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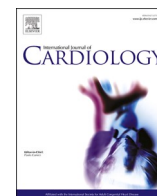
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Coronary artery- and aortic valve calcifications in patients with Philadelphia-negative myeloproliferative neoplasms

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ABSTRACT

Background: Patients with the hematological cancers Philadelphia-negative Myeloproliferative Neoplasms (MPNs) have an increased risk of cardiovascular disease. However, whether MPNs have an increased burden of cardiac calcification has not been thoroughly investigated. Our aim is to investigate whether patients with MPNs have an increased burden of cardiac calcification that could help explain their increased risk of cardiovascular disease.

Methods and results: We recruited 161 patients (mean age 65 years, 52% men) with an MPN diagnosis between 2016 and 2018. Coronary artery calcium score (CACS) and aortic valve calcification (AVC) were measured by cardiac computer tomography, and detailed information on cardiovascular risk factors was recorded. MPNs were matched on age and sex, with 805 controls from the Copenhagen General Population Study. A CACS > 400 was present in 26% of MPNs and 19% of controls ($p = 0.031$). AVC was present in 58% of MPNs and 34% of controls ($p < 0.0001$). After adjustment for cardiovascular risk factors, the odds ratio (OR) of a CACS > 400 was 1.9 (95% CI 1.2–3.1, $p = 0.008$) in MPNs compared to controls, and the OR of AVC was 4.4 (95% CI 2.9–6.9, $p < 0.0001$) in MPNs compared to controls.

Conclusion: Patients with MPNs have a significantly higher prevalence of a CACS > 400 and AVC, compared to controls from the general population. The association between MPN and a CACS > 400 or AVC remains significant after adjustment for cardiovascular risk factors. These novel data support the hypothesis that MPNs have an increased burden of cardiac calcifications, independent of other cardiovascular risk factors.

Abbreviations: MPNs, Philadelphia-negative chronic myeloproliferative neoplasms; ET, essential thrombocythemia; PV, polycythemia vera; MF, myelofibrosis; CACS, coronary artery calcium score; AVC, calcification of the aortic valve; CVD, cardiovascular diseases; IHD, ischemic heart disease; DM, diabetes mellitus; BMI, Body Mass Index.

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¹ This author takes responsibility for all aspects of the reliability and freedom from bias of the data presented and their discussed interpretation.

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1. Introduction

The Philadelphia-negative chronic myeloproliferative neoplasms (MPNs) essential thrombocythemia (ET), polycythemia vera (PV), and myelofibrosis (MF) are chronic hematological cancers caused by an acquired genetic defect in the pluripotent stem cell in the bone marrow. Three genetic mutations have been identified as driver mutations in MPNs. The most common genetic mutation is the JAK2V617F-mutation, affecting 98% of PV patients and 50–60% of ET and MF patients. [1] In ET and MF, most of the remaining patients have the CALR mutation (20–30%) or the MPL mutation (2–5%) [1]. All three driver mutations of MPNs affect proteins in the JAK-STAT pathway. It is not known what triggers the mutations that leads to proliferation of myeloid cells (erythrocytes, platelets and/or leucocytes), but chronic inflammation is considered to be an important pathogenic factor in the development and sustenance of MPNs [2,3].

A substantial part of the morbidity and mortality rates seen in MPN patients are attributed to thrombotic and thromboembolic complications. Acute myocardial infarctions and strokes are common arterial manifestations [4,5]. The increased risk of thrombi is partly explained by increased peripheral myeloid cell count and local inflammatory activation of endothelium, platelets and leucocytes [6]. Whether development of atherosclerosis plays a part in this increased risk has not been thoroughly examined, although it is well known that MPN patients have increased systemic inflammation, and that inflammation leads to development of atherosclerosis [2,3,7,8].

Atherosclerotic plaque burden in the coronary arteries is a manifestation of atherosclerosis and can be assessed by the coronary artery calcium score (CACS) using cardiac computer tomography (CT). CACS is a measure of both size and density of calcified plaques in the coronary arteries [9]. Increased CACS is an independent risk factor for future cardiovascular diseases (CVD) [10,11]. The American College of Cardiology guidelines states that a CACS >400 entails a high risk of CVD, while a CACS of 101–399 entails an intermediate risk of CVD. [10] In the European Society of Cardiology 2016 guidelines a CACS >300 indicates an increase in CVD risk [11].

Cardiac CT is also used to assess calcification of the aortic valve (AVC), a process that shares many similarities with the development of cardiac atherosclerosis [12]. Measurement of AVC is most commonly used to assess aortic valve stenosis in patients where echocardiographic measurements are inconclusive, but AVC also reflects systemic chronic inflammation and atherosclerotic burden [12–14].

Several studies show that CACS is an independent prognostic predictor of CVD in both symptomatic and non-symptomatic CVD patients [15–18]. However, only two small studies from a single cohort have investigated CACS levels in ET, a subgroup of MPN patients [19,20]. No studies have investigated AVC in MPNs. In this study, we aim to investigate whether MPN patients have an increased burden of cardiac calcification that could help explain their increased risk of CVD, by comparing CACS and AVC in a cohort of MPN patients with a matched cohort from the Copenhagen General Population Study (CGPS).

2. Methods

2.1. Study cohort

2.1.1. MPNs

We recruited MPN patients from a specialized hematological outpatient clinic at Zealand University Hospital in Denmark, between 2016 and 2018. Eligible patients with a diagnosis of PV, ET, or MF according to the WHO 2008 criteria of MPN classification were included [21]. MPNs were divided into subgroups of MPN-disease (ET, PV, or MF) according to their first confirmed MPN diagnosis. 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.

2.1.2. Controls

MPNs were exact-matched 1:5 on age and sex with participants from CGPS. The CGPS is a large ongoing population study, and participants are considered representative of the general Danish population [22]. Cardiac CT has been implemented from 2010 [22]. Inclusion criteria for cardiac CT in CGPS were age ≥ 40 with normal kidney function (s-creatinine $<100 \mu\text{mol/L}$).

2.2. Coronary artery calcium score and measurement of aortic- and mitral valve calcification

Cardiac CT was performed as a low-dose electrocardiogram (ECG)-gated non-contrast examination. Areas with a tissue density above the attenuation threshold of 130 Hounsfield units were automatically identified, and manually assessed. A semi-automatic algorithm was used to calculate a total CACS summarizing the calcium score of the three major coronary arteries, by the Agatston method [9]. The individual calcium scores of the mitral- and aortic valves were calculated to establish presence of aortic- and mitral valve calcification. In MPNs a 256-slice CT-scanner (Phillips iCT 256), with 2.5 mm slice thickness was used. In the CGPS cardiac CT was performed using a 320-multidetector CT scanner (Aquilion One, Toshiba Medical Systems, Japan) and for the non-contrast analyses the images were reconstructed in 3.0 mm slice thickness [23,24].

2.3. Participant characteristics

In both groups, participants completed a detailed questionnaire and information on diabetes mellitus (DM), hypertension, hypercholesterolemia, prior stroke or ischemic heart disease (IHD), medication, smoking, and alcohol consumption was registered in order to outline the patients cardiovascular risk profile. If patients displayed symptoms of cardiac disease at the time of examination, they were referred to their local department of cardiology for further evaluation. Data from these follow-ups were not accessible to this study. Blood samples were drawn for selected blood analyses. Systolic and diastolic blood pressures were measured with the patient in a supine position, after a period of minimum 15 min of rest. Body Mass Index (BMI) was calculated from self-reported values of height and weight.

Hypertension was defined by 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 such as statins. Estimated glomerular filtration rate (eGFR) was calculated by the CKD-EPI formula [25].

2.4. Statistics

Continuous data were reported as mean and standard deviation (SD) or median with interquartile range (IQR) as appropriate. Categorical data were reported as count and percentage. Comparison between groups was performed using Students' *t*-test or Mann-Whitney *U* test for continuous data and for categorical data we used χ^2 -test or Fishers' test as appropriate. Two-sided *p*-values were presented.

To examine the association between MPN disease and CACS we performed both logistic and linear regression analyses. Our primary analyses were univariate and multivariate logistic regression analyses on the association with CACS >400, and on the association with AVC > 0. We performed supplemental multivariate logistic regression analyses on CACS >0 and CACS >300. Results were presented as odds ratio (OR) with 95% confidence interval (CI).

As CACS were not normally distributed, we logarithmically transformed the CACS after adding 1 to all values, as many participants had a CACS of zero. We performed supplemental linear regression models on $\log(\text{CACS}+1)$. Exponentiation of the coefficients was performed to interpret the impact of the independent variables on the association with CACS, reported as percentage difference in CACS with 95% CI. Thus,

results are inferred as the percent-wise difference in CACS that would occur if all other covariates were unchanged, except for the covariate of interest. All multivariate models were adjusted for age, sex, IHD, stroke, hypertension, hypercholesterolemia, DM, smoking, and obesity. A p -value below 0.05 was considered statistically significant. We used SAS 9.4 for Windows (SAS Institute Inc. NC, USA) for all statistical analysis.

2.4.1. 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) [26]. The CGPS was approved by the Ethics Committee of the Capital Region of Denmark (H-KF-01-144/01). Informed consent was obtained from all subjects prior to examination.

3. Results

3.1. Patient inclusion and patient characteristics

From a total of 197 MPN patients screened, 161 MPN patients (mean age 65 years \pm 10, 52% men) completed the cardiac CT and were matched 1:5 with participants from CGPS, resulting in 805 controls (Fig. 1). PV was by far the largest subgroup of MPNs (95 patients, 59%), followed by 38 patients (24%) with ET and 28 (17%) patients with MF. Hypertension and hypercholesterolemia were frequent in both MPNs and controls (63% vs 67%, $p = 0.42$ and 56% vs 77%, $p < 0.0001$, respectively), as well as a history of smoking (52% vs 58%, $p = 0.16$) (Table 1). There were more active smokers among controls than MPNs (11% vs 4%, $p = 0.008$) and controls had a higher total cholesterol level (5.5 mmol/l, SD 1.0 vs. 4.6 mmol/l, SD 1.4, $p < 0.0001$) (Table 1). MPNs had a higher frequency of use of acetylsalicylic acid (83% vs. 14%, $p <$

0.0001) and statins (26% vs 19%, $p = 0.038$) (Table 1). Stroke was registered in 24% of MPNs and 5% of controls ($p < 0.0001$), while IHD was registered in 8% of MPNs and 9% of controls ($p = 0.45$) (Table 1).

3.2. CACS and prevalence of calcium deposits in various parts of the heart

The range of CACS in the total study population ($n = 966$) was 0 to 7067, with a median CACS of 35 (IQR 0–453) in the MPNs and 35 (IQR 0–236) in the control group ($p = 0.17$). A CACS >400 was significantly more frequent in MPNs compared with controls (26% vs 19%, $p = 0.031$) (Fig. 2). In the MPN subgroups, a CACS >400 was recorded in 9 ET patients (24%), 22 PV patients (23%), and 11 MF patients (39%). There was no statistically significant difference between the subgroups' prevalence of a CACS >400 ($p = 0.22$). AVC was present in 58% of MPNs and 34% of controls ($p < 0.0001$), and mitral valve calcification was present in 17% of MPNs and 19% of controls ($p = 0.71$) (Fig. 2 and supplemental table 1). Frequency of calcified plaques in the individual coronary arteries are displayed in Supplemental Table 1. In the MPN subgroups, AVC was present in 21 ET patients (55%), 53 PV patients (56%), and 19 MF patients (68%), with no significant difference between the subgroups ($p = 0.20$).

A univariate analysis of MPN disease vs. controls on CACS >400 showed an OR of 1.52 (95% CI 1.04–2.29, $p = 0.031$) (Supplemental Table 2). After adjusting for cardiovascular risk factors, the MPN disease was still associated with a CACS >400 compared with controls (OR of 1.92 [95% CI 1.19–3.10], $p = 0.008$) (Fig. 3a). In addition, age, male sex, hypercholesterolemia, and prior IHD were significantly associated with CACS >400 (Fig. 3a). In a sensitivity analysis, using a CACS >300 threshold, we found similar results, with an OR of 2.10 (95% CI 1.34–3.30, $p = 0.001$) in MPNs (Supplemental Table 3).

The univariate logistic regression model on AVC and MPN disease

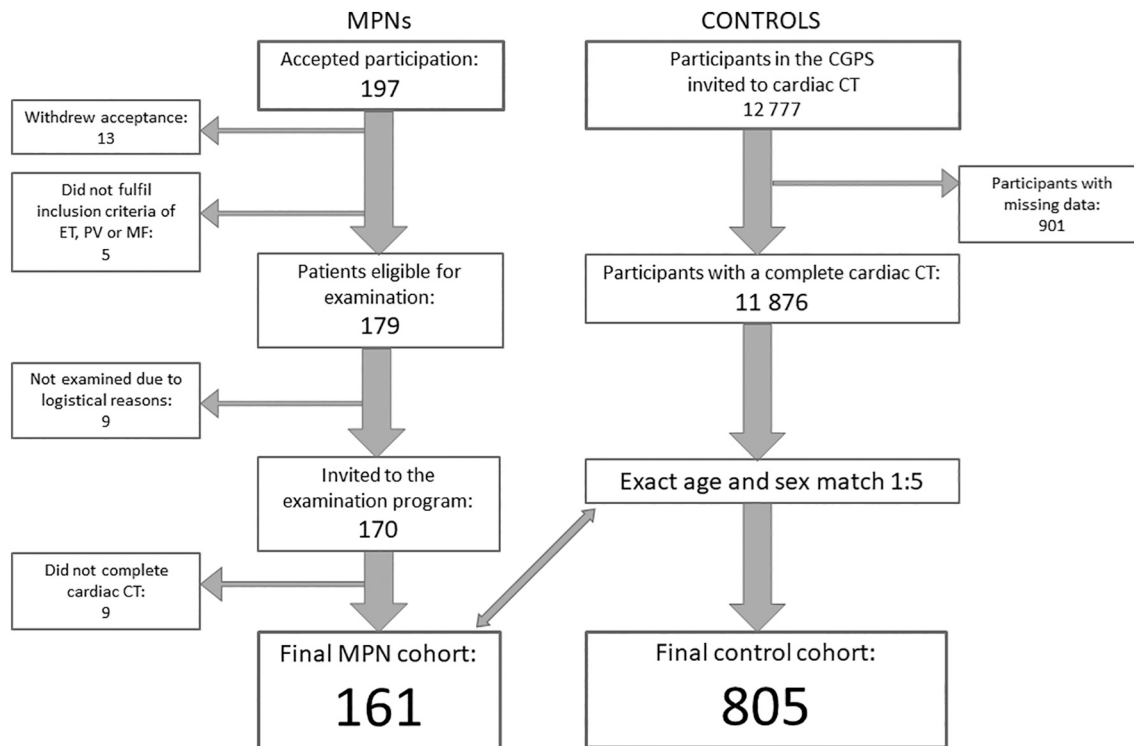


Fig. 1. Selection of study participants.

The figure illustrates a flowchart of the selection of MPNs and controls.

Among the identified 197 patients with MPN, 13 withdrew acceptance, 5 did not meet the inclusion criteria for ET, PV or MF, 9 were not able to participate in the physical examinations, and 9 did not complete cardiac CT. After the inclusion and examination of MPNs, these were matched with controls from CGPS. CGPS, Copenhagen General Population Study; CT, Computer Tomography; ET, Essential thrombocythemia; MF, myelofibrosis; MPN, Philadelphia-negative myeloproliferative neoplasms; PV, Polycythemia Vera.

Table 1
Characteristics of MPNs and controls.

	MPNs (n = 161)	Controls (n = 805)	P-value
Age (years, mean ± SD)	65.5 ± 10.5	65.5 ± 10.3	0.98
Male sex, N (%)	84 (52)	416 (52)	0.91
Ischemic heart disease, N (%)	12 (8)	75 (9)	0.45
Stroke, N (%)	38 (24)	40 (5)	<0.0001
Diabetes Mellitus, N (%)	11 (7)	40 (5)	0.34
Hypertension, N (%)	102 (63)	534 (67)	0.42
Hypercholesterolemia, N (%)	90 (56)	615 (77)	<0.0001
Systolic blood pressure (mmHg), mean ± SD	140 ± 19	146 ± 22	0.0047
Diastolic blood pressure (mmHg), mean ± SD	79 ± 10	86 ± 12	<0.0001
Smoking ^a , N (%)	84 (52)	461 (58)	0.16
Package years among smokers, mean ± SD	22.5 ± 20	21.3 ± 17.4	0.84
Alcohol >14 units/week, N (%)	41 (26)	217 (27)	0.69
BMI (kg/m ²), mean ± SD	25.8 ± 4.1	26.5 ± 4.3	0.077
Obesity ^b , N (%)	27 (17)	144 (18)	0.73
Acetylsalicylic Acid, N (%)	134 (83)	114 (14)	<0.0001
Statins, N (%)	42 (26)	150 (19)	0.038
Total Cholesterol (mmol/L), mean ± SD	4.6 ± 1.4	5.5 ± 1.0	<0.0001
Cholesterol HDL (mmol/L), mean ± SD	1.5 ± 0.6	1.6 ± 0.5	0.0033
Cholesterol LDL (mmol/L), mean ± SD	2.4 ± 1.2	3.1 ± 0.9	<0.0001
eGFR (mL/min/1.73m ²), mean ± SD	80 ± 17	79 ± 13	0.68
Creatinine (μmol/L), mean ± SD	81 ± 23	79 ± 13	0.25
hs-CRP (mg/L), mean ± SD	3.4 ± 9.3	2.1 ± 3.3	0.60

eGFR, estimated glomerular filtration rate; HDL, High Density Lipoprotein; hs-CRP, High sensitivity C-Reactive Protein; LDL, Low density lipoprotein; SD, Standard deviation.

^a A history of ever smoking.

^b Defined as a BMI > 30 kg/m².

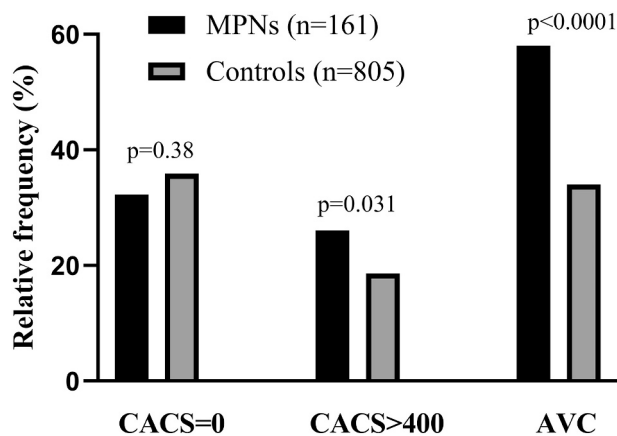


Fig. 2. Distribution of CACS and AVC in MPNs and controls.

The figure shows the distribution of CACS and AVC in MPNs and controls. The prevalence (%) of MPNs ($n = 161$) and controls ($n = 805$) is illustrated for CACS = 0, CACS>400 and AVC. AVC, aortic valve calcification; CACS, coronary artery calcium score; MPN, Philadelphia-negative myeloproliferative neoplasms.

showed an OR of 2.61 (95% CI 1.85–3.68, $p < 0.0001$). In the adjusted multivariate logistic regression model the OR of AVC in MPNs was 4.44 (95% CI 2.86–6.88, $p < 0.0001$) (Fig. 3b). Age, male sex and hypercholesterolemia were also significantly associated with AVC after adjustment (Fig. 3b).

Coronary calcified plaques (CACS>0) were common in both MPN patients ($n = 109$, 68%) and controls ($n = 516$, 64%). After adjustment for cardiovascular risk factors, there was no difference between MPNs and controls (Supplemental Fig. 1). However, age, male sex, hypertension, hypercholesterolemia, and smoking (ever) were significantly

associated with a CACS>0. (Supplemental Fig. 1).

In a multivariate linear regression model on logCACS+1, MPNs had a 52% (95% CI 1%–127%, $p = 0.044$) higher CACS compared with the control group. In addition, age, male sex, hypertension, hypercholesterolemia, prior IHD, DM, and a smoking (ever) were significantly associated with a higher CACS after adjustment (Supplemental Table 4).

4. Discussion

In this study, we investigated the atherosclerotic burden in a cohort of MPN patients and compared them to a cohort from the general population. The key findings were: (i) MPN patients had a significantly higher prevalence of CACS>400 and AVC compared to controls from the general population; (ii) MPNs were significantly associated with a CACS>400 and with AVC, even after adjustment for cardiovascular risk factors.

Even though the link between chronic inflammation and the development of atherosclerosis is well documented, it has not been thoroughly investigated if the increased risk of CVD in MPNs is associated with an increased prevalence of atherosclerotic plaques or cardiac calcification [7,8]. We examined the relationship between CACS and MPNs in several ways. Our primary focus was on CACS>400 and AVC. As mentioned in the American guidelines, a threshold of CACS>400 is clinically relevant and associated with an increased risk of CVD [10]. CACS have prognostic value on all-cause mortality, as shown in a study of 25,253 asymptomatic patients with a mean follow-up of 6.8 years, where patients with a CACS of 400–699 had a relative risk of all-cause death of 5.78 (95% CI 3.00–11.16) compared with those with a CACS of 0 [27]. The relative risk was even greater for higher CACS values [27]. We found that MPN patients had a higher prevalence of CACS>400 compared to the general population, and that the MPNs had a significantly higher OR for CACS>400 even after adjustment for traditional cardiovascular risk factors. To comply with the European 2016 guidelines we also tested if the OR of MPN on CACS changed when the cut-off level of CACS was lowered to 300 [11]. The results were consistent with our primary analysis.

As for AVC, it has been shown to predict all-cause mortality independently of CACS and severe aortic stenosis, and also predict non-fatal myocardial infarctions and non-fatal cerebrovascular events in a cohort of 1529 individuals with a moderate- to high CVD risk [28]. In a cohort of 6685 participants free of clinical CVD from the Multi-Ethnic Study of Atherosclerosis, participants with AVC had a significantly higher risk of cardiovascular events and cardiovascular mortality compared to participants with no AVC [29]. Our results of increased prevalence and odds of CACS>400 and AVC in MPNs are novel information, and could suggest that CACS and AVC might be important predictors of both future CVD and all-cause mortality in MPN patients.

There are currently only two published studies on MPNs and CACS; one cross-sectional study and one follow-up study on the same small cohort of 40 ET patients [19,20]. In these studies, CACS was one of several different variables used to compare the ET patients and controls, revealing no difference in crude CACS (median CACS 0.1 [IQR 0–16.85] vs. 0 [IQR 0–8.55], $p = 0.26$) [19,20]. Patients and controls were matched on age and sex, as well as some classic risk factors, but matching was with some reservations and the authors did not perform multivariate analyses to adjust [19]. However, they did find that patients with ET had a significantly higher prevalence of CACS >160 compared to controls [19]. After a four year follow-up time, there was no significant difference in how much CACS changed over time between the ET patients and controls [20].

Our study showed that MPNs as a group have increased prevalence of cardiac calcification, both in the coronary arteries and the aortic valve, and this is the first time this has been shown for MPNs in general. Our results support the assumption that the increased risk of CVD in MPNs could in part be due to a higher burden of cardiac calcification. We examined the association between MPN and the crude CACS

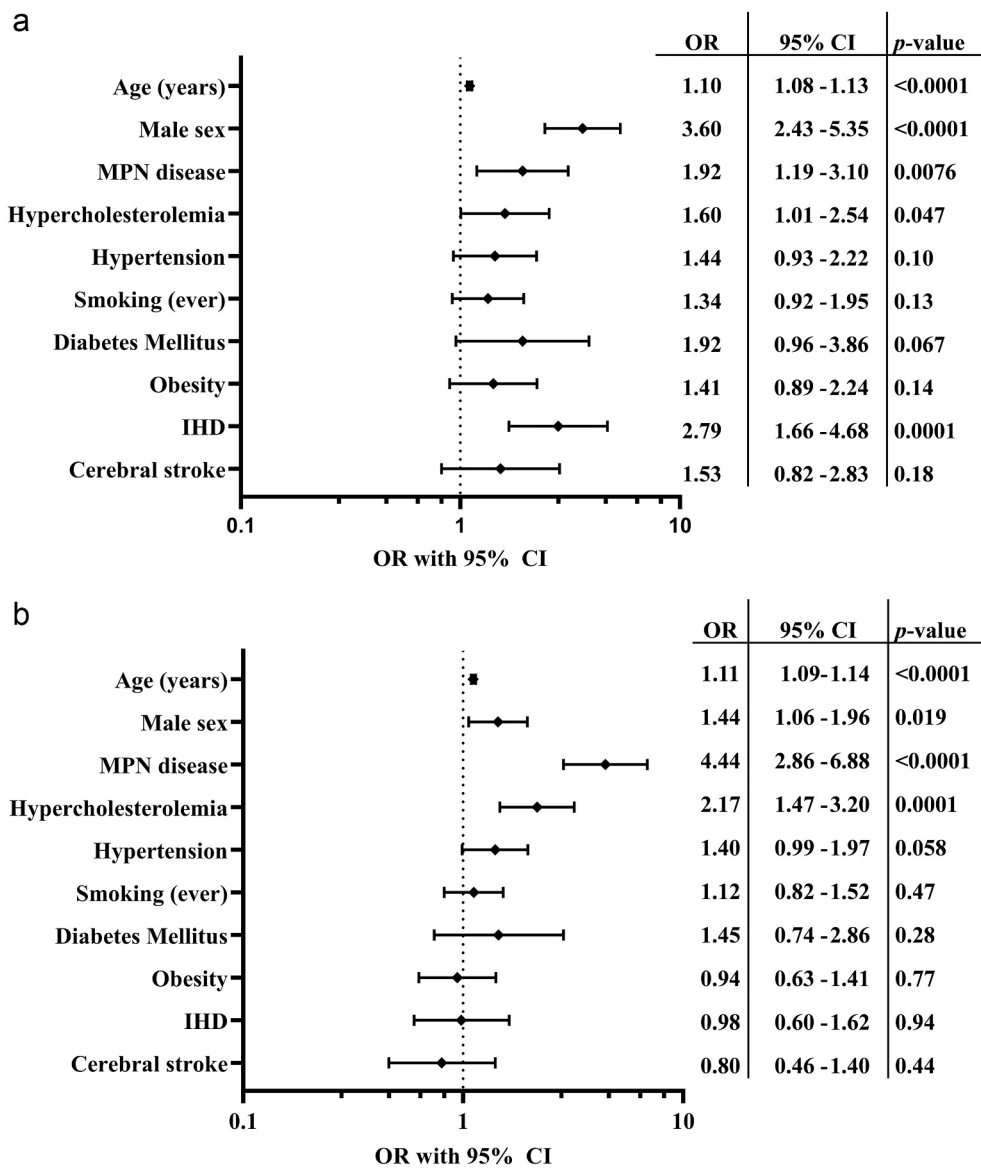


Fig. 3. Association of cardiovascular risk factors with a) CACS>400 and b) AVC. Multivariable logistic regression analyses of the association between MPN and a) CACS>400 and b) AVC. The forest plot illustrates the odds ratio of a) CACS>400 and b) AVC in MPNs compared with controls, after adjustment for age, sex, and cardiovascular risk factors. The results are presented as OR with 95% CI, and a two-sided p-value <0.05 is considered significant. CACS, coronary artery calcium score; CI, confidence interval; IHD, ischemic heart disease; MPN, Philadelphia-negative myeloproliferative neoplasms; OR, odds ratio.

(logCACS+1), to test the robustness of our hypothesis, and MPN disease was significantly associated with a higher crude CACS after adjustment. However, pre-determined cut-off risk values for CACS are more commonly used to guide clinical decisions, either as a general cut-off limit, or as comparison with predicted sex and age adjusted limit [10,11,30].

We found that coronary calcified plaques (CACS>0) were common in both MPNs and controls. Many studies have reported a lower risk of future CVD in patients with no signs of coronary calcifications (CACS = 0) compared to individuals with a similar Framingham risk score and signs of coronary calcifications (CACS>0) [31–33]. A CACS = 0 has been proposed as a risk modifier to reclassify patients at intermediate risk of CVD into a lower CVD risk category and postpone the initiation of statin therapy [18]. To establish whether a CACS = 0 can be used to reclassify individual MPN patients to a lower risk category of CVD, prospective studies are needed.

We found that patients with MPN disease show similar prevalence of CACS as patients with diabetes mellitus. Schurgin et al. found that 25.9% of 139 diabetic patients had a CACS>400, which is very close to our finding of 26% of 161 MPNs [34]. It is well established that diabetic patients have increased risk of both atherosclerosis and CVD mortality,

and preventive measures are recommended accordingly, such as strict blood pressure control, early initiation of statin treatment, and to some extent the use of acetylsalicylic acid [35]. Patients with acknowledged MPN disease, especially the subtypes ET and PV, are recommended preventive acetylsalicylic acid (Aspirin) as a standard treatment [36]. In our cohort, statin use was also more frequent in MPNs than controls, possibly due to the higher prevalence of stroke. As CVD is a substantial part of MPNs morbidity and mortality, preventive measures such as strict blood pressure control, changes in alcohol and smoking habits, weight reduction or lipid lowering treatment should have a more prominent place in the MPN guidelines [36]. Further, MPN patients may benefit from statin treatment at an early stage, if they have signs of CACS, since statins both have a lipid lowering effect as well as an anti-inflammatory effect [37,38].

4.1. Strengths and limitations

Our study has several strengths and limitations. This is the largest study of CACS data in patients with MPN and the first study of AVC in MPNs, with patients prospectively included from a highly specialized hematological outpatient clinic with detailed phenotyping. Our findings

are consistent across several statistical approaches, which supports the assumption of an increased burden of cardiac calcifications in MPNs. We did not include echocardiographic data on myocardial function or aortic valve sclerosis/stenosis, as these data were not accessible for the control group. Echocardiographic data could have added important information on how our findings affect the structural and dynamic functions of the heart. A possible limitation of our study is that we treated the MPNs as a single cohort. Some may argue that the subtypes of MPNs are too different to pool in to a single cohort, while others argue they are a continuum of the same disease. We did not find a significant difference in CACS >400 between the subtypes, which supports our choice of analysing the MPNs as one cohort. However, there was a trend towards higher prevalence of AVC and CACS >400 in the MF group, and we cannot rule out that the non-significant difference between subgroups is solely due to a lack of power. Furthermore, MPNs and controls were not examined simultaneously, but they were exact-matched on age and sex. Different CT scanners were used, as well as a slight difference in slice thickness, which could influence our results, but the difference in slice thickness was only 0.5 mm, and thus it is unlikely to have major impact on CACS. Our study does not provide proof of causality or whether the increased CACS in MPNs have prognostic value. Prospective studies are necessary to provide this kind of information.

5. Conclusion

Patients with MPNs have a significantly higher prevalence of a CACS >400 and AVC, compared to controls from the general population. The association between MPN and a CACS >400 or AVC remains significant after adjustment for cardiovascular risk factors. These novel data support the hypothesis that MPNs have an increased burden of cardiac calcifications, independent of other cardiovascular risk factors. However, prospective studies are needed to establish the causal impact of increased CACS and AVC on CVD in MPNs.

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Conflicts of interest

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Appendix A. Supplementary data

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