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Research Paper

Patients with myeloproliferative neoplasms and high levels of systemic inflammation develop age-related macular degeneration

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ABSTRACT

Background: Epidemiological data show that myeloproliferative neoplasms (MPNs) are associated with increased risk of neovascular age-related macular degeneration (AMD). However, knowledge about the retinal findings in these patients is lacking. This study was conducted to examine retinal ageing and the prevalence of a hallmark of AMD; drusen, in patients with MPNs. Further, we examine the role of chronic systemic inflammation, considered central in both AMD and MPNs.

Methods: In this single-centre cross-sectional study, we consecutively enrolled 200 patients with MPNs. The study was divided into three substudies. Firstly, we obtained colour fundus photographs from all patients to evaluate and compare the prevalence of drusen with the published estimates from three large population-based studies. Secondly, to evaluate age-related changes in the various retinal layers, optical coherence tomography images were obtained from 150 of the patients and compared to a healthy control group, from a previous study. Thirdly, venous blood was sampled from 63 patients to determine the JAK2V617F allele burden and neutrophil-to-lymphocyte ratio (NLR), a marker of systemic inflammation, in MPN patients with and without drusen.

Findings: Patients with MPNs had an increased risk of having large drusen compared to the three population-based studies OR 5·7 (95%CI, 4·1–8·0), OR 6·0 (95%CI, 4·2–8·4) and OR 7·0 (95%CI, 5·0–9·7). Also, we found that the retinal site of drusen accumulation - the Bruch's-membrane-retinal-pigment-epithelium-complex was thicker compared to healthy controls, 0·43μm (95%CI 0·17–0·71, p = 0·0014), but there was no sign of accelerated retinal ageing in terms of thinning of the neuroretina. Further, we found that MPN patients with drusen had a higher level of systemic inflammation than MPN patients with no drusen (p = 0·0383).

Interpretation: Patients with MPNs suffer from accelerated accumulation of subretinal drusen and therefore AMD from an earlier age than healthy individuals. We find that the retinal changes are located only between the neuroretina and the choroidal bloodstream. Further, we find that the drusen accumulation is associated with a higher JAK2V617F allele burden and a higher NLR, suggesting that low-grade chronic inflammation is a part of the pathogenesis of drusen formation and AMD.

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1. Introduction

The Philadelphia-negative myeloproliferative neoplasms (MPNs) are acquired clonal stem cell neoplasms and include essential thrombocythaemia (ET), polycythaemia vera (PV), and primary myelofibrosis (PMF). These diseases are characterised by an excess production of one or more of the mature blood cells from the myeloid lineage, and they cause massive systemic inflammation as well as neoangiogenesis and fibrosis in the bone marrow [1–3]. Recently it has been shown that patients with MPNs are at increased risk of neovascular age-related macular degeneration (AMD) [4], a progressive degenerative disease of the retina, causing loss of central vision. Worldwide AMD accounts for 8.7% of legal blindness, and it is the most common cause of visual impairment in the western world [5]. The hallmark sign of AMD is drusen. Drusen consist of cellular debris located between the retinal pigment epithelium (RPE) and Bruch's membrane. AMD-associated lesions: drusen and pigmentary abnormalities, characterise the early stages of AMD. In addition, the late stages

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Research in context

Evidence before this study

A most recent register study has reported that patients with myeloproliferative neoplasms (MPNs) have a higher risk of neovascular age-related macular degeneration (AMD) than an age- and sex-matched control group. However, this study did not provide any insight into the retinal findings in these patients. We are unaware if patients with MPNs are more susceptible to choroidal neovascularisation, or if this group of patients are victims of accelerated retinal ageing. Age-related macular degeneration (AMD) is a progressive, degenerative retinal disease, causing loss of central vision and accordingly the leading cause of blindness in the western world. The prevalence of AMD is expected to increase drastically due to a change in demographics. The disease is a major burden both to the individual afflicted and to the health care systems worldwide.

Despite extensive research, the exact pathophysiology of AMD remains unknown. In recent years evidence has emerged, demonstrating the role of the immune system and chronic inflammation in the pathogenesis of the disease. Importantly, we recently conducted a systematic review showing that neutrophil-to-lymphocyte ratio (NLR), a marker of systemic inflammation, is elevated in patients with AMD.

Chronic inflammation is a driving force for the clonal evolution and disease progression in MPNs, and these patients have important similarities with patients with AMD such as inflammation, angiogenesis, and fibrosis.

Treatment with anti-VEGF has improved visual outcomes for patients with the neovascular/"wet" form of late AMD, but no cure is available, and there are no treatment options for the "dry" form of late AMD (geographic atrophy).

Added value of this study

This study shows that patients with MPNs have a significantly higher prevalence of drusen and consequently AMD from a younger age than persons without MPNs. Drusen is cellular debris located between the retinal pigment-epithelium (RPE) and Bruch's membrane (BM). In this study, we find that patients with MPNs have a thicker RPE-BM-complex than a healthy control group, further supporting that accumulation is located below the outer retina but does not involve the neuroretina, as in normal retinal ageing. Finally, we find that neutrophil-to-lymphocyte ratio (NLR), a marker of systemic inflammation, is higher in MPN patients with drusen compared to those without drusen.

This information is of great importance for the individual MPN patient who might benefit from retinal screening examination, but our results also provide important insights into the development of AMD.

Implications of all the available evidence

We found an increased prevalence of drusen and AMD and also discovered that MPN patients with drusen have a higher NLR than MPN patients with normal retinal ageing changes. These results combined with the knowledge that chronic inflammation is a driving force for the development of MPNs, adds proof to the concept of chronic inflammation and immune dysregulation as shared mechanisms for the development of drusen, AMD and MPNs. Further studies are needed to elucidate the underlying causal association between AMD and MPNs and the factors eliciting drusen formation in patients with MPNs. Studying this group of patients has the potential to reveal novel aspects of the pathogenesis of AMD, and hopefully pave the way for a treatment option for AMD, which reduces drusen formation and progression to the debilitating late stage.

of AMD have either characteristic neovascular lesions or sharply demarcated areas of retinal atrophy, defined as neovascular AMD and geographic atrophy (GA), respectively [6]. Despite extensive research, the exact pathophysiology of AMD remains unknown. Certain risk factors are known such as age, cigarette smoking and genetic susceptibility [7,8]. In recent years evidence have emerged demonstrating the role of the immune system, including inflammation, in the pathogenesis of the disease [9–18]. Since MPNs cause massive inflammation and a recent register study has reported that patients with MPNs have a significantly higher risk of neovascular AMD [4], it is intriguing to investigate these patients in the context of AMD.

The two disease groups, MPNs and AMD, have important similarities such as inflammation, angiogenesis, and fibrosis. However, knowledge of the retinal status in patients with MPNs is lacking. We are unaware if MPNs are merely associated with angiogenesis, or if the occurrence of other AMD-associated lesions is also increased in these patients. With this study, we investigated an unselected cohort of patients with MPNs. As the first substudy, we evaluated fundus photographs to assess AMD-associated lesions and thereby the prevalence of different AMD-stages. In substudy two, we further investigated the retinas of these patients with optical coherence tomography (OCT) images to evaluate the age-related changes in the structural layers. In substudy three, we examined the role of chronic inflammation in patients with MPNs, through measurement of the neutrophil-to-lymphocyte ratio (NLR), a marker of systemic inflammation, previously shown to be elevated in patients with AMD [19]. Further, we assessed the JAK2V617F allele burden as a marker of inflammation, since the JAK2V617F mutation is seen as a key driver of MPN-associated chronic inflammation [20].

2. Methods

2.1. Study design and participants

This cross-sectional study was approved by the Ethics Committee in Region Zealand, Denmark (SJ-588, SJ-679), and each patient provided oral and written informed consent. The eligibility criterion was a diagnosis of a Philadelphia-negative MPN (WHO2016 criteria) [21]. Patients at the Department of Haematology, Zealand University Hospital (ZUH), were invited to participate in the study. Patients were consecutively enrolled for substudy one until we reached 200, and inclusion was conducted between January 2017 and October 2019. Of the 200 patients, 150 were enrolled in substudy two to match the number in the healthy control group. Finally, 63 of the patients not receiving immunomodulating treatment were enrolled in substudy three.

In substudy one, drusen prevalence data were compared to the published estimates from three population-based studies; the Beaver Dam Eye Study (BDES), The Blue Mountains Eye Study (BMES) and The Rotterdam Study (RS) [22–24].

In substudy two, we compared layers in the retina with a healthy aged control group of 150 patients. The control group was from a previous study by Harris et al., and characteristics and inclusion criteria are described thoroughly elsewhere [25]. This control group did not have fundus photographs taken and could not be used as controls for substudy one.

In substudy three NLR and the key driver of MPN-associated chronic inflammation JAK2V617F mutation was compared between MPN patients with drusen and those without.

2.2. Imaging and grading method

All participants underwent an examination at the Department of Ophthalmology, ZUH and had their pupils dilated with tropicamide 1% before examination. For substudy one, we obtained a stereoscopic 45° colour fundus photograph centred on the macula (model TRG-NW8, Topcon). Each fundus photograph was graded using a simplified version of the Wisconsin age-related maculopathy grading system (WARMGS) [26] (supplementary material 1.1). This allows comparisons to several studies, including the population-based studies used in this study. We chose to compare our results to populations of European ancestry since ethnicity differences have been reported [5].

We applied the classification system introduced by the Beckman Initiative for Macular Research Classification Committee [27] using drusen size and presence of pigmentary abnormalities to classify AMD (supplementary material 1.3).

One investigator (CL) graded all images, and another investigator (MKN) re-graded 80 images to test intergrader agreement.

The types of drusen present were categorised in three groups according to the description from Spaide and Curcio; soft drusen, cuticular drusen and subretinal drusenoid deposits [28] (supplementary material 1.1).

For substudy two, we obtained optical coherence tomography images (SD-OCT, Heidelberg Engineering, Heidelberg, Germany) from 150 of the patients. The images were examined in Heidelberg Eye Explorer version 1.9.10.0. We used the automated segmentation and the thickness profile part of the software to measure the thickness of the different retinal layers, and segmentations was checked manually (supplementary material 1.2).

The neuroretina is the inner part of the retina and can be subdivided into several layers. The retinal pigment epithelium (RPE) is a monolayer of pigmented cells in the outer retina, essential for homoeostasis of the retina and vision. The Bruch's membrane (BM) is the innermost layer of the choroid were the fenestrated capillaries of the eyes are located. The inner part of the BM forms the basement membrane of the RPE (supplementary material 1.2). The thickness of the neuroretina and RPE-BM-complex were compared to values measured from a healthy aged control group of 150 patients [25].

2.3. Blood sampling

For substudy three, venous blood from antecubital veins was sampled from 63 patients, not receiving immunomodulating treatment - 35 of the patients had drusen, and 28 had normal ageing changes. The blood was sampled in an ethylenediaminetetraacetic acid-coated (EDTA) tube and analysed on Sysmex KX-21NTM (Sysmex Corporation, Kobe, Japan), to measure white blood cells count; lymphocytes count, and percentage; neutrophils count, and percentage; and monocytes–basophils–eosinophils mixed count and percentage. Sample volume for the count was 50 μl. NLR was calculated by dividing the absolute neutrophil count by the absolute lymphocyte count. The JAK2V617F mutation analysis was performed on peripheral blood EDTA anticoagulated blood with highly sensitive real-time quantitative PCR on an ABI Prism7900HT (Applied Biosystems, Foster City, CA, USA), on fluorescence-activated cell sorted (FACS) monocytes, lymphocytes and granulocytes on a FACSVantage (BD Biosciences). DNA was extracted using a MagnaPure robot (Roche Diagnostics, Mannheim, Germany) according to the manufacturer's protocol.

2.4. Outcomes

The main outcomes were drusen size (largest present), pigmentary abnormalities, early-, intermediate- and late AMD, neuroretinal- and RPE-BM thickness, NLR and JAK2V617F allele burden. Secondary outcomes were area-covered-by drusen; drusen count, and drusen type.

2.5. Statistical analysis

To detect a difference of at least 20%, at a 5% significance level and a power of 80%, the total sample size required is 140–210 [29]. We aimed for 200 in substudy one and 150 in substudy two. Power calculations for substudy three were based upon previous immunologic studies of neovascular AMD were calculations show a sample size of minimum 26 in each group is necessary [30].

We analysed the data using the SAS statistical software package (SAS ver. 9.4; SAS Institute Inc.).

In substudy one, the age-associated prevalence rates were compared with the estimates from the population studies by using the Chi-squared test. To assess interobserver-agreement in image-grading, we calculated Cohen's kappa coefficient [31]. We found strong agreement for drusen size (weighted kappa=0·87) and drusen count (weighted kappa=0·86), moderate agreement for drusen area (weighted kappa=0·79) and weak agreement for pigment abnormalities (weighted kappa=0·47).

In substudy two and three, linear regression models with age as exposure variable were used to investigate the relationship between the continuous outcome variables; layer thicknesses of the retina, NLR and JAK2V617F allele burden. Non-normal data were transformed. Where outcome was independent of age, a two-sided t-test was used for normally distributed data and Wilcoxon rank-sum test for non-normal data. Otherwise, analysis of covariance (ANCOVA) was used to estimate differences between the two regression lines corresponding to groups.

2.6. Role of the funding source

The funding sources had no role in the design and conduct of the study; collection, analysis, and interpretation of the data; preparation, review, or approval of the manuscript; or the decision to submit the manuscript for publication. The corresponding author confirms that she had full access to all the data in the study and had final responsibility for the decision to submit for publication.

3. Results

3.1. Study population

For substudy one, 200 patients were enrolled. One was excluded post hoc since the diagnosis of MPN was later questioned. Eight patients were excluded due to poor image quality of the obtained fundus photographs. As a result, 191 patients had gradable photographs and were included for analyses.

For substudy two, we obtained OCT images for 150 of the patients. Seven patients with late AMD and four with poor image quality were excluded. As a result, 139 were included for further analyses. The healthy control group used in substudy two had no ocular or systemic diseases known to influence the macula (cancer, blood diseases, severe hypertension or diabetes) and they had normal macular ageing changes (no or few drusen). For more information on the control group see Harris et al. [25]

For substudy three, we collected blood samples from 63 of the patients.

Patient characteristics are shown in Table 1. The majority of the patients suffered from PV and to a lesser extent ET and PMF. Most patients had the JAK2V617F mutation, associated with MPNs. The patients received different treatments for their MPNs; Peginterferon alfa-2a, Ruxolitinib or a combination, hydroxyurea, anagrelide, acetylsalicylic acid or other drugs with anticoagulant effect, and statins.

Table 1
Characteristics of the participants in substudy 1, 2 and 3.

	Substudy 1		Substudy 2 MPN patients		Substudy 2 Control group		Substudy 3 Patients with drusen		Substudy 3 Patients without drusen	
Patients included, no.	191		139		150		35		28	
Age, years mean (SD)	64·8	(10·9)	65·2	(10·5)	70·6	(9·5)	69·2	(9·2)	66·0	(11·5)
BMI, kg/m^2 mean (SD)	25·8	(4·5)	25·9	(4·6)	26·1	(4·9)	25·5	(4·3)	27·7	(6·3)
Sex, n (%)										
Females	95	(49·7)	74	(53·2)	92	(61)	15	(42·9)	17	(60·7)
Males	96	(50·3)	65	(46·8)	58	(39)	20	(57.1)	11	(39·3)
Smoking, n (%)										
Never	89	(46·6)	68	(48·9)	83	(55)	16	(47·1)	12	(42·9)
Former	88	(46·1)	64	(46·1)	-*	-*	18	(52·9)	14	(50·0)
Smoker	14	(7·3)	7	(5·0)	-*	-*	1	(3·0)	2	(7·1)
MPN Diagnosis, n (%)										
PV	108	(56·5)	78	(56·1)			26	(74·3)	14	(50·0)
ET	48	(25·1)	39	(28·1)			6	(17·1)	11	(39·3)
PreMF	3	(1·6)	1	(0·7)			0	(0·0)	1	(3·6)
PMF	32	(16·8)	21	(15·1)			3	(8·6)	2	(7·1)
Mutation, n (%)										
JAK2V617F	165	(86·4)	120	(86·3)			31	(91.3)	23	(82·2)
CALR	16	(8·4)	13	(9·4)			1	(2·9)	3	(10·7)
MPL	1	(0·5)	1	(0·7)			1	(2·9)	0	(0·0)
Triple Negative	9	(4·7)	5	(3·6)			1	(2·9)	2	(7·1)

PV: Polycythaemia vera, ET: Essential thrombocythemia, PreMF: Pre-myelofibrosis, PMF: primary myelofibrosis JAK2V617F: mutation in the JAK2 gene, CALR: calreticulin gene, MPL: MPL gene, gene encoding the thrombopoietin receptor.
* The control group in substudy two was from a previous study and smoking status was categorised in "ever smokers" - 67 participants (45%) and "never smokers" – 83 participants (55%). Therefore, "smokers" and "former" smokers are included in the category "ever smokers".

It was not possible for us to assess the potential effect of treatment on retinal changes since detailed information regarding treatment, was not available. We did not have information on previous treatment or for how long the treatments had been administered.

3.2. Substudy 1 – AMD-associated lesions and AMD-stages

Drusen size (largest present): For MPN patients younger than 54 years, the most frequent finding was small drusen less than 63μm. With increasing age, the prevalence of larger drusen increased, and the prevalence of small drusen correspondingly decreased. Large drusen greater than 125μm increased from 10% in patients younger than 45 years to 45% in patients 75–84 years (Fig. 1).

Drusen count: Only 6·4% of patients with MPNs had no drusen in the macular area, 34·2% had between one to nine drusen, and 59·4% had ten or more drusen. The number of drusen increased with increasing age. Ten or more drusen were seen in 20·0% of patients younger than 45 years and 71·8% of the patients aged 75–84 years.

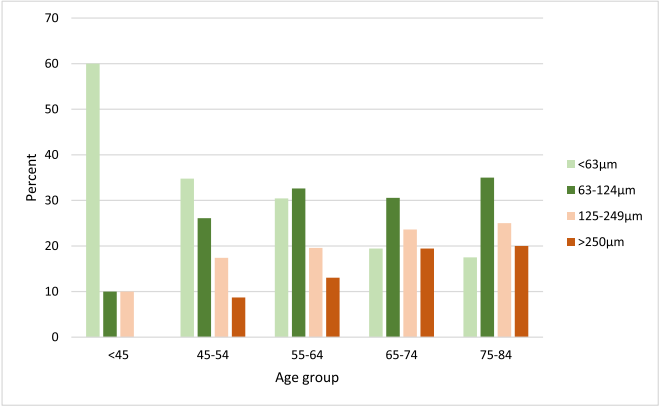

Fig. 1. Age-specific distribution of maximum drusen size within a radius of 3000 μm from the fovea of the worst eye in patients with myeloproliferative neoplasms.

Drusen type: For patients with drusen larger than 63 μm (129 patients), the drusen-type were primarily soft drusen (91·1%). In 11 patients, both soft drusen and one of the two types, cuticular drusen or subretinal drusenoid deposits, were also found.

Drusen area: The area-covered-by drusen within the grading-grid was greater than 0·069 mm^2 in 49·7% of the patients, greater than 0·146 mm^2 for 26·5%, greater than 0·487 mm^2 for 12·4%, greater than 1·27 mm^2 for 4·9% and greater than 2·5 mm^2 for 2·2% of the patients.

Pigmentary abnormalities: Of the 184 patients without late AMD, we found pigmentary abnormalities in 25 cases (13·6%; CI 9·4–19·3%). Nine had increased pigment, nine had hypopigmentation, and seven had combined types. Accordingly, 16 patients (8·7%) had increased pigment, and 16 patients (8·7%) had decreased pigment. None of the patients younger than 45 years had pigmentary abnormalities. For the age groups 45–54, 55–64, 65–74 and 75–84 years, the prevalence was 13·6%, 4·3%, 17·1% and 22·2%, respectively. Except for the age group 55–64 years, pigment abnormalities were increasing with age.

Presence of AMD: Table 2 shows the prevalence of AMD stages. The prevalence of intermediate and late AMD increased with age. The prevalence of early AMD increased with age in patients up to 55–64 years. For patients older than 65 years, the prevalence of early AMD decreases as the prevalence of intermediate and late AMD increases. Late AMD was seen in seven patients (3·7%; CI, 1·8%–7·4%), six had geographic atrophy, and one had neovascular AMD. In patients younger than 45 years and patients aged 55–64 years, none had late AMD. For the age groups 45–54 years, 65–74 years and 75–84 years, the prevalence was 4·4%, 2·8% and 10·0%, respectively.

3.3. Results compared to prevalence rates from population studies

Drusen size: Fig. 2 compares the prevalence of large drusen (>125 μm) between patients with MPNs and the population studies. The figure further shows that patients with MPNs at the age of 45–54 years have the same prevalence of large drusen as a population that is 75–84 years old, indicating that patients with MPNs develop intermediate AMD at an earlier age. The prevalence rates of large drusen were significantly higher in patients with MPNs (p-

Table 2
Age-specific prevalence of all stages of age-related macular degeneration (AMD) in patients with MPNs and the prevalence rates of late AMD in the same age groups from The Beaver Dam Eye Study, The Blue Mountains Eye Study and the Rotterdam Eye Study.

	Patients with MPN			Beaver Dam Eye Study	Blue Mountains Eye Study	Rotterdam Study
Age group	Early AMD % (95%CI)	Intermediate AMD % (95%CI)	Late AMD % (95%CI)	Late AMD %	Late AMD %	Late AMD %
<45	10·0 (1·8–40·4)	10·0 (1·8–40·4)	0·0	–	–	–
45–54	13·0 (4·5–32·1)	26·1 (12·6–46·5)	4·4 (0·8–21·0)	0·1[1]	(0·0)[3]	–[4]
55–64	30·4 (19·1–44·8)	32·6 (20·9–47·0)	0·0	0·6	(0·2)	(0·2)
65–74	18·1 (10·9–28·5)	44·4 (33·5–55·9)	2·8 (0·8–9·6)	1·4	(0·7)	(0·8)
75–84	22·5 (12·3–37·5)	40·0 (26·4–55·4)	10·0 (4·0–23·1)	7·1[2]	(5·4)	(3·7)
Total	20·9 (15·8–27·3)	36·6 (30·1–43·7)	3·7 (1·8–7·4)	1·6	(1·4)	(1·7)

MPN: myeloproliferative neoplasms.
The age groups in The Beaver Dam Eye Study and The Blue Mountains Eye study are different from the age groups in this study: [1]age group 43–54 years. [2]age group 75–86 years. [3]age group 49–54 years. [4]The Rotterdam Study did not include patients younger than 55 years.
Since the classification of the earlier stages of AMD is different in our study compared to the population-based studies, it is not possible to compare these stages.

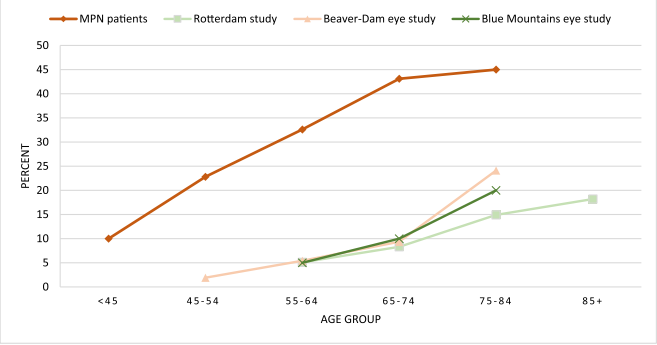

Fig. 2. Comparison of the prevalence of drusen >125 μm as the largest drusen present within a 3000 μm radius of the fovea between patients with Myeloproliferative neoplasms and three large population-based studies (Beaver Dam Eye Study, Blue Mountains Eye Study and Rotterdam Study).

values<0.001). For patients 55–84 years, the odds ratio (OR) of having large drusen for MPN patients were 5·7 (95%CI: 4·1–8·0), 6·0 (95%CI: 4·2–8·4) and 7·0 (95%CI: 5·0–9·7) compared to the BDES, BMES and RS population, respectively. Age-specific odds ratios are given in Table 3.

Only RS gives the exact prevalence of drusen size 63–125 μm; 35·7%, 43·2%, 41·0% in the age groups 55–64 years, 65–74 years and 75–84 years, respectively. The comparable prevalence in MPN patients was 32·6%, 31·4% and 38·9%. There was no significant difference between MPN patients and the RS population in intermediate-sized drusen (P = 0.11).

Drusen area: The area-covered-by drusen within the grading-grid was greater than 0·069 mm^2 in 49·7% of the patients and greater than 1·27 mm^2 for 4·9% of the patients. These areas correspond to approximately to 0·3% and 4·5% of the macula area. In BMES similar numbers are given. Drusen covered more than 0·2% in 15·3% of the participants and more than 4·7% in 6·2% of the participants.

Pigmentary abnormalities: To compare with BDES and BMES, we excluded the age group younger than 45 years and patients with late AMD. Twenty-five (14·4%) of the MPN patients had hyperpigmentation, hypopigmentation or both. In the BDES and BMES, the prevalence of any pigmentary abnormality was 13·1% and 11·9% (data was not available to exclude patients aged 85+ in the BDES). There were no significant differences between the prevalence of any pigmentary abnormalities in patients with MPNs and the BDES (OR 1·1, CI 0·7–1·7) or the BMES (1·2, CI 0·8–1·9).

Excluding further the age-group 45–54 years to compare with the RS population, the prevalence of any pigmentary abnormalities in patients with MPNs was 14·5%. The prevalence in the RS study was 7·0% (age group 85+ excluded). Patients with MPNs had a significantly higher risk of pigmentary abnormalities compared to the RS population (OR 2·2, CI 1·4–3·5).

In patients with MPNs, 8·7% had hyperpigmentation, and 8·7% had hypopigmentation. These numbers for hyper- and hypopigmentation was for BDES 12·2% and 8·3%, BMES 12·1% and 5·8%, RS 5·9% and 4·4%.

Patients with MPNs had a significantly higher risk of having hypopigmentation compared to the BMES (OR 1·8, CI 1·0–3·0) and a significantly higher risk of both hyper- and hypopigmentation

Table 3
Age-specific odds ratios for large drusen >125 μm within a 3000 μm radius of the fovea for MPN patients compared to the Beaver Dam Eye Study, The Blue Mountains Eye Study and The Rotterdam Study.

	Beaver dam Eye Study		Blue Mountains Eye Study[b]		Rotterdam Study[c]	
Age group	OR (CI)	p-value	OR	p-value	OR	*p*-value
45–54	17·3 (6·4–47·1)	<0·0001	–	–	–	–
55–64	8·5 (4·4–16·5)	<0·0001	9·1 (4·7–17·8)	<0·0001	9·3 (4·9–17·5)	<0·0001
65–74	7·5 (4·5–12·5)	<0·0001	6·8 (4·1–11·2)	<0·0001	8·3 (5·1–13·5)	<0·0001
75–84	2·6 (1·3–4·9)	0·0055	3·2 (1·7–6·3)	0·0005	4·7 (2·5–8·8)	<0·0001
Total	7·3 (5·3–10·1)[a]	<0·0001	6·0 (4·2–8·4)	<0·0001	7·0 (5·0–9·7)	<0·0001

[a] If the age group 45–54 is left out OR is 5·7 (4·1–8·0) as given in the main text.
[b] The prevalence of large drusen in The Blue Mountains Eye Study was not reported as exact numbers but are given as "less than 5%" for participants younger than 65 years, "10%" for participants 65–74 years, and "almost 20%" for those 75 years or older. We used these numbers as exact numbers for the age groups 55–64 years, 65–74 and 75–84 years to compare large drusen between the BMES population and patients with MPNs.
[c] The Rotterdam Study did not include patients younger than 55 years.

compared to the RS population (OR 1·8, CI 1·1–3·1) (OR 2·0, CI 1·1–3·6).

Late AMD: Patients with MPNs had a significantly higher prevalence of late AMD compared to BDES, BMES and RS (Table 2) (p-values=0·0176, 0·0063, 0·0079). Patients with MPNs have a higher risk of late AMD compared to BDES (OR 2·5 [CI 1·1–5·5]), BMES (OR 2·9 [CI 1·3–6·6]) and RS (OR 2·9 [CI 1·3–6·8]). Only one patient with MPN had neovascular AMD, and six had geographic atrophy (GA), which is opposite to the population studies where neovascular AMD was found to have a higher prevalence than GA.

3.4. Substudy 2 – retinal thickness

The thicknesses of the retinal layers were approximately normally distributed. The RPE-BM layer was 0·43μm (ANCOVA, 95%CI 0·17–0·71, p = 0·0014) thicker in patients with MPNs compared to the healthy control group. Mean RPE-BM thickness in the macula for patients with MPNs was 14·39μm (95%CI 14·19–14·58) compared to 13·96μm (95%CI 13·75–14·16) for the healthy control group (which was older than the MPN patients, p-value <0·0001)

There was no significant difference in neuroretinal macular thickness between patients with MPNs and the healthy control group (ANCOVA, p-value=0·2990). Mean neuroretinal macular thickness in patients with MPNs was 286·55 μm (95%CI 283·85–289·25) compared to 288·95μm (95%CI 286·54–291·36) for the healthy controls.

We also evaluated each sublayer of the neuroretina in the centre-, inner and outer subfield independently (supplementary material 2).

3.5. Substudy 3 – chronic systemic inflammation and drusen formation

Neutrophil-to-lymphocyte ratio was not normally distributed and log-transformed. Linear regression lines were fitted for each group and showed that NLR was not dependent on age. We found that MPN patients with drusen had a significantly higher NLR than MPN patients without drusen, estimated difference 1·37 (T-test, 95%CI 1·02–1·86, p = 0·0383).

The JAK2V617F allele burden was non-normally distributed and did not fit an approximately normal distribution with transformation. Linear regression showed that the allele burden was not dependent on age. We found a higher allele burden of JAK2V617F among patients with MPNs and drusen (Wilcoxon rank-sum test, P = 0·0754). Thirty-three out of 35 patients (94·3%) with MPNs and drusen had the JAK2V617F mutation, and the median allele burden was 33·00 [IQR 11·00–56·00]. Only 24 out of 28 of the patients (85·7%) without drusen had the JAK2V617F mutation, and the median allele burden was 17·50 [IQR 5·55–28·50].

4. Discussion

In this study of patients with Philadelphia-negative MPNs, we found a higher prevalence of large drusen and AMD compared to estimates from large population-based studies. In addition, the retinal changes appear at an earlier age in patients with MPNs. Our data support the findings in a register study by Bak et Al [4], showing that MPN patients are at increased risk of having neovascular AMD. Further, we find that MPNs are not only related to neovascularization but also to the subretinal accumulation and the formation of drusen. We found no difference in the neuroretina suggesting that the retinal changes are not due to overall accelerated ageing of the retina, but rather to the accumulation of debris between the neuroretina and the choroidal bloodstream.

Studies of the natural history have shown that the risk of developing late AMD increases with advancing age and that the number and size of drusen also possess predictive value. Drusen volume, bilateral drusen and pigment abnormalities further increase the risk of late AMD [32,33]. Patients with MPNs have large drusen in their macular area, and a higher prevalence of late AMD compared to population studies. In our study, we found a higher occurrence of GA than neovascular AMD. The register study by Bak et al. reports an increased risk of neovascular AMD, but our results indicate that GA could be even more prevalent.

Numerous studies have shown that low-grade chronic inflammation is involved in the pathogenesis of AMD [9–11,34–39] and chronic inflammation and immune deregulation are common features between AMD and MPNs [1,9,45,46,11,35,38,40–44]. Thus, chronic inflammation has been proposed as both a trigger and a driver of clonal evolution in MPNs [47]. The neoplastic clone is a major source of inflammatory cytokines, released into the systemic circulation, and contributing to the symptom burden in patients with MPNs, and the inflammation-mediated comorbidities, including cerebral- and cardiovascular diseases, the increased risk of autoimmune diseases and second cancer [47,48]. Similar to MPNs, AMD has been associated with systemic diseases characterised by immune modulation or inflammation, such as diabetes, cardiovascular disease and AIDS [7,49–51]. Rozing et al. have proposed a two-level hypothesis of the development of age-related degenerative diseases, including AMD [18]. The hypothesis includes two steps: the first step is an accumulation of retinal damage due to ageing, and the second step is the following inflammatory host response to these damages. Both steps should be present to develop AMD. In patients with MPNs, inflammation plays a pivotal role in disease pathogenesis and therefore possesses a massive “second step” contribution to the development of AMD. NLR is a good and relatively stable indicator of subclinical systemic inflammation [52], and we found that MPN patients with drusen have a significantly higher NLR and a tendency of higher JAK2-allele burden than MPN patients without drusen, further supporting a role for chronic inflammation in drusen formation and the pathogenesis of AMD.

In this context, it is intriguing to consider chronic inflammation as a major contributing common factor in disease pathogenesis between AMD and MPNs. Using the MPNs as a Human Inflammation Model [1,53] comparative molecular, genomic and immunological studies between drusen/AMD and MPNs are envisaged to unravel novel insights into disease-promoting mechanisms and how to modify disease evolution by targeting the common denominator for disease evolution and progression – chronic inflammation.

Another feature that occur during development of AMD is accumulation of mononuclear phagocytes in the subretinal space (between the photoreceptor’s outer segments and the RPE). The subretinal space is normally immune privileged and when mononuclear phagocytes occasionally reach the space in healthy individuals, these are eliminated by the immune-suppressive properties of the RPE. Animal models show that the mononuclear cells that infiltrate the subretinal space are likely resident microglia but also blood derived, monocyte derived inflammatory macrophages [54]. In donor eyes of patients with late AMD, the same mononuclear cells have been found to accumulate [55].

Microglia respond to neuronal damage, and they increase in number and change morphology due to injury, ageing and disease. Microglia release inflammatory mediators and phagocytose and remove cellular debris [56,57]. The components of drusen have been shown to retain microglia in the subretinal space and thereby sustain the inflammatory response which turns destructive and can influence photoreceptor and RPE integrity [58].

The chronic pro-inflammatory environment in patients with MPNs could also contribute to the overactivation of microglia by promoting blood-derived inflammatory mononuclear phagocytes with characteristics that lead to resisted elimination and excessive recruitment in the subretinal space [54].

Important limitations must be kept in mind when interpreting the results. Firstly, patients were voluntarily referred for ophthalmological examination, and we have no information on the patients who

refused to be a part of the study. This might lead to selection bias. Nonparticipation may be more common among those without vision-related complaints, but nonparticipation could also be related to more severe disease and therefore, more advanced AMD. The life expectancy in patients with MPNs is lower than the general population [59]. The result of this could be that only patients with mild MPN disease reach old age. Consequently, this could lead to an underrepresentation of older patients with severe disease and accordingly, a lower estimation of late AMD in these age groups.

Secondly, there was a difference in MPN diagnosis, duration of disease, and treatment regime. This is likely to influence retinal findings. A newly diagnosed patient may not have had the time to develop drusen compared to a patient who has had the diagnosis for many years. The medical treatment could also affect drusen formation due to immune-modulating effects. The idea that treatment can influence drusen presence is not new. In patients treated with statins, drusen regression has been reported [60–62]. Since cardiovascular diseases are common in MPNs patients they are often being treated with statins. Many of the patients in our study also receive immunomodulating treatment, and this may prevent some of the patients with intermediate AMD and GA from turning in to neovascular AMD, a possible explanation of the high occurrence of GA in our study.

In conclusion, this study demonstrates that patients with MPNs have a significantly higher prevalence of drusen and consequently AMD from a younger age than persons without MPNs. Further, the MPN patients with drusen have a higher level of chronic systemic inflammation compared to patients without drusen. These findings add evidence to the concept that chronic systemic inflammation is involved in the pathogenesis of AMD. However, additional studies - including the very early stages of MPN development - are needed to elucidate the underlying causal association between AMD and MPNs and the factors eliciting drusen/AMD in patients with MPNs. Studying patients with MPNs have the potential to reveal novel aspects on the pathogenesis of AMD.

Contributors

All named authors conceptualised the study. CL led the data collection and data analysis with inputs from MKN. CL led the interpretation of data and wrote the first draft of the manuscript with inputs from MKN, HCH, and TLS. All named authors contributed to the critical revision of the manuscript. CL obtained funding with help from TLS. HCH and TLS led study supervision.

All named authors read and approved the final draft.

Data statement

The dataset used for the statistical analyses are available from the corresponding author (CL), upon request."

Declaration of Competing Interest

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Supplementary materials

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