)	3 (8)	.14
Polycythaemia vera, *N* (%)	2 (8)	22 (67)	21 (62)	24 (70)	26 (72)	.0005
Myelofibrosis, *N* (%)	14 (58)	1 (3)	1 (3)	5 (15)	7 (20)	.0004
Duration of MPN disease (years, median IQR)	4 (2.5–8)	6 (4–10)	6.5 (3–10)	6.5 (2–10)	9 (3.5–18)	.12
Haemoglobin (mmol/L, mean ± SD)	7.75 ± 0.97	8.59 ± 1.12	8.41 ± 0.98	8.33 ± 0.88	7.73 ± 1.11	.0009
Haematocrit fraction (mean ± SD)	0.38 ± 0.05	0.42 ± 0.05	0.41 ± 0.04	0.42 ± 0.04	0.39 ± 0.05	.0040
Platelet count ($\times 10^9$/L, mean ± SD)	406 ± 220	313 ± 110	343 ± 180	324 ± 167	288 ± 174	.17
Leucocyte count ($\times 10^9$/L, median, IQR)	5.0 (4.2–8.5)	6.0 (4.3–6.6)	5.3 (4.3–6.7)	5.6 (4.8–7.1)	5.9 (4.3–11.8)	.67
Hydroxycarbamid, *N* (%)	7 (29)	10 (30)	7 (21)	12 (35)	5 (14)	.26
Ruxolitinib, *N* (%)	1 (4)	3 (9)	6 (18)	6 (18)	20 (56)	<.0001
Peginterferon alfa-2a, *N* (%)	9 (38)	12 (36)	15 (44)	15 (44)	18 (50)	.80
Anagrelide, *N* (%)	2 (8)	1 (3)	4 (12)	1 (3)	0	.15
No MPN specific treatment[a], *N* (%)	6 (25)	9 (27)	6 (18)	4 (12)	4 (11)	.31
Single treatment strategy[a], *N* (%)	17 (71)	22 (67)	24 (71)	26 (76)	21 (58)	.58
Combination treatment[a], *N* (%)	1 (4)	2 (6)	4 (12)	4 (12)	11 (31)	.029
Time from diagnosis to *Initial-JAK2*[b] (years, median, IQR)	–	3 (0–6)	4.5 (0–8)	3 (0–6)	5 (0–14)	.097
Time from *Initial-JAK2*[b] to study (years, median, IQR)	–	4 (2–4)	3 (1–4)	3 (1–4)	3 (3–4)	.18
CALR mutation positives, *N*	17	–	–	–	–	–
MPL mutation positives, *N*	2	–	–	–	–	–

Abbreviations: AVC, aortic valve calcification; CACS, coronary artery calcium score; IQR, interquartile range; MPN, myeloproliferative neoplasms; *N*, number of patients; SD, standard deviation.

[a]Hydroxycarbamid, ruxolitinib, anagrelide, and/or peginterferon alfa-2a.

[b]*Initial-JAK2* = first quantification of *JAK2V617F* variant allele frequency after diagnosis.

In the multivariate logistic regression analysis, the only group with significant association with a CACS>400 was the *Initial-JAK2* Q4 group (OR 15.95, 95% CI 2.13–119.53, $p = .0070$), while the association with a CACS >400 in the other quartile groups did not differ significantly from the *JAK2V617F* mutation-negative patients (Figure 2A). The sensitivity analyses did not change the overall results, that an *Initial-JAK2* in Q4 was associated with a CACS >400, regardless of how we adjusted (Figure S1a–d). The association between a CACS >400 and being in the Q4 was also confirmed in analyses on the *Followup-JAK2* quartile groups, where both Q3 and Q4 were significantly associated with a CACS >400 after adjustment (Table S2).

3.2 | AVCs and the *JAK2V617F VAF*

The median AVC score was 27 (IQR 0–172). The highest median AVC score was found in patients with a *VAF* in Q4, with a median AVC score in the *Initial-JAK2* Q4 of 119 (IQR 1–394) and a median AVC score in the *Followup-JAK2* Q4 of 119 (IQR 24–381) (Tables 2 and S1). AVC was present in 93 (58%) of the patients with MPNs, in 27 (75%) of patients with MPNs with an *Initial-JAK2* in Q4, and in 24 (83%) of patients with MPNs with a *Followup-JAK2* in Q4 (Tables 2 and S1). Seven (4.4%) patients had an AVC score indicating severe AVC; six were *JAK2V617F*

TABLE 2 Overview of CACS and AVC according to initial *JAK2V617F* variant allele frequency.[a]

Cardiac calcifications	*JAK2V617F* mutation negatives	*Initial-JAK2 VAF* Q1	*Initial-JAK2 VAF* Q2	*Initial-JAK2 VAF* Q3	*Initial-JAK2 VAF* Q4	*p* Value
N	24	33	34	34	36	
CACS, median (IQR)	13 (0–212)	20 (0–375)	14 (0–152)	25 (0–307)	569 (54–1863)	.0013
CACS >400, *N* (%)	4 (17)	7 (21)	5 (15)	6 (18)	20 (56)	.0007
AVC, *N* (%)	12 (50)	18 (55)	17 (50)	19 (56)	27 (75)	.19
Severe AVC, *N* (%)	1 (4)	1 (3)	1 (3)	1 (3)	3 (8)	.86
AVC score, median (IQR)	24 (0–130)	8 (0–131)	3 (0–122)	24 (0–206)	119 (1–394)	.066

Abbreviations: AVC, aortic valve calcification; CACS, coronary artery calcium score; IQR, interquartile range; *N*, number of patients; VAF, *JAK2V617F* variant allele frequency.
[a]First *VAF* quantified after diagnosis.

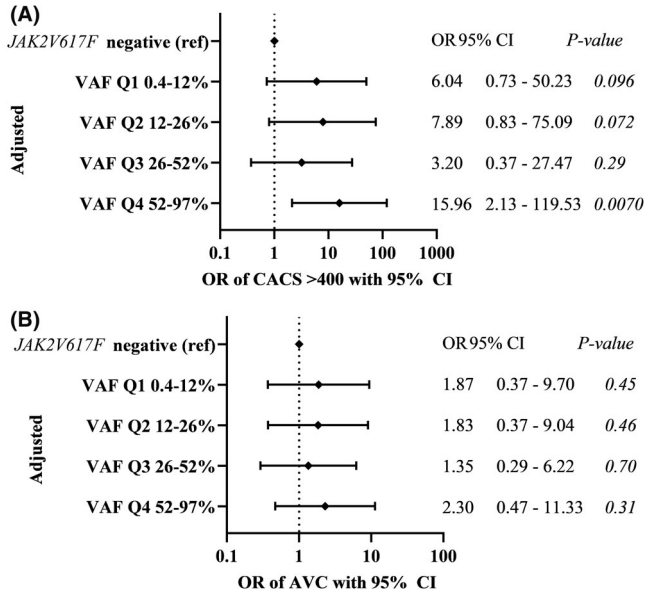

FIGURE 2 Associations between *JAK2V617F* allele variant frequency and CACS >400 or AVC in patients with MPNs. (A) The odds ratios of having a CACS >400 in each quartile group of *VAF*, compared to patients with MPNs who are *JAK2V617F* mutation negative. (B) The odds ratios of having AVC in each quartile group of *VAF*, compared to patients with MPNs who are *JAK2V617F* mutation negative. Both models have been adjusted for age, sex, ischaemic heart disease, stroke, diagnosis (ET, PV, and MF) and having >1 additional cardiovascular risk factor (hypertension, hypercholesterolemia, diabetes mellitus, smoking, family history of IHD or stroke, and/or obesity). AVC, aortic valve calcifications; CACS, coronary artery calcium score; CI, confidence interval; ET, essential thrombocythaemia; IHD, ischaemic heart disease; MF, myelofibrosis; MPN, myeloproliferative neoplasms; OR, odds ratio; PV, polycythaemia vera; *VAF*, *JAK2V617F* variant allele frequency.

mutation-positive, two had an *Initial-JAK2* in Q4, and three had a *Followup-JAK2* in Q4.

The multivariate logistic regression analysis on *Initial-JAK2* Q4 and the presence of AVC showed that none of the *Initial-JAK2* quartile groups differed significantly from the *JAK2V617F* mutation-negative patients when investigating associations with the presence of AVC (Figure 2B). The sensitivity analyses on *Initial-JAK2* did not alter our overall results, and neither did the analyses on the *Followup-JAK2* quartile groups (Figure S2a–d and Table S2).

Characteristics and logistic regression analyses on the individual subgroups of MPNs (ET, PV, and MF) are presented in Tables S3–S5.

4 | DISCUSSION

The primary findings in this study are; (1) There is a significant association between a *VAF* in the upper quartile range and severe cardiac atherosclerosis defined as a CACS >400 in patients with MPNs, (2) there is not a significant association between *VAF* and AVC, regardless of *VAF* quartile group.

4.1 | The *JAK2V617F* mutation

The *JAK2V617F* mutation was first described in 2005.[14] It is considered one of three driver mutations of MPNs, and is found in most patients with PV, and approximately 50% of patients with ET and MF.[15] The mutation is not only found in the classic MPNs but also in some other myeloid neoplasms, as well as in clonal haematopoiesis of indeterminate potential (CHIP), who are free of overt myeloid disease.[15] The *JAK2V617 VAF* is usually higher in patients with PV and MF, and lower in patients with ET, where it rarely exceeds 50%. When the mutation is found in patients without MPNs, the VAF is usually very low (<2%).[15] In our cohort, we have three patients who were registered with ET at inclusion and therefore are listed as ET in this paper. These three patients have later been reclassified as having pre-MF or post-ET/PV MF, showing how real-world classifications can be difficult before all pieces of the diagnostic puzzle are present. We have chosen to look at the *JAK2V617F VAF* in this study, as it is a well-described mutation, and the mutation most commonly found in the classic MPNs, but we are well aware that how the *JAK2V617F* mutation is related to the development of atherosclerosis in MPNs is still a fairly new area of research. The discovery of CHIP and how CHIP is associated with increased risk of cardiovascular disease in patients without myeloid disease will hopefully shed more light on the role of the mutation in atherosclerotic plaque formation, both in

patients with CHIP where the VAF is low, and in MPNs who have a higher VAF.[16,17]

4.2 | Coronary atherosclerosis

The pathophysiology of arterial thrombosis in patients with MPNs is highly complex and combines both classical risk factors of arterial thrombosis, as well as MPN-specific risk factors.[3,4] Animal studies have shown increased development of atherosclerotic plaques, and increased prevalence of plaque instability, in mice with the *JAK2V617F* mutations compared to mice with wild type *JAK2*.[18] The pathophysiological mechanisms linking the *JAK2V617F* mutation and the formation of atherosclerotic plaques in non-MPNs are not fully understood, but increased leucocyte binding to the endothelium, and formation of neutrophil extracellular traps are some of the possible processes involved.[17]

In a recent review on the pathogenesis of cardiovascular events in patients with MPNs, it is stated that 'atherosclerosis seems to be favoured by *JAK2V717F* blood cells, but that there is a need for more data on MPN and atherosclerosis in human cohorts'.[3] To our knowledge, only three studies on patients with MPNs and coronary atherosclerosis have been published, making this a relatively new research territory, even though the link was proposed years ago.[7,19–21] However, none of these studies have investigated the relationship between *VAF* and coronary atherosclerosis. The studies have shown that there is an increased prevalence of severe coronary atherosclerotic plaques in patients with MPNs compared to controls from the general population and that there is a lack of correlation between the Framingham risk score and the coronary calcium burden in *JAK2V617F* positive ET patients.[7,20] An association between higher *VAF* and arterial thrombosis in *JAK2V617F* positive PV and ET patients have been reported, but other studies have not found this association.[22–25] We are the first to show that a higher *VAF* is associated with higher levels of calcified coronary plaques and severe coronary atherosclerosis in patients with MPNs, which might contribute to the increased risk of arterial thrombosis in these patients.[22–27] Although our results cannot show causality between *VAF* and severe coronary atherosclerosis, we hypothesize that *VAF* could be important to guide if MPN patients should receive treatment directed at preventing atherosclerotic disease, but further studies designed to show causality and risk modification are needed.

4.3 | Aortic valve calcification

The development of AVC in the general population is known to be multifactorial and involve haemomechanics, such as changes in shear stress on the valve, systemic inflammation and local molecular signalling, that can all be affected in patients with MPNs, regardless of mutation status.[28] We are not aware of studies investigating if the *JAK2V617F* mutation or *VAF* is associated with AVC or aortic stenosis in patients with MPNs, and we have not found studies investigating *JAK2V617F* mutations in non-MPN patients with aortic valve stenosis. As our previous study showed that MPN patients have a higher prevalence of AVC compared to the general population, we found it relevant to investigate if there was an association between AVC and *VAF* in patients with MPNs.[7] There was, however, no such association, regardless of the *VAF* quartile group. Our previous findings call for future studies on MPNs and aortic valve disease, investigating whether patients with MPNs might benefit from screening with either cardiac CT or echocardiography, to unmask early signs of aortic valve disease. However, it does not seem that *VAF* would be a useful tool in selecting which patients could benefit from such screening.

4.4 | Limitations

Although our study brings novel information about how *VAF* is associated with cardiac calcifications in patients with MPNs, it has some limitations. First, we are not able to show causality, only associations, as we have no follow-up data on the cohort, and we cannot know for certain whether a higher AVC score or CACS affects mortality in patients with MPNs. Second, we have limited statistical power, especially when performing subgroup analyses, why our focus is on the MPN cohort as a whole. Third, we do not have access to the cohort's blood counts at the time of diagnosis, nor how long they have received their current MPN treatment regime. This would have added interesting information on the cohort characteristics, but it is not likely to have changed our overall result.

4.5 | Conclusion

The main findings of this study are that there is a significant association between a *VAF* in the upper quartile (≥52%) and severe coronary calcifications in patients with MPNs, measured as a CACS >400. A higher *VAF* level is not associated with the presence of AVC.

AUTHOR CONTRIBUTIONS

Camilla Nordheim Solli, Sandra Chamat-Hedemand, and Niels Eske Bruun: Design of the project, analysis of data, draft of the manuscript. **Hanne Elming:** Design of the project, analysis of data, critical revision of data, reviewing and editing the manuscript. **Vibe Skov:** Design of the project, extraction of data, critical revision of data, reviewing and editing the manuscript. **Anh Ngo, Lasse Kjær, Anders Lindholm Sørensen, Hans Hasselbalch, and Christina Ellervik:** Design of the project, critical revision of data, reviewing and editing the manuscript. All authors have read and approved the final manuscript.

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CONFLICT OF INTEREST STATEMENT

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SUPPORTING INFORMATION

Additional supporting information can be found online in the Supporting Information section at the end of this article.

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